THE USE OF PUPILLOMETRY, SEROLOGY, ETHNICITY AND IMAGING IN THE DIAGNOSIS OF OPTIC NEURITIS

Mithu Storoni

UNIVERSITY COLLEGE LONDON

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DECLARATIONS

Chapter 1:

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The construction of the pupillometer used was undertaken by Mr. Fion D. Bremner and Professor Stephen Smith (deceased) of the National Hospital of Neurology and Neurosurgery, Queen Square, London.

Chapter 3:

The construction of the testing equipment setup and its calibration was undertaken by Professor John L. Barbur and by Mr Wei Bi, both of the Applied Vision Research Centre, City University, London. Patient testing was undertaken in collaboration with Mr Wei Bi. The results of this chapter have been presented at the Association for Research in Vision and Ophthalmology (ARVO) annual conference, in Fort Lauderdale, Florida in May 2011.

Chapter 5:

The construction of the testing equipment was undertaken by Professors Sei-ichi Tsujimura of Kagoshima University, Japan and John Barbur of the Applied Vision Research Centre, City University, London. Its calibration was undertaken by Professor Sei-ichi Tsujimura of Kagoshima University, Japan and Mr Wei Bi of the Applied Vision Research Centre, City University, London. Testing was carried out in collaboration with Mr Wei Bi of the Applied Vision Research Centre, City University, London who detrended the results using MatLab©.

Chapter 7:

Dr Axel Petzold of the UCL Institute of Neurology, Neuroimmunology & CSF Laboratory, Queen Square, London, carried out the serum laboratory analysis for GFAP using an assay technique which has been published before. (Petzold A, et al. (2004) An ELISA for glial fibrillary acidic protein. J Immunol Meth, 287:169–177) Excerpts of this chapter have been published in:

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Chapter 9:

The MRI images of the patients were assessed independently by two Neuroradiologists (Drs Indran Davagnanam and Mark Radon, both of the Dept. of Neuroradiology, Queen Square, London), as part of the project. Dr Mark Radon provided valuable assistance with the statistical analysis carried out within this chapter. The results from this chapter were presented at the European Neuro-ophthalmology society meeting (EUNOS) in Barcelona, Spain in 2011 and at the NANOS conference in Texas, U.S.A. in February 2012.

I, Mithu Storoni, confirm that the work presented in this thesis is my own.

Where information has been derived from other sources, I confirm that this has been indicated either within this section or within the remainder of this thesis.

Mithu Storoni

ABSTRACT

‘Acute isolated optic neuritis' may be the first manifestation of both Multiple Sclerosis (MS) and Neuromyelitis Optica (NMO). Twenty percent of patients with MS in western Europe present with optic neuritis as their first relapse (Mcdonald & Compston, 2006). NMO has been recently found to be more common amongst the Caucasian population of northern Europe than previously believed (Asgari et al, 2011). Patients with NMO may experience a long temporal delay after acute isolated optic neuritis before another relapse occurs, which can help to confirm the diagnosis (Wingerchuk et al, 2007). In such cases an episode of optic neuritis caused by NMO may be indistinguishable from optic neuritis caused by MS.

This thesis explores differences in the manifestation of optic neuritis caused by MS and that caused by NMO and evaluates four ways in which the two aetiologies may be identified from one another: pupillometry, serum glial fibrillary acidic protein analysis, ethnic background considerations and MRI findings in the context of the visual pathways.

The thesis begins by assessing the potential role of pupillometry in the diagnosis of optic nerve disease; eventually investigating its potential in discriminating between MS related optic neuritis and NMO related optic neuritis. The results of the first part of the thesis indicate the usefulness of pupillometry in patients with optic neuritis who show poor recovery, when tested in a chronic setting. Three further ways of differentiating optic neuritis caused by MS and NMO in an acute setting are then pursued. First, the measurement of serum Glial Fibrillary Acidic protein (GFAP) is

shown to be a useful potential indicator of the presence of NMO. Second, the ethnic background of a patient is found to correlate with the risk of NMO. Third, the Magnetic Resonance (MR) image of the visual pathway of patients with optic neuritis from the two aetiologies is found to differ with regard to the lesion extent and the lesion site. The four investigative approaches tested in this thesis (pupillometry, serology testing for GFAP, assessment of ethnic background and MR image) can be combined to offer a patient with isolated optic neuritis of unknown cause a likelihood of suffering from NMO. The latter three methods may be used to assess the risk of NMO in a patient presenting acutely with optic neuritis in the absence of any other sign of underlying disease, and may allow for the appropriate management of this condition.

LITERATURE REVIEW OF PUPILLOMETRY

The history of the pupillary light reflex in its use as an indicator of disease

The use of the pupillary response in the management of eye disease was first documented in the 2nd century CE when cataract couching was popular practice. Galen described the use of the pupil to predict which patients will benefit from this procedure by performing a cover test on patients focussing at a distance, although the precise relationship of the pupil to the optic nerve was yet unknown (Thompson & Corbett, 1991). The relationship between disease in the optic nerve and an impaired pupil response to light was established in the nineteenth century, after the invention of the ophthalmoscope. Hirschberg (1884) described a case of subacute visual loss and a normal fundal examination which was dismissed as hysteria until an asymmetry in the pupillary light response resulted in a diagnosis of retrobulbar optic neuritis. Twenty years following this report, Gunn (1902) published a series of similar cases in which pupillary escape to a sustained bright light stimulus was used to differentiate organic from inorganic visual loss. By the middle of the twentieth century, examination of the pupillary response to light had become a routine part of the clinical evaluation of optic nerve function. Kestenbaum (1946) referred to this as the '*Marcus Gunn pupil sign'*, and suggested a technique by which this afferent pupillary defect could be quantified.

The Relative Afferent Pupillary Defect.

The pupillary response test evolved further over subsequent decades. Levatin (1959) suggested rapidly alternating a bright light stimulus between the two eyes of a patient with unilateral optic nerve disease, observing for miosis in response to the stimulation

of one eye and mydriasis in response to the other. Thompson (1966, 1976) called this a *relative afferent pupillary defect* (RAPD) and concluded that its presence indicates unilateral or asymmetric disease of the retina or optic nerve regardless of the appearances on fundoscopy. Further studies (Thompson & Jiang, 1987; Borchert & Daun,1988) refined the technique with the emphasis on alignment along the visual axis, consistency of retinal bleaching, the avoidance of afterimages, and the control of accommodation.

The test for an RAPD became quantifiable with the use of graduated neutral density filters (Thompson et al, 1981), crossed polarizing filters (Rosenberg & Oliva, 1990; Ramsay et al, 1995) or Bagolini filters (McCormick et al, 2002). The use of filters was shown to be of great value in the detection of subtle pupillomotor asymmetry, where a 3dB filter may unmask underlying disease (Thompson 1981).

The relationship between the pupillary response and other tests of optic nerve function has since been demonstrated (Thompson et al, 1982) although the absence of a precise correlation was shown with the advent of automated threshold perimetry when the relationship between the size of an RAPD in patients with unilateral optic neuropathy was shown to only moderately correlate ($r = 0.69$) with the inter-eye difference in mean threshold (Johnson et al, 1988).

This result was replicated elsewhere (Kardon et al, 1993), however the correlation was shown to vary with disease aetiology with greater correlation occurring in idiopathic intracranial hypertension ($r = 0.89$) and compressive optic neuropathy ($r =$

0.79) and lesser correlation in ischaemic optic neuropathy (r = 0.43) and optic neuritis (r = 0.38).

In clinical use, the swinging flashlight test used to detect an RAPD is a relative measure and relies on the presence of asymmetric disease. A false positive RAPD may arise from a physiological pupillomotor asymmetry in healthy subjects (Kawasaki et al, 1996) , anisocoria (Lam & Thompson, 1999a) and media opacities (Lam & Thompson, 1999b).

The use of Pupillometry in research

The modern era of pupil research began with the development of infrared video pupillometry (IRVP) in 1958 allowing the continuous measurement of pupil size (Lowenstein & Loewenfeld, 1958). Edge-detection techniques are employed to monitor the dimensions of the pupil (usually area or diameter) in real time, with temporal and spatial resolutions determined by camera specifications. Outcome variables such as response amplitude, response latency and velocity of pupillary constriction/dilation are analysed under controlled stimulus conditions. IRVP permits the absolute measurement of the pupillary light reflex (PLR) and may be used in bilateral disease. Its superior sensitivity in comparison with the clinical swinging flashlight test has been demonstrated using latency measurements in resolved retrobulbar optic neuritis (Alexandridis et al, 1981).

The pupil response to transient stimuli

In response to a transient white light stimulus (duration 50-500 msec) in a healthy subject, a short latency period is followed by pupil constriction from a baseline diameter (RD) which reaches a maximum constriction velocity (V_{max}) before slowing to a point of maximum miosis (R_{max}) and redilating. The outcome measures derived from this waveform include latency, amplitude (RD-R_{max}) and V_{max}.

In healthy subjects the latency of the PLR following a bright stimulus is limited by delay at the neuro-effector junction between post-ganglionic parasympathetic nerve fibres and sphincter muscle fibres within the iris. The PLR latency has been shown to bear an inverse relationship with the size of the effective afferent signal. Loewenfeld (1999) suggested the use of PLR latency in place of PLR amplitude as a measure of effective afferent signal hence avoiding the confounding influence of the mechanical properties of the iris. The use of latency in the measurement of optic nerve disease has been effectively demonstrated in selective optic neuropathies (Lowenstein & Loewenfeld, 1958) and shown in some cases to supersede Goldmann perimetry in the detection of disease (van Diemen et al, 1992; Lüdtke et al, 1999; Alexandridis et al, 1981). However, latency measurements may be limited by the temporal and spatial frequency resolution of infra-red video cameras and recognition of the precise onset of pupillary contraction may be subjective in the case of a noisy trace.

The amplitude of the PLR in healthy subjects shows a sigmoid relationship with stimulus intensity (Loewenfeld 1999). At low light intensities, the response is indistinguishable from noise and the response becomes linear as the intensity

increases, reaching a maximal plateau dictated by iris mechanical properties. The value of the absolute amplitude of contraction is related to the baseline pupil diameter (Usui & Stark, 1982).

The usefulness of other aspects of the PLR waveform have been evaluated in the detection of optic nerve disease. Bergamin et al (2003) used waveform-partitioning techniques to estimate the sensitivity and specificity of components of the PLR in unilateral retinal and optic nerve disease. They concluded that the best response parameter for the diagnosis of asymmetric disease was the change in pupil size from the point of maximal contraction velocity until the point of peak contraction ('window IV' measurement). At an arbitrary sensitivity level of 96%, the false-positive rate was shown to be 27.3% when using this measure compared to 38.5% when using amplitude measurements. At an arbitrary specificity level of 96% the false-negative rate was 9.6% compared with 20.3% for amplitude measurements. However, the accuracy of this measurement was inconsistent across the disease spectrum.

The pupil response to sustained stimuli

Marcus Gunn (1904) was the first to document the occurrence of pupillary escape in retrobulbar neuritis, in response to a sustained bright light stimulus. Pupillary escape was found to be a normal response to a sustained low intensity light stimulus in normal cases by Lowenstein & Loewenfeld (1959) and Thompson (1966) and was shown to be influenced by the pupil diameter at baseline (Sun & Stark, 1983). Cox (1992) measured pupillary escape in 14 patients with afferent visual defects and did not find a significant difference between patients and normal controls.

The sustained pupillary response has been proven useful in the distinction of central visual field defects from peripheral visual field defects. Bergamin and Kardon (2002) studied the response of the pupil to sustained light stimulation and defined a 'phasic' component of the pupil's response to a sustained stimulus as the 'change in size from the pupil's baseline size to the point of maximal pupil contraction'. The amplitude of the sustained pupillary response was calculated using the amplitude of pupil contraction from the baseline pupil diameter to the average pupil size during the 2-5 second interval of continuous light stimulation. This sustained component of the response was then compared with the 'phasic' response as a ratio, and this relation was examined in the context of normal subjects and patients with peripheral or central visual field loss. A statistically significant greater reduction of the sustained reaction component in proportion to the phasic component was found in eyes with visual field loss in the centre compared to the periphery.

The pupil response to perimetric stimuli:

Early pupillometric studies (summarised by Loewenfeld, 1966) involved manual presentation of light stimuli along the horizontal meridian. Recent studies have used automated visual field analysers such as the Octopus and the Humphrey to present stimuli (Fankhauser & Flammer, 1990; Kardon et al, 1991) . Stimulation at or near fixation has been shown to produce larger pupillary responses than stimulation at greater eccentricities (Harms & Grundlagen, 1949; Burke & Ogle, 1964; Bresky & Charles, 1969). In addition, the nasal retina shows greater sensitivity than the temporal retina, and the inferior more the superior. The largest pupillary response has

been shown to occur from stimulation of the supero-temporal quadrant in visual space and the smallest from the infero-nasal quadrant. With increasing age there is an overall reduction in the amplitude of pupil responses at all stimulus locations and the central 'peak' of sensitivity is less pronounced (Schmid et al, 2004).

The pupil response to isoluminant stimuli

The pupil has been shown to respond to isoluminant stimuli such as sinusoidal gratings, checkerboard reversals and chromatic stimuli (Slooter et al, 1985; Ukai 1985; Young et al, 1980; Barbur, 1991).The amplitude of the pupillary grating response depends on the spatial frequency of the grating stimulus. This relationship may be used as a means of estimating visual acuity in preverbal children. Comparison of behaviourally assessed visual acuity with the pupillary response to gratings in infants aged 1 month showed a good correlation (Cocker et al, 1994).

Barbur et al (1994) used the method of dynamic random luminance contrast (LC) masking to hide chromatic signals within isoluminant noise. A pure pupil response to colour in normal trichromats is of smaller amplitude than the luminance response and shows considerable inter-individual variability. The pupil appears to show least response to chromatic modulation along the blue-yellow axis. Response latencies to isoluminant stimuli (grating or chromatic modulation) are significantly longer than those following luminance stimuli (Barbur et al, 1998). This increased latency has been interpreted as evidence that pupil responses to colour and grating stimuli require central cortical processing as the extent of the latency prolongation is consistent with

the estimated response time if V1 were involved in grating responses and V4 in colour responses.

The potential for the use of the pupil's response to colour has been demonstrated in the context of optic neuritis. Moro et al (2007) tested 14 patients with unilateral optic neuritis (five of whom were later diagnosed with Multiple Sclerosis) and 15 control subjects. The patients were tested for luminance and colour responses 3 to 60 weeks (mean 29 weeks) after the onset of optic neuritis and a large proportion of the patients showed near normal Visual Acuity (6/6 or better) and good visual field sensitivity. The majority of these patients showed some pupillary deficit compared to the control group with longer mean latencies and a reduction in mean amplitude.

Current developments in pupillometric research

According to the 'classical' view light of the neural circuitry that underlies the PLR, light entering the eye activates rods and cones which in turn generate afferent impulses in the retinal ganglion cells (RGC). This afferent signal travels along the optic nerve. At the chiasm, nasal fibres decussate and crossed and uncrossed signals are conveyed to the olivary pretectal nuclei of the midbrain along branches of the same retino-geniculate projections that mediate conscious visual perception. Recent developments which include the discovery of a new class of photoreceptor in the eye and the observation of pupillo-visual dissociation in some diseases of the optic nerve challenge this classical view. These will be discussed.

1. A new class of photoreceptor

The assumption that intact rods and cones are necessary for the PLR was first questioned by Keeler in 1927 when he bred a pure strain of mice lacking the 'sensory elements' in the eye. Histology confirmed incomplete development of the outer layers of the retina. Despite appearing perceptually blind in behavioural experiments, the mice demonstrated pupillary responses to transient light stimuli, which were of smaller amplitudes and longer latencies than those measured in normal control mice. Further studies on this topic continued almost half a century later. Using murine models for outer retinal degeneration (rd/rd), Foster et al (1991) confirmed the persistence of both the PLR and circadian rhythms after widespread damage to the rods and cones. Immuno-staining studies suggested the presence of a few viable rods and cones, and the experiment was repeated with 'knock-out' techniques to breed mice completely lacking in rods and cones. In 1999, both Freedman et al and Lucas et al confirmed the persistence of a circadian rhythm in rodless coneless mice ($^{0}R^{0}C$). Removal of the eye had already been shown to abolish both pupil responses and circadian entrainment (Nelson & Zucker, 1981). The presence of an additional non-rod non-cone photoreceptor, thought to be a sub-class of retinal ganglion cells (Intrinsically photosensitive retinal ganglion cells or IPRGCs) with 'intrinsic photosensitivity' was now recognised.

The photopigment within the photoreceptor was identified as 'melanopsin' (Provencio et al, 1998), a vitamin A-derived pigment. Subsequent studies on rod, cone and melanopsin knock-out mice ($^{0}R^{0}C^{0}M$) confirmed the role of melanopsin in the PLR and circadian entrainment. Mice with rods and cones but no M-RGCs (^{0}M) displayed an identical PLR to the wild type at low irradiances, but at high irradiances the PLR

was attenuated, suggesting a complementary partnership the three receptor types. The presence of IPRGCs in human and macaque retinae was subsequently confirmed. IPRGCs were found to form 0.2% of the total ganglion cell population in flat mounts of the entire retina and their density was greatest around the fovea with 20-25 cells per mm^2, thinning out to 3-5 cells per mm^2 peripherally.

Measurements with the pharmacological blockade of non-IPRGC photoreception revealed the characteristics of this new class of photoreceptor (Gamlin et al, 2007). The spectral sensitivity curve of melanopsin was shown to have peak sensitivity at 482nm.

Under physiological conditions the IPRGC response interacts with rod and/or cone mediated signals in ways which depend on the incoming stimulus wavelength (Dacey et al, 2005). Under photopic conditions, activating cones and melanopsin as well as activating only cones has been shown to elicit full pupilloconstriction. With pharmacological blockade of rods and cones, the PLR latency was shown to increase, and sustained pupilloconstriction after light cessation was only present with a stimulus which activated both cones and melanopsin. In vivo human tests have shown that pupillary constriction persists after exposure to a 10s light stimulus presented at 493nm but not at 612nm for the same irradiance (Gamlin et al, 2007).

Studies of IPRGCs indicate that the melanopsin signal predominantly mediates the sustained pupil constriction following long-duration light stimuli. In doing so, it contributes to maintaining the pupil in its steady-state during continuous illumination (Tsujimura et al, 2010).

2. Pupillo-visual dissociation

Along with the establishment of the pupillary light response as a valid measure of optic nerve function throughout this century, came the observation that in certain diseases of the optic nerve, the pupil response to light showed no correlation to the presence of disease (Wakakura et al, 1995; Bremner et al, 2001). This has been predominantly reported in the setting of Dominant Optic Atrophy (DOA) and Leber's Hereditary Optic Neuropathy (LHON). Although this phenomenon has been challenged the discovery of the IPRGCs has offered the possibility that separate pathways for visual perception and the pupillary response exist and the occurrence of pupillo-visual dissociation now appears more feasible. A disparity between pupil and visual measurements may have diagnostic potential, by demonstrating differing susceptibilities of separate groups of ganglion cells to disease.

3. Role of the visual cortex

Although earlier studies confirmed the significance of the midbrain in the pupil light reflex it was subsequently shown that loss of human or primate striate cortex is known to diminish the pupil light response (Brindley et al, 1969; Barbur, 1991; Weiskrantz et al, 1998). Patients suffering from scotomas or hemianopias secondary to post-geniculate cortical lesions display abnormal pupil light reflexes to small test stimuli presented within their scotomas (Barbur et al, 1998). Whereas stimuli of small size and luminance contrast produced responses that were either absent or reduced in amplitude, stimuli involving large light flux increments resulted in little or no difference in the pupil light response between the blind and sighted hemifields. It has also been reported that the pupil's response to colour can be abolished in cerebral

achromatopsia (Barbur, 2004). Tests carried out on patients with a non-functioning pre-tectum showed pupil colour responses to be within the normal range, although both the transient and sustained pupil responses to light were gravely diminished. These observations suggest a circuitry with a direct input from V2,V3, V4 & MT in the cortex to the Edinger-Westphal nucleus (EW). These inputs can bypass the olivary pretectal nucleus. The pupil light reflex response is thought to involve two components. The steady state component determines the response to rapid large increments in light flux. The transient component reflects new changes in luminance contrast.

A new model for the pupil pathway circuitry has been proposed which includes a separate pathway for the neurones responsible for the steady-state response and one for neurones mediating the transient response projecting onto the olivary pretectal nucleus (OPN) which then connects to the EW (Barbur, 2004). Visual cortex area V1 relays onto the projection of the transient neurons into the OPN.

Conclusions

The three recent developments in pupillometric research detailed above and their potential use in the diagnosis of optic nerve disease will be explored further in this thesis. The application of pupillometry in optic nerve disease is investigated in three stages, using three different purpose-built pupillometer setups.

Stage one

In the first stage, a Maxwellian-view pupillometer is used to assess the nature of the pupil response to light in normal control subjects in order to ascertain the variability and repeatability of each aspect of the pupillary wave function and identify potential measures of optic nerve function which may be used to detect disease. The results of this stage of study suggest a high degree of inter-individual variability which poses a conundrum in the use of the particular setup in the assessment of optic nerve function.

Stage two

As a result of establishing that the measurement of the standard pupillary light response is perhaps too variable to be useful in assessing disease, in the next stage of study, the pupillary response to chromatic stimuli, grating stimuli and luminance is assessed in the setting of a disease where there is known to be selective sparing of the pupillary response. This stage of the study explores the precise nature of dissociation of the pupil response and whether all channels of the pupillary response (the chromatic, grating and luminance channels) are equally affected. This stage *indirectly* assesses the integrity and the role of the IPRGC pathway by testing the parvocellular and magnocellular ganglion cell pathways in parallel with pupillometry in disease sparing the pupil response. The results of this study indicate that pupillovisual dissociation does not uniformly affect all pupil channels, leading to the conclusion that at the measured irradiance and wavelength levels, the input signal for the pupil response to colour and grating stimuli travel along the parvocellular ganglion cell pathway, whereas the input signal for the luminance response traverses a non-P non-M ganglion cell pathway which is spared in Leber's Hereditary Optic Neuropathy. The positive results of this experiment indicate that the measurement of separate

pupillary channels in optic nerve disease may offer a greater complexity of information about optic nerve function, than simply measuring the pupillary response to white light, where inter-individual variability may mask pathological results.

Stage three

In the third experiment, a purpose-built pupillometer is used to *directly* stimulate rods, cones and the IPRGC channel. The pupillary chromatic response, luminance response and IPRGC response are first assessed in normal control patients using the new pupillometer setup. These are then assessed in patients matched for visual loss with optic neuritis secondary to Neuromyelitis Optica (NMO) and Multiple Sclerosis (MS). The aim of the experiment is to assess whether each disease affects each ganglion cell response component differently, leading to a potential use of pupillometry in being able to identify the underlying aetiology of optic neuritis.

THE PUPIL RESPONSE IN NORMAL CONTROL SUBJECTS

Aim

The aim of this study was to investigate the variability of the pupil light reflex obtained in healthy individuals with a purpose-built Maxwellian-view Pupillometer.

Introduction

The stimulus commonly used in pupillometric research may be either transient or sustained. When using a sustained stimulus, Maxwellian optics are preferable in order for the entire stimulus to continue entering the eye regardless of the size of the pupil. Maxwellian optics are also advantageous when providing a transient stimulus, as the same stimulus enters every eye regardless of the starting pupil diameter. A Maxwellian optical system is achieved by making the stimulus converge to a small (≤ 1mm) disc of light focussed in the plane of the pupil. Previous studies examining the variability of the pupillary light response have involved the use of both Maxwellian and non-Maxwellian view pupillometers.

Műller-Jensen and Hagenah (1976) used an infra-red light based image sensor to measure the pupillary light reflex in 101 healthy volunteers ranging in age from 15 to 89 years to a light stimulus presented at 4 different light intensities. Their findings showed the most stable parameters with least intra-individual variability to be the latency and the time taken to maximal contraction.

Ellis (1981) used the Whittaker Series 1800 binocular infrared television pupillometer to stimulate the pupils of 19 healthy volunteers with a 100ms white light Maxwellian

stimulus delivered at a maximal intensity of 550 cd/m². Six intensity settings were used and the maximal intensity was attenuated with a series of 1log unit neutral density filters. Ellis established 95% confidence intervals for the relationships between amplitude of constriction and latency, amplitude of constriction and maximal constriction velocity, stimulus intensity and maximal dilatation velocity and various other relationships.

Murray et al (1991) used the Pupilscan type 6 instrument from Fairville Medical Optics to study the repeatability of pupil response measurements in eight volunteer subjects. The Pupilscan instrument used a solid-state image sensor with a temporal resolution of 20Hz and a spatial resolution of 0.05mm. A square wave stimulus pulse lasting 0.2seconds and at 270 lux intensity was used at a predominant wavelength of 565nm. A significant daily variation in minimum and final diameters, maximum diameter change and constriction velocity was shown.

Fosnaugh et al (1992) studied the circadian pattern of dynamic pupillary reflex measurements using a 100millisecond light stimulus with an intensity of 20lumnes/sq ft. A small but statistically significant diurnal variation was found. The inter-subject variability in the pupillary response to light was found to be greater than the intra-subject variability.

Bär et al (2005) tested 90 healthy subjects for evidence of the laterality of the pupil light reflex along with measurements of test-retest and short term/long-term variability. A Compact Integrated Pupillograph system was used for this, incorporating image detection software operating under infra-red illumination at a

frequency of 16.67 Hz. Re-testing the pupil light reflex after 10 mins revealed significant differences in the latency time (which decreased) , the constriction velocity (which increased) and in the relative constriction amplitude (which increased). Repetition of testing 1 week later under the same conditions as the first test (e.g. time of day, dark adaptation), did not show significant differences when compared to the first test.

In this study, I used a purpose-built, Maxwellian-view, binocular pupillometer to study the variation of components of the pupillary light reflex occurring within the normal healthy population.

Methods

Subjects

A total of thirty-four healthy volunteers, ranging in age from 21 years to 52 years (seventeen males and seventeen females) were prospectively recruited for the study. The age distribution of the volunteers is shown in Figure 1.

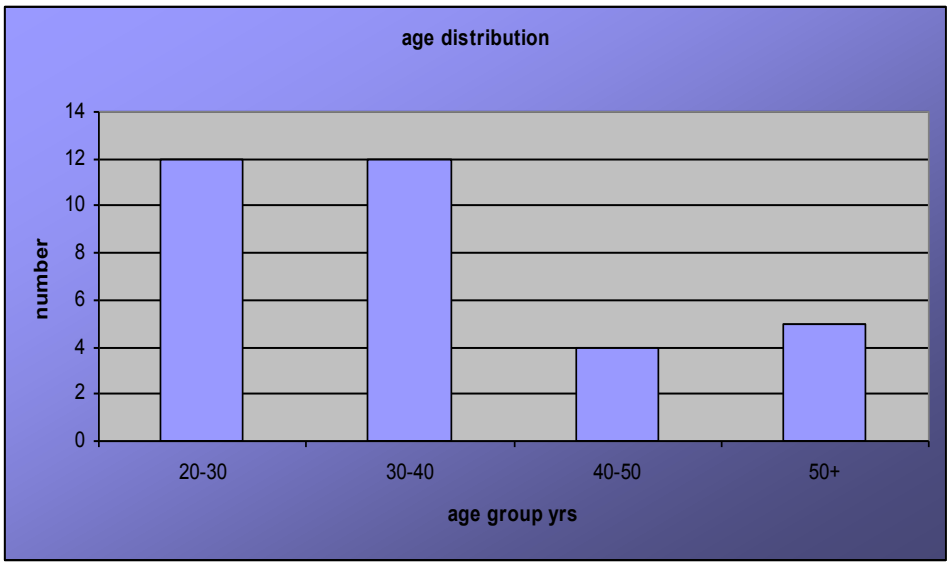

Figure 1
The age distribution of the volunteers in this study

Of these volunteers, 9 volunteers were tested on 5 different occasions. 25 volunteers were tested on only one occasion. When carrying out repeat testing, no rules of temporal separation or time of day were followed. The tests were repeated with a break of as little as 45 minutes and as long as a couple of months.

The exclusion criteria were as follows:

- History of eye disease/injuries or operations
- Lack of Binocular single vision
- Abnormal appearance of optic discs on slit lamp examination
- Best corrected visual acuity worse than 6/9 on a Snellen chart
- Autonomic nervous system or Central nervous system-targeting medication.
- Known autoimmune, inflammatory or neurological medical conditions, including diabetes mellitus. Past history of such illness.
- Cognitive deficit
- Age under 18 years.

The volunteers were given a reference number, by which they are referred. Their numbers begin with a T. The numbers do not always observe a particular order, and hence protect anonymity.

Equipment

A purpose-built Maxwellian view binocular pupillometer was used in this study. The pupillometer is composed of a pair of LED based white light sources and infra-red cameras, each designed to simultaneously test and record from each eye independently. The LED based white light source projects a 2mm disc of light onto

the pupil plane. The intensity of the light projected at this point was measured to be 2.4 lux in the right eye and 2.64 lux in the left eye using a hand-held photometer. It was assumed that this disc of light illuminated a retinal area of approximately 10 degrees, corresponding to the macular zone.

The volunteer was asked to place his/her face upon a chin rest, similar in design to that found on all slit-lamps. Each camera separately captures the image of each pupil using a low intensity invisible infra-red light source and the pupil images are displayed on a monitor screen, such that the quality of these images may be instantaneously recognised. Once the pupil image is accurately recognised, a software programme (written in Labview©) calculates the pupil area. The diameter of a perfect circle with this area is back-calculated by the software and the change in this diameter during the pupillary reflex process is shown by the pupillometric trace. Thus the resulting display of pupil diameter measurements is based on the diameter of this perfect circle and not the absolute vertical or horizontal diameter of the actual pupil. The perfect circle that is estimated by the software appears as a red circle occupying the approximate margin the of the pupil image on the monitor. The contrast, brightness and resolution of the cameras may be manually altered to aid this process. The camera may be moved in x, y and z directions in order to allow for a better image. The cameras for each eye are manipulated independently. The cameras and software calculate the absolute measurement of the pupils. There is no allowance for the distance of the eyes from the camera; however, the camera's depth of focus is reasonably shallow, and the operator attempts to maintain constancy in this distance as far as is possible. The volunteer was instructed to maintain fixation on a small red light target 6 metres away. The light stimulus series was shone into one eye.

Stimulus paradigm

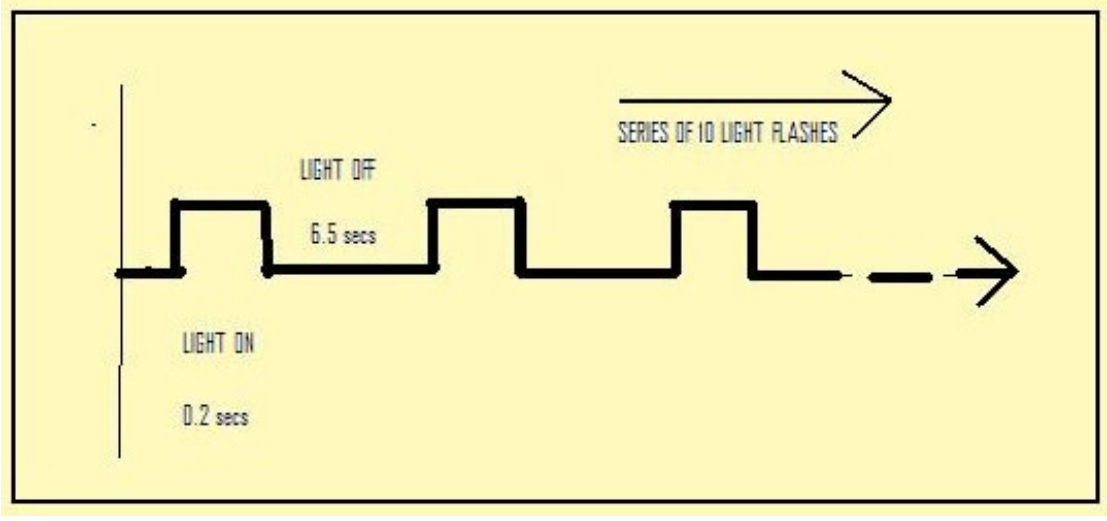

Figure 2

The stimulus paradigm: The stimulus involved a square wave light step lasting 200 milliseconds, offered ten times in each series, and separated by 6.5 seconds.

The subject was dark adapted for one minute. The light stimulus series was then presented to either the right or the left eye. The stimulus involved a series of ten light square steps, each lasting 200 milliseconds and separated by 6.5 seconds (as shown in Figure 2). No background illumination was used. The volunteer was asked to count to 4 before blinking after each light flash in order to make the experience as comfortable as possible. After one eye was stimulated with the series, the room lights were switched back on for a couple of minutes before the dark adaptation procedure was repeated. The other eye was then stimulated. The order of the eyes being stimulated was decided at random. The volunteer's face and the pupillometer were covered with a lightproof screen to prevent stray light from entering the eyes.

A transient stimulus was chosen instead of a longer sustained stimulus. A high intensity light signal has been previously shown to be necessary in order to elicit a clear, sustained pupillary response and our pilot tests suggested our stimulus was not bright enough for this purpose. The duration of the stimulus was chosen to be 200 ms in line with similar, published studies. The highest light intensity stimulus available was used in order to reduce noise as far as possible. Only the direct response has been used in this chapter.

Analysis

The response of the pupil to the light stimulus was interpreted, as described above, by Labview© software. Once the pupil's movement was transformed into a trace recording, specific parameters were deduced from the resulting trace. A typical pupillographic trace of the test series based on the stimulus paradigm described above is shown in figure 3.

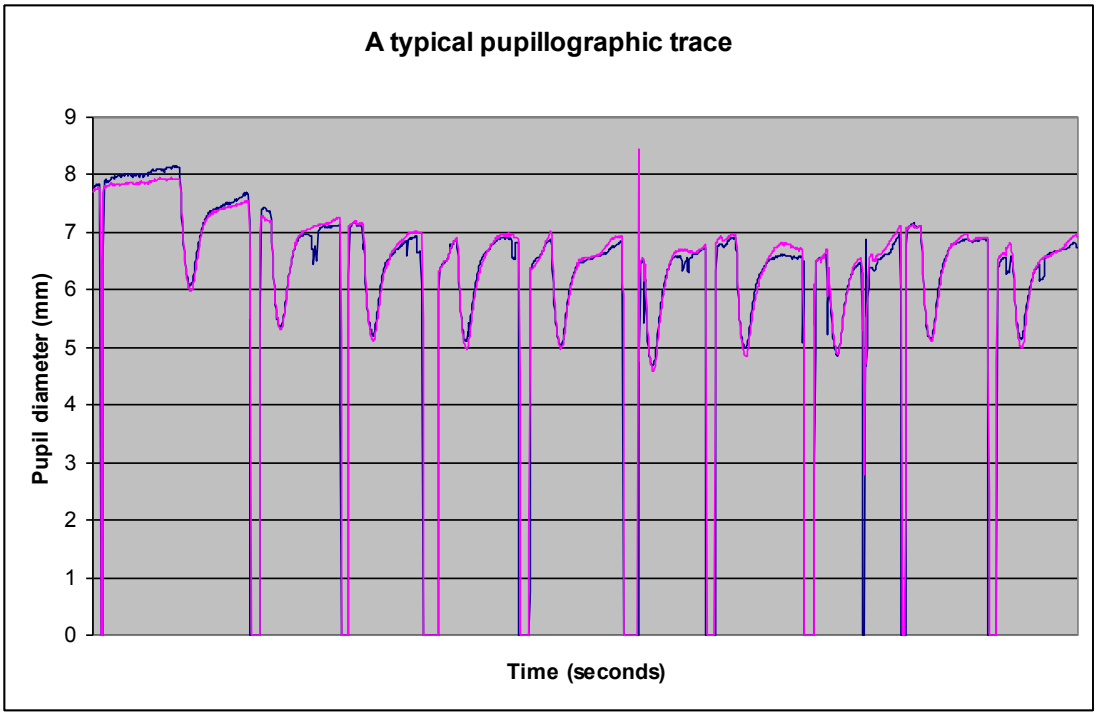

Figure 3

A typical pupillographic trace during the stimulation of the right pupil in a healthy volunteer: The lighter shade (pink) demonstrates the size of the right pupil. The darker trace (blue) corresponds to the left pupil. The vertical lines reaching y=0 show the software's interpretation of blinks.

The pupillographic software may occasionally lose track of the change in pupil diameter which manifests as a segment of excessively rapid change in the pupil size or as a sudden deviation from the expected pupil trace. Pupil curves displaying such obvious inaccuracies were omitted from analysis.

Bergamin et al (2003) used waveform-partitioning techniques to estimate the sensitivity and specificity of various components of the PLR in unilateral retinal and optic nerve disease (using the healthy fellow eyes as controls). The components they tested included a 'window IV' measurement which they described as the decrement in

pupillary diameter between the point at which the maximal constriction velocity occurs, and that point at which constriction is at its maximal. They subsequently concluded this was the best response parameter for the diagnosis of asymmetric disease in the anterior visual pathway.

In accordance with the findings of Bergamin (2003), three parameters within the pupil response curve were identified:

1. ***The maximal change in pupil diameter (AA).***
2. ***The decrement in diameter (DCV) from the baseline diameter until the point at which the maximal constriction velocity (CV) is reached.***
3. ***The change in diameter between the diameter at the point of maximal constriction velocity (CV) and the point of maximal pupil constriction. This may be calculated by subtracting DCV from AA and is hence denoted AA-DCV.***

These parameters are illustrated in figure 4.

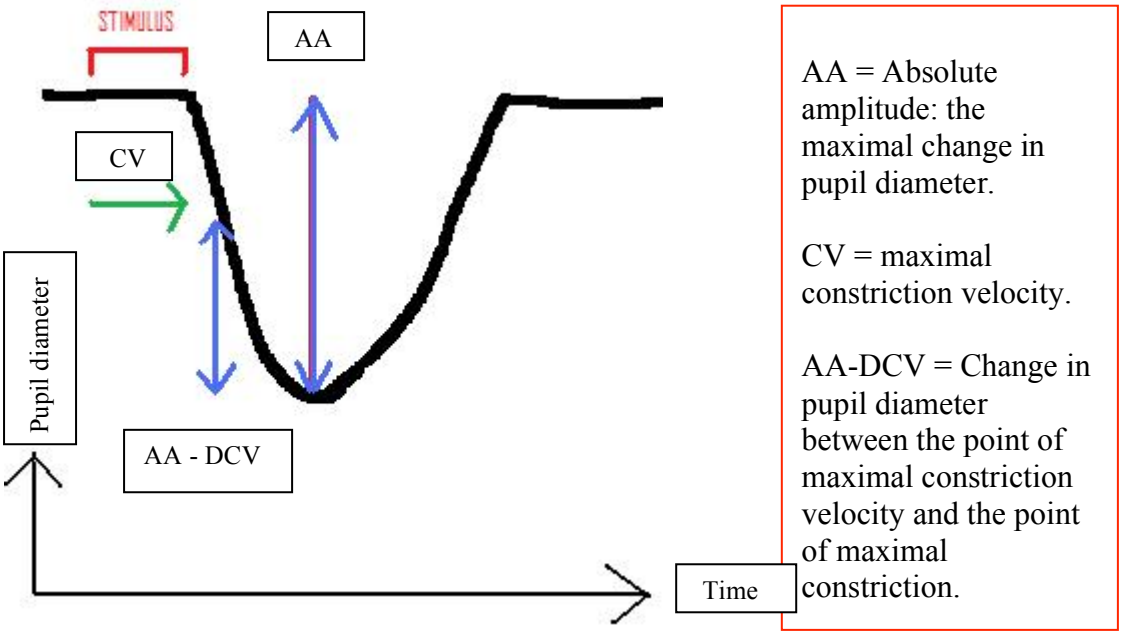

Figure 4

The typical shape of the pupil light reflex to a transient stimulus. The parameters studied are shown.

Statistical Analysis

The spread of the measurements was assessed using the ratio of their mean to their standard deviation (Co-efficient of variation = CoV). Only information from the right eye was used.

1. Intra-individual, intra-test CoV

   In each volunteer (n=34), the first five valid readings (from the series of ten) were used from the first (or only) occasion of testing, because there was a variation in the number of valid readings achieved in each volunteer but almost all individuals provided at least five valid readings.

2. Intra individual, inter-test CoV

   Information from nine volunteers who underwent repeated testing was used. The mean values for AA, CV and AA-DCV were first calculated for each occasion. The CoV of these values across the multiple occasions were then calculated.

3. Inter-individual CoV

   The data from the twenty-five volunteers who did not undergo repeated testing was used for this part of the analysis. First the mean values of AA, CV and AA-DCV during the test stimulus series were calculated. The CoV of this value across all 25 volunteers was then calculated.

Results

Relationship between parameters

The relationship between AA, CV and (AA-DCV) across the volunteers is shown in figures 5,6 and 7. Figure 7 shows the line of best fit between AA and (AA-DCV) to have an R^2 value of 0.9 (1 d.p.) suggesting a good linear relationship.

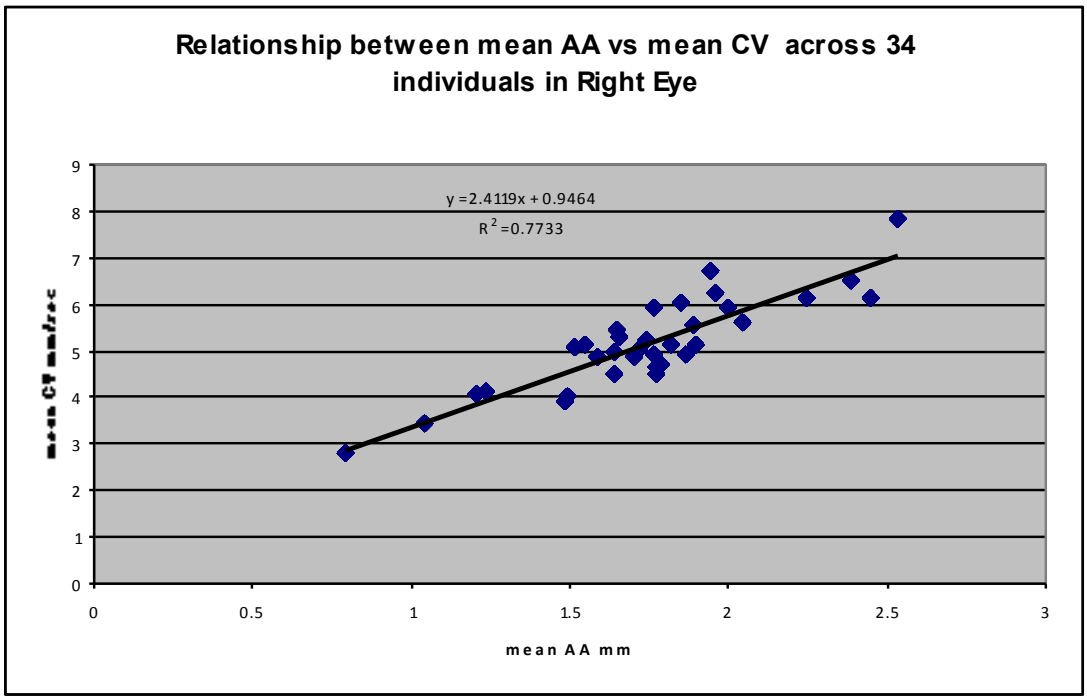

Figure 5

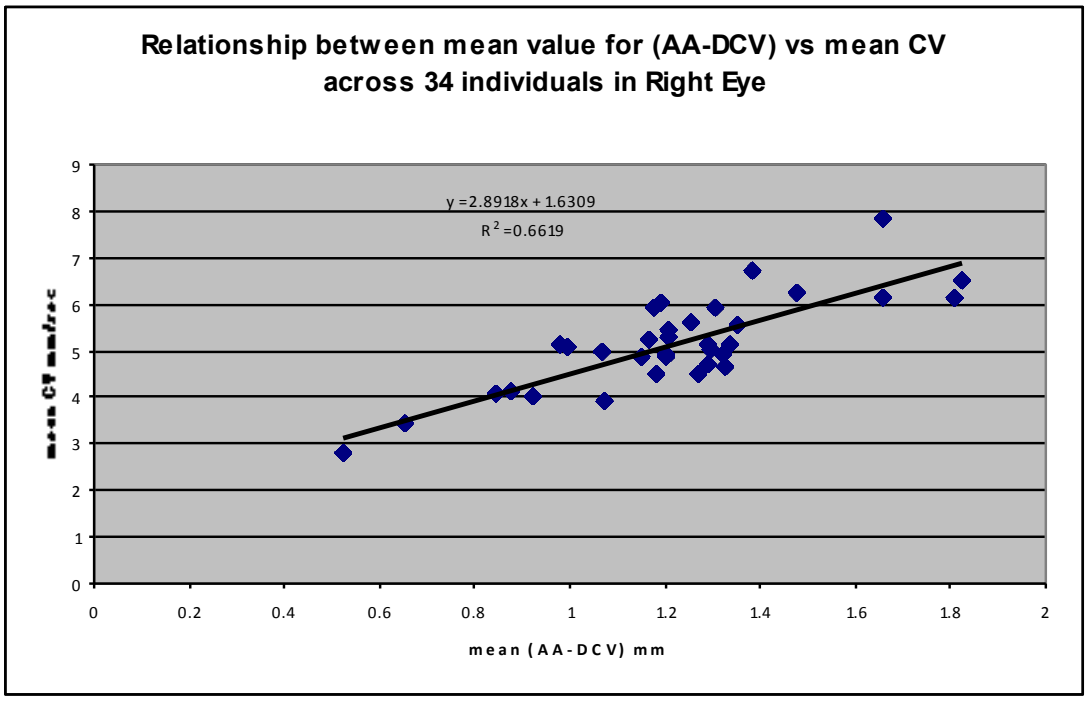

Figure 6

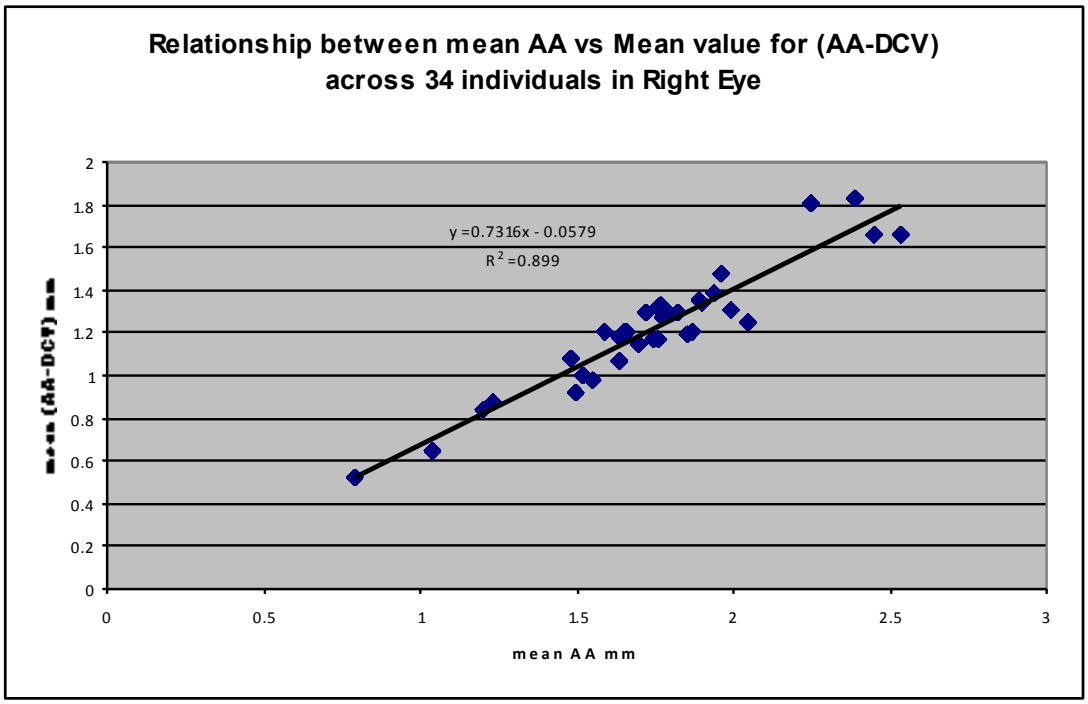

Figure 7

Variation

The absolute amplitude (AA), the maximal constriction velocity (CV) and the change in pupil diameter from the point of maximal constriction velocity to the point of maximal constriction (AA-DCV) were assessed for variation by the calculation of the Coefficicient of variation (CoV). The values of intra-individual intra-test (WTWI), intra-individual, inter-test (BTWI) and inter-individual (BTBI) Coefficients of Variation (CoV) for the parameters of AA, CV and DCV are shown in figure 8.

1. Intra-test CoV

For each volunteer (n=34), within-test coefficient of variation (CoV) was calculated by taking the first 5 valid readings during the test series, where relevant, on the first occasion of testing.

2. Intra-individual, inter-test CoV

The data of the nine volunteers who underwent repeated testing on four (volunteer T22) or five separate occasions was used for this analysis. First the mean value of AA, CV and AA-DCV for each test was calculated for each individual. Then the CoV of these means across the four or five occasions was calculated in each individual. The inter-test CoV is therefore a CoV of mean values over all volunteers.

3. Inter-Individual CoV

The data of the 25 volunteers, who did not undergo repeated testing, was used for this part of the analysis. The mean value of AA, CV and AA-DCV acquired over the single test series was first recorded for each individual. The CoV of this across all 25 volunteers was then calculated. Like in (2), this is also a calculation of CoV based on mean values.

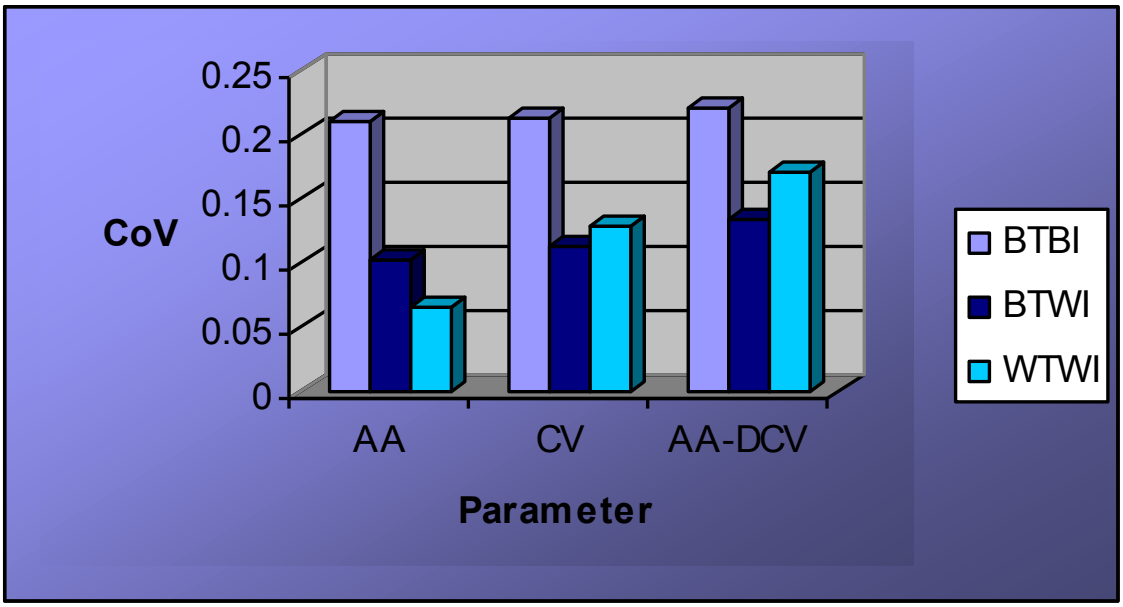

Figure 8
BTBI= inter-individual CoV. WTWI= Intra-individual intra-test CoV. BTWI = Intra-individual inter-test CoV.

The parameter AA was found to show the least spread and overall, the least spread was acquired intra-individually within the same test, as would be expected. The Intra-individual inter-test CoV values of CV and AA-DCV are over 10%. The spread of all three parameters was similar when assessed across individuals.

<u>The relationship between resting diameter and maximal constriction amplitude</u>

The results from the right eye of all volunteers were pooled to study the relationship between resting diameter and amplitude of constriction. This relationship has been

explored multiple times in the past and previous studies were assimilated by Usui and Stark in their 1982 publication. Here, they proposed a model to define the relationship of pupillary diameter with the maximal amplitude of constriction achievable. They proposed a relationship of ***y= 2.794*(x-1.17)^2*(x+0.174)^2/((x-2.11)*(x+1.11)*(x^2-1.01*x+0.436))*** where y is the amplitude of constriction in mm and x is the baseline pupil diameter in mm.

If this model were to be shifted upwards by 1 mm and the relevant segment applied to my results, a moderately compatible picture may be observed, as shown in figure 9.

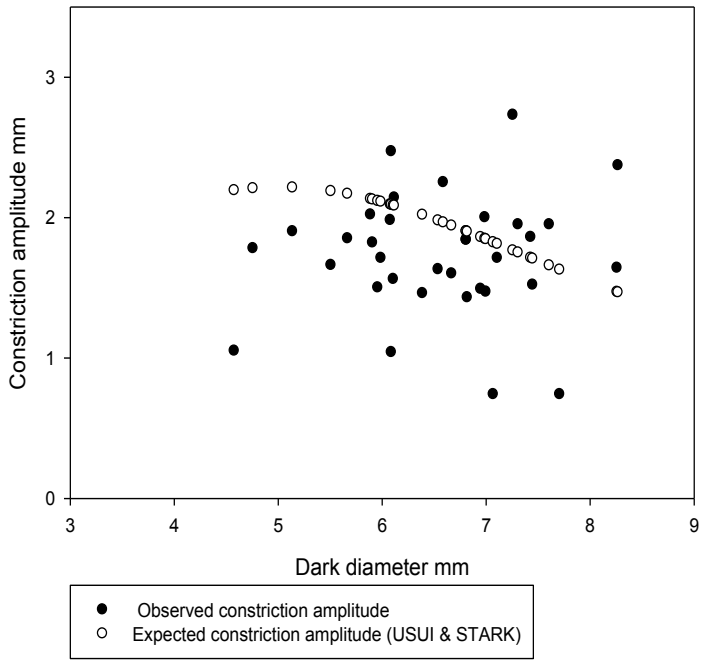

Figure 9
Usui and Sark's predicted relationship of diameter and amplitude of constriction translated superiorly by 1mm then superimposed on the data from each individual's first pupil light reflex from the series of ten reflexes in the first test series

Laterality

Different cameras were used to capture the left and right pupil image and a slightly different intensity of incident stimulus was used for either eye, hence a comparison was made of the results of the left and right eye from 8 of the 9 volunteers who underwent testing on multiple occasions. In every case, only the direct response was studied. The absolute amplitude (AA) was studied for this purpose because it had shown the least spread (CoV) of all three variables. The left and right eyes were not tested simultaneously, but they were tested at a similar time. One of the nine volunteers was omitted from analysis because his/her results from the left eye were incomplete. In 6 of the volunteers, the mean results from each of 5 tests were used, in 2 of the volunteers the mean results from each of 4 tests were analysed.

The difference between each test set carried out in the left and right eyes (5 sets in 6 volunteers and 4 tests in 2 volunteers) was analysed. In one volunteer, there was no statistically significant difference between the results of AA of the left and right eyes. In 7 out of the 8 volunteers, there was a statistically significant difference between the left and right eyes on at least one occasion. In 4 volunteers, there was a difference at the 1% significance level. Table 1 shows the P-values of the paired t-test results when comparing left with right eye where a difference at the 5% significance level was found.

Subject	Total number of tests	Number of tests with different results between right and left eyes	P values of the t-test in the cases where a difference exists between left and right
T2	5	1	P=0.011
T6	5	2	P=0.02, P<0.001
T7	5	2	P=0.005, P<0.001
T9	4	2	P=0.026,P=0.043
T18	5	2	P=0.016,P=0.001
T19	5	0	
T22	4	2	P<0.001, P<0.001
T31	5	2	P=0.01, P<0.001

Table 1

The total number of tests with a difference between the left and right pupils and the P-value where a P-value ≤ 0.05 was found is shown.

Discussion

Bergamin et al (2003) suggested that the diagnosis of asymmetric disease was better achieved by studying the contraction phase of the pupil reflex, rather than the re-dilation phase. Moreover, when the contraction phase was divided into an early phase and a late phase, the late phase was more diagnostic compared with the entire phase of

contraction amplitude (onset to peak amplitude). They found that the change in pupil size measured between the time at which maximum contraction velocity occurs and the time to peak contraction provided the best response parameter for objective diagnosis of asymmetric disease of the anterior visual pathway. They thus concluded that disease of the afferent visual system selectively affects one segment of the pupil light reflex more than other segments.

After repeating this study on a larger sample size, 5% confidence intervals may be established. If one segment of the pupil reflex waveform is selectively affected by optic nerve disease more than another, then the ratio between parameters such as AA and CV is likely to be altered in these patients, and their results will fall outside the 95% confidence intervals. This ratio may have the potential to be a marker for the presence or absence of optic nerve disease.

AA appears to have the least spread of results when compared with CV and AA-DCV. However, the CoV almost doubles from its intra-test intra-individual value (0.067) to reach the inter-test, intra-individual value (0.102), and this value doubles again to reach the inter-individual value (0.209).

Yasukouchi et al (2007) measured the CoV of the PLR, which they defined as the change in pupil diameter in response to light (equivalent to our AA) across twenty healthy male volunteers. They found that the fluctuation in CoV became more extensive as the intensity of the light source stimulus decreased. This fluctuation became marked at an illuminance level of 30 lux and below. At illuminance levels of 1,3,30 and 600 lux, the CoV was 51.5%, 45%, 28.4% and 6.2% respectively.

Ellis (1981) found that at the highest level of stimulus intensity (55a candles/m²), the variation of the amplitude of the response was consistently small. At the lowest intensity, there was a larger and inconsistent variability in the amplitude.

Fosnaugh et al (1991) tested ten volunteer healthy male subjects with a light stimulus of intensity 20 lumens/sq ft and of 100ms duration, with a background ambient light of 4foot candles. When looking at AA, they found an intra-individual inter-test CoV of 13.5% and an inter-individual CoV of 32.4%. When studying the constriction velocity (CV) these values were 13.2% and 26% respectively. Fosnaugh et al's results therefore showed a wider spread than the results of this study.

Ogbuehi (2006) carried out intra-ocular pressure (IOP) testing on the right eye of 60 healthy individuals in order to compare the results of Goldmann applanation tonometry with non-contact tonometry using the Topcon CT80 tonometer. The measurement was repeated three times using each machine and the whole process was repeated after one week. One-Way repeated Measures ANOVA showed an absence of a difference between the IOP readings across the four sessions. The Coefficient of Variation (CoV) of IOP readings calculated by Goldmann tonometry across individuals was found to be 17.2% (2.3/13.6). We may compare this with our value for the CoV of the absolute amplitude across individuals of 20.1%.

Although the CoVs obtained from this study are comparable to that from the Ogbuehi study, this spread may be more acceptable in the context of IOP measurements used

as a screening test with other variables taken into consideration, rather than in the context of pupil measurements used as a standalone diagnostic test.

The presence of disease within peripheral nerves may be diagnosed in the clinical setting with 'nerve conduction studies' where the conduction velocities of particular nerves are reduced as a direct *consequence* of nerve disease. This is analogous to the reduction in the size of pupil reflex parameters caused by the presence of optic nerve disease. Bleasel and Tuck (1991) investigated the variability of nerve conduction velocities by taking repeated measurements of motor and sensory conduction velocity (MCV and SCV). They found the coefficient of variation for MCV and SCV ranged from 2.0% to 6.7%. These values are far smaller than the values obtained in this study. The difference may be explained by the multiple synapses involved in the pupil reflex pathway and the delay caused by the neuro-muscular junction on the iris, which do not affect MCV and SCV measurements. In addition, higher centres affect the gain of the pupil reflex pathway.

Fosnaugh et al (1992) studied the effects of time-of-day, variable ambient light and occlusion of the contralateral on thirteen healthy volunteers. Multivariate analysis of variance (ANOVA) showed that the constriction velocity was not affected by time-of-day. The constriction amplitude was found to significantly decrease as a function of time of day, with the average decrease being 0.07mm. If this amount is seen within the context of this study, this may be deemed to exert an extremely small influence, as the intra-individual inter-test differences in amplitude were often well above this value. (See Figures 8 to 16). Fosnaugh et al found a fluctuation in pupil diameter over the course of the day with a 6.7% reduction from 6am to 12pm, and a 2.4% increase

between 6pm and midnight. Bär et al (2005) carried out one-way ANOVA in their 'First test series' looking at the effect of time-of-day on intra-individual inter-test differences. They found statistically significant intra-individual inter-test differences in all parameters. The parameters that they studied included *constriction velocity*: the gradient of the pupillographic trace in the constriction phase at the 40%-80% interval of the amplitude and *relative amplitude:* the ratio of absolute amplitude to baseline diameter. Yu et al (2007) found no effect of time-of-day on the constriction amplitude but they did find a 10% increase in the pupil resting diameter when this was measured in the evening. This may explain the effect of time-of-day on the *relative amplitude* measured by Bär et al (2005). This conflict in the literature has meant that there are no solid conclusions on the exact effect of time-of-day, so we do not know exactly what adjustments need to be made to our measurements in order to take this effect into account

There may be many other reasons behind the variability. It is beyond the scope of this chapter to explain all the causes. Lowenstein and Loewenfeld showed in 1961 that personal alertness levels can significantly change the pupillary response curve. They also showed that this emotional factor determined how the pupil reflex curve altered in the context of complete background darkness and dim background illumination. In the more fatiguable person, the presence of dim background illumination created a starkly differing pupillary response curve, when compared with the response curve attained on a background of complete darkness. In the less fatiguable person, this difference was barely perceptible. Nagai et al (2002) showed that trait anxiety was significantly predicted by parameters of the pupillary light reflex, which also mirrored state anxiety. The parameters studied included the pupillary diameter at maximal

pupillary constriction, the ratio of the constriction amplitude to the resting diameter and the constriction velocity. They found that high trait anxiety decreased the amplitude of the pupillary light reflex. The intra-individual inter-test differences may therefore result from a variation in the autonomic balance of the individual from day to day. In addition, if there were small variations in the level of background illumination from one visit to the next, this would have caused a variation in the results, which would have differed according to the arousal level of the individual being tested.

When tested repeatedly on four or five separate occasions, none of the eight volunteers consistently demonstrated a statistically significant difference in the constriction amplitude between the two sides. It follows therefore, that in none of these volunteers, the pupillary constriction to light was consistently stronger on one side than on the other. In one volunteer (T18) there was a statistically significant difference in constriction amplitude on two out of three occasions. On both of these occasions, the same side showed larger constriction amplitude.

These results challenge the work of Bär et al (2005) who pooled the data from both eyes of 34 healthy volunteers who underwent testing both at different times of the day and on different days. Analysis of variance (ANOVA) confirmed significant differences between the left and right eyes across many parameters including relative amplitude. They found parasympathetic parameters including relative amplitude significantly more pronounced for the right pupil and they suggested the hemispheric lateralization of autonomic function as a possible reason.

The difference between the study of Bär et al (2005) and this study may be explained by their use of the parameter of *relative amplitude* instead of amplitude. They concluded in their study that different parameters of the pupil light reflex were likely to be influenced by diurnal variation in different ways, depending on whether the left or right eye was being studied. Multivariate ANOVA showed that the latency time for the pupil light reflex was shorter in the evening in the right eye, whereas the diameter of the left pupil was smaller in the evening. In using the ratio of the absolute amplitude and resting diameter, this interaction becomes combined, and may result in the difference with the values from this study. Another possible explanation for the difference between the results of Bär et al (2005) and the results of this study may lie in the use of different cameras for the left and right eyes. A systematic error may result in a consistently higher or lower reading of the values from one eye, and thus may apparently mask a potential difference.

This work has provided information about the boundaries of normal pupil function. This boundary is rather wide and it poses a challenge in the identification of subtle changes in the presence of early disease. The next stage of the study will aim to investigate the nature of changes in the pupillary light reflex brought on by optic nerve disease. If these changes fall outside the boundaries of normality, then the chances of developing a monocular pupillometric test, for the diagnosis of optic nerve disease, will be greatly increased. The white light stimulus used in this setup has been shown to produce a response which has a large variability. Increasing the complexity of the stimulus may allow for the improved detection of optic nerve pathology.

In the next stage, a complex pupillary stimulus will be used to assess the different pupillary channels in the context of established optic nerve disease. The specific testing of each pupillary channel (chromatic, luminance and grating) may offer more information regarding the extent of disease in the optic nerve.

TESTING SEPARATE CHANNELS OF THE PUPILLARY RESPONSE IN ESTABLISHED DISEASE:

An investigation into the presence of pupillovisual dissocation and channel dissociation in Leber's Hereditary Optic Neuropathy

Light resulting in pupil constriction may directly stimulate the melanopsin pigment within intrinsically photosensitive retinal ganglion cells (IPRGCs) as demonstrated by Gamlin et al (2007). This direct pathway is thought to be responsible for the static pupil response to light (Tsujimura et al, 2010). IPRGCs have also been shown to receive input from both rods and cones and show colour opponency (Dacey et al, 2005; McDougal et al, 2010) and these rod/cone dependent pupillary responses require the integrity of the IPRGC pathway (Güler et al, 2008). Since the discovery of IPRGCs in 1991, mounting evidence points to IPRGCs being the final common pathway required for any visual signal to result in a pupil response.

The mammalian pupil has been shown to respond to different modalities of stimuli (Barbur et a, 1992; Gamlin et al, 1998). Barbur et al (1998) used latency measurements for the pupillary response to luminance, chromatic and grating signals to ascertain the level of cortical input for each modality. Pupil responses to a change in spatial structure or a colour stimulus were found to be almost identical, but the latency of both were approximately 40 ms longer than the latency to a luminance stimulus. This was evidence of the need for subcortical processing of the luminance signal, but not of the other stimulus modalities which were likely to require cortical involvement resulting in the prolonged latency. Further evidence of cortical involvement in the presence of lesions in cortical area V4 can entirely abolish the

pupillary response to a chromatic stimulus presented within the foveal region (Barbur 2004). The pupillary responses to both grating and chromatic stimuli have been shown to be preserved in mammals with lesions of the striate cortex, suggesting its redundancy in this context (Weiskrantz et al, 1998).

The role of the IPRGCs within the context of different stimulus modalities is unknown.

A study of pupillovisual dissociation across different stimulus modalities

Reports of cases where patients with Leber's Hereditary Optic Neuropathy (LHON) were being diagnosed with psychogenic visual loss first alerted the medical community to the possibility that the relationship between the pupillary light response and the presence of optic nerve disease may be more complex than had hitherto been understood (Nakanishi et al, 1994; Thompson, 1966).

Leber's Hereditary Optic Neuropathy was first described by Theodore Leber (1840-1917) who described a distinctive pattern of visual loss which was predominantly seen amongst young males, inherited maternally in a non-Mendelian pattern and was not accompanied by other neurological deficit (Leber, 1871; Bell, 1931). It is now known that 95% of LHON patients carry one of three mutations within genes encoding the subunits of complex 1 of the mitochondrial respiratory chain: G3460A, G11778A, and T14484C (Mackey et al, 1996). The prevalence of LHON has been estimated to be 1 in 25 000 in the north east of England (Chinnery et al, 2000). Patients first present with a subacute, painless clouding of vision in one eye which does not recover. Although fundal examination may reveal abnormal retinal

vasculature and nerve fibre layer swelling, the fundal appearance may be normal in up to 20% of cases in the acute phase (Riordan-Eva & Harding, 1985). In parallel diseases of the optic nerve where the fundal appearance is normal (such as retrobulbar optic neuritis) the pupillary light response can be used to confirm the presence of optic nerve disease. This technique was found to be fallible in patients with LHON when their pupillary light response was not found to corroborate their complaint of visual loss (Nakanishi et al, 1994).

Wakakura and Yokoe (1995) assessed the pupillary response to light using infra-red pupillometry in 13 patients with LHON and compared the results with 23 eyes diagnosed with idiopathic optic neuritis and 19 eyes diagnosed with anterior ischaemic optic neuropathy. All patients had comparable visual deficit. A significant reduction in constriction amplitude was found across all patients apart from those with LHON, when compared with controls.

Since the publication of this study, consecutive reports contradicted and then confirmed the presence of a 'pupillo-visual dissociation' within patients with LHON. Jacobsen et al (1998) examined the pupillary response in 10 patients with LHON and failed to find pupillo-visual dissociation. Luedtke et al in March 2009 challenged Wakakura and Yokoe's study by reporting the presence of statistically significant abnormal constriction amplitudes in 40 patients with LHON disputing the existence of pupillo-visual dissociation. Bremner et al in October 1999 found that although the pupillary responses were abnormal in LHON patients, they were better than would be inferred from other measures of visual function.

The discovery that intrinsically photosensitive retinal ganglion cells (IPRGCs) play a key role in mediating the pupillary light reflex and are a separate class of ganglion cell offered weight to the possibility that separate channels may convey image forming and non-image forming visual information. One channel pathway could therefore be affected by disease more than another. A recent study in a mouse model reported that a loss of 80% of IPRGCs is required before non-image visual information is compromised (Goz et al, 2008).

La Morgia et al (2010) provided the first histological proof of relative IPRGC sparing in LHON. The group found the proportion of IPRGCs amongst all ganglion cells in a 58 year old control patient to be 1.5% compared with 0.9% in an 85 year old with a peak density at the parafoveal zone. In a case of severe LHON, this proportion rose to 38.5%, despite a strong decline in the overall number of ganglion cells. The distribution of the IPRGCs remained the same.

With definitive histological evidence of IPRGC sparing in LHON, patients with LHON present an ideal opportunity to assess pupillovisual dissociation across the different pupillary stimulus modalities. The extent of pupillovisual dissociation across modalities in LHON has not yet been explored. It is not known if IPRGCs convey chromatic and grating signals resulting in a pupillary response. The absence of a response to these modalities in the presence of cortical lesions would suggest a non-retinohypothalamic pathway is responsible for mediating this information. This notion is contradictory to recent work suggesting that IPRGCs are the final common pathway for all information culminating in a pupillary response.

Aim:

To investigate whether all pupillary signal channels (luminance, grating and colour) are equally spared in LHON-associated pupillo-visual dissociation.

Methods

This study was carried out in two stages. In the first stage, the integrity of the parvocellular and magnocellular ganglion cell pathways in each LHON patient (Rod-> M-cell and Cone->P-cell) was tested to confirm the presence of extensive destruction in each which eliminated the possibility that these pathways were carrying information to the cortex to allow a pupillary chromatic and grating response to take place. In the second stage, the patients' pupillary responses to luminance, chromatic and grating stimuli were tested. Figure 1 shows the schematic pathway being tested.

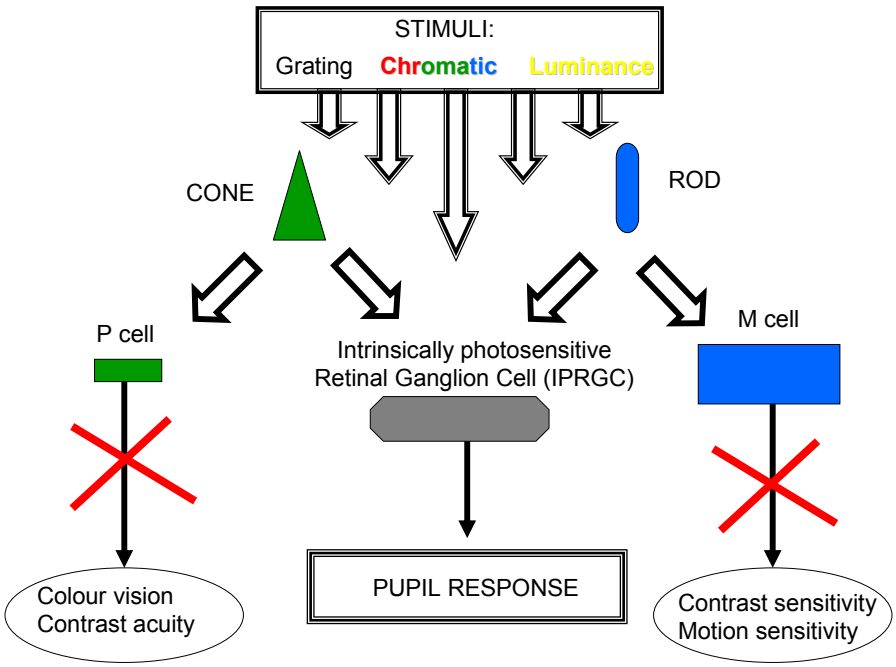

Figure 1

Patients

Three patients suffering from LHON were recruited for this study, each at a different stage of visual disability from the illness. All patients suffered from central visual field loss affecting at least the central 10 degrees of visual field. Goldmann perimetry was carried out on all patients prior to testing. All patients were shown to have good photoreceptor function from electro-diagnostic testing. The pupillary traces from five normal age and gender-matched control subjects were also obtained.

Testing

All testing was carried out targeting the central 10 degrees of visual field. The magnocellular pathway was assessed using a test for motion detection and contrast sensitivity. The parvocellular pathway was tested using the Colour Assessment and Diagnosis (CAD) test and high contrast visual acuity testing.

Motion detection testing

An achromatic stimulus composed of a 5x5 square array of same achromatic checks moving diagonally in a randomly selected direction at a constant speed of 4˚/s in a 15 x 15 square array which subtend 8.5^{o} x 8.5^{o} was presented in a uniform background using a Multiscan 500PS Trinitron monitor (Sony Corporation, Tokyo, Japan) using a frame rate of 75Hz. The background subtended an area of 30^{o} x 24^{o}, the background chromaticity in CIE co-ordinates was 0.305, 0.323 and the background luminance level was 24cd/m^2. The 15 x 15 achromatic square array that acted as random luminance modulation scintillated their luminance above and below the background luminance level dynamically but the overall luminance of these checks were the same

as the background. The threshold of detection of the moving target was measured using interleaved staircases with three random luminance noise levels, i.e., 6%, 12% and 24%. The observer was asked to press one of four buttons arranged as a square in a forced-choice procedure, to demonstrate the direction of movement of the square.

Measurement of the contrast sensitivity and visual acuity

An Landolt C test was employed in the contrast sensitivity and visual acuity measurement. The stimulus was presented in the centre of a uniform background field of 26cd/m^2 for 600ms in both experiments. The observer's task was to press one of the four buttons located on the corners (top left, top right, bottom left and bottom right) of a button box to indicate the gap of the stimulus. In the contrast sensitivity test, the gap of the Landolt C was fixed at 73.2^o and a staircase method was used to measure the threshold of the contrast that an observer needs to detect the gap direction. In the visual acuity test, the contrast of the Landolt C was fixed at -100% and a staircase method was used to measure the threshold of the gap size that an observer needs to detect the gap direction.

Measurement of the colour sensitivity

The CAD test was employed to measure the chromatic sensitivity. An isoluminant 5x5 square array of checks, of which 21 are coloured, moving diagonally in a randomly selected direction at a constant speed of 4˚/s in a 15 x 15 square array of achromatic checks and was presented in the centre of a 26cd/m^2 (0.305, 0.323) background field that subtends a 28^o x 23^o visual angle using a Multiscan 500PS Trinitron monitor (Sony Corporation, Tokyo, Japan) using a frame rate of 75Hz. The achromatic checks scintillated their luminance above and below the background

randomly but the overall mean luminance was the same as the background. These checks acted as spatio-temporal luminance noise which minimized any luminance detection from the colour defined stimulus. The stimulus colour was defined by CIE co-ordinates and sixteen different directions in CIE space were employed. The threshold along each direction was measured using 16 interleaved staircases. The observer was asked to press one of four buttons arranged as a square in a forced-choice procedure, to demonstrate the direction of movement of the square.

Pupillometry

The 'worse' eye of each patient was tested under monocular viewing conditions. All patients were dark adapted for 10 minutes. An abrupt onset-offset square-wave stimulus of 400ms duration was presented on a uniform background field of 12 cd/m² and a chromaticity of CIE coordinates 0.298, 0.335 which was placed at a viewing distance of 70cm. The pupil diameter was continuously measured for 6 second for each presentation. Three stimulus modalities were presented in the shape of a 10˚ circular disc.

1. An achromatic luminance stimulus which had a contrast of 171% of the background level.
2. An achromatic square wave grating at a spatial frequency of 5.5cycles/degree, equiluminant with the background level.
3. Two chromatic stimuli (bluish and reddish) which were isoluminant with the background level. The cone contrast angles for an ‘average’ observer at the background chromaticity level (CIE coordinates 0.298, 0.335) are shown in elliptical space at a chromatic distance (CD) of 0.004 and 0.012 in the figure

below. The ratios of rod, L, M and S cone contrast levels at each chromatic angle are shown. We chose a red stimulus at a chromatic angle of 298° and a chromatic distance of 0.15. At this CD and chromatic angle, there was zero rod contrast. The ratios of contrast between the different cone classes and the rods can be discerned from figure 2 below. At this angle there is no rod contrast, some L-cone contrast (0.04%), some S-cone contrast (0.2%) and -0.08% of M-cone contrast. We chose a blue stimulus at a chromatic angle of 240° and a CD of 0.25. At this CD and chromatic angle, there was 171% rod contrast. The ratios of contrast between the different cone classes and the rods can be discerned from figure 1. At this angle, there is 0.36% rod contrast, no L or M- cone contrast and 0.08% S cone contrast, due to the proximity in spectral sensitivity between the rod and the S cone. This stimulus was a Rod/S-cone signal.

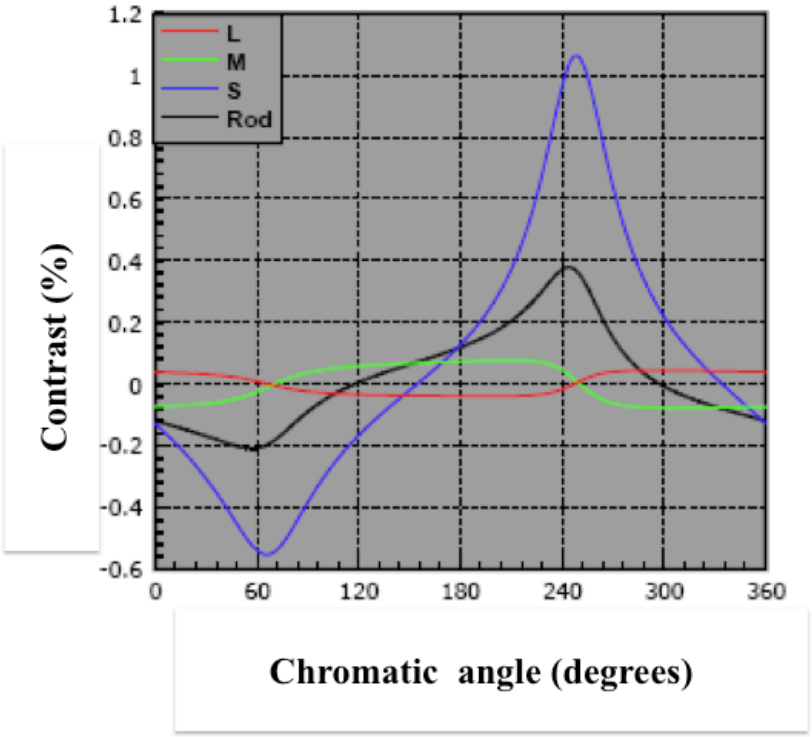

Figure 2.

Cone contrast angles for 'average' observer at background chromaticity level.

The stimulus presentation was interleaved using a Latin-square method and the mean pupil response trace was obtained by an average of 32 measurements per stimulus. The four stimulus modalities are demonstrated in figure 3 below.

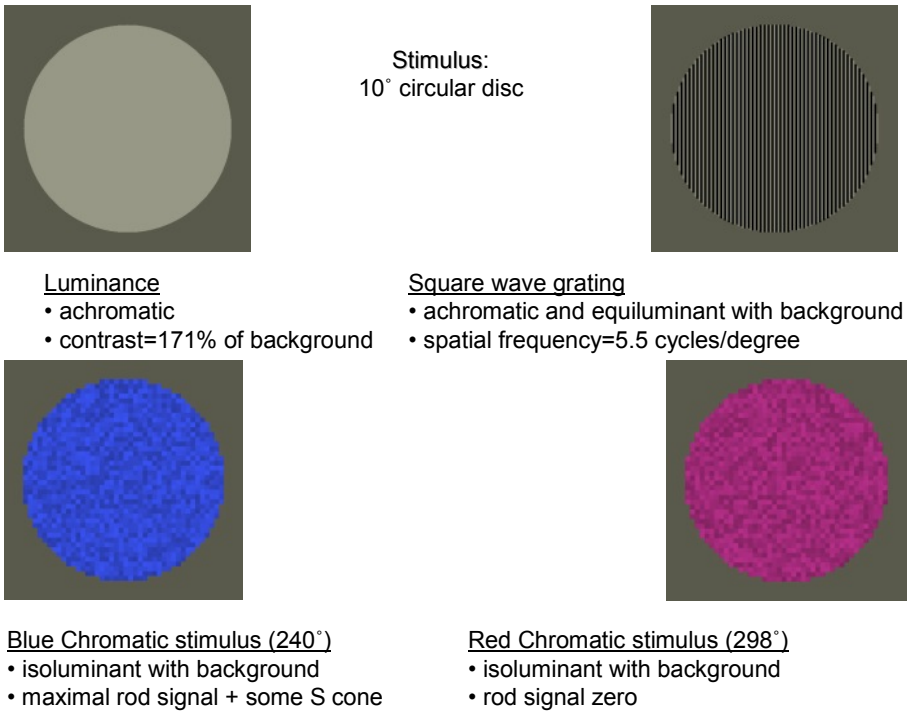

Figure 3

Results

The demographic details, visual acuities, onset of visual loss and treament details of each patient is shown in table 1.

	Patient one	Patient two	Patient three
Age	19	61	58
Gender	m	F	M
Onset	6 months ago	21 months ago	48 months ago
Leber's mutation	14484	14484	11778
Visual Acuity	6/36	1/120	Hand Movements only
Treatment	On 800mg OD Coenzyme Q10 for 1 month	On 1200mg OD Coenzyme Q10 for 8 months	None

Table 1

M pathway assessment

The results of motion testing in all three patients in comparison to a normal control are shown in figure 4 below.

Results of Motion test performance by LHON patients 1, 2 and 3 compared to a control patient.

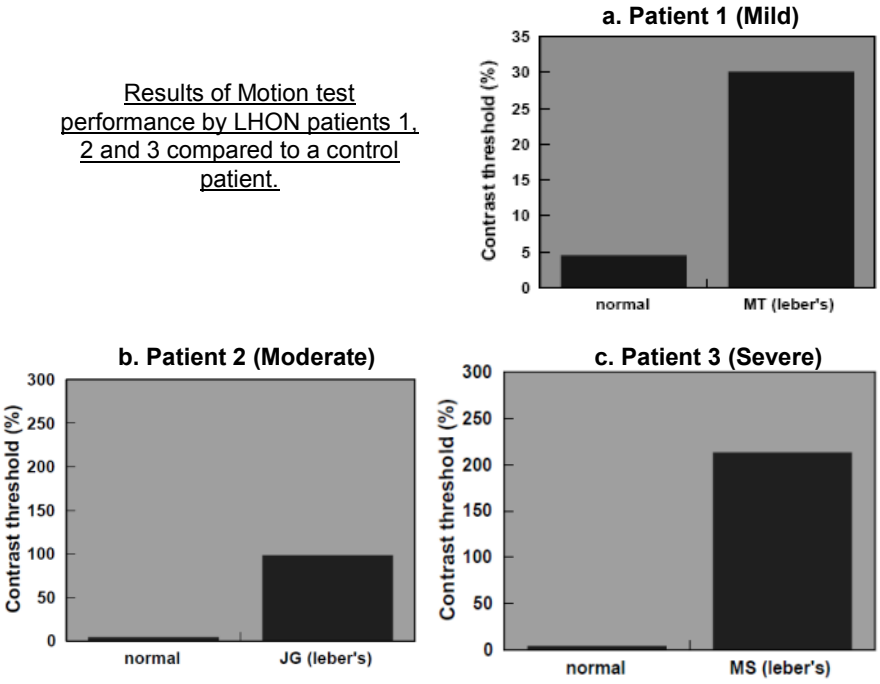

Figure 4.

Cone contrast angles for 'average' observer at background chromaticity level

Patient 1 showed a motion detection threshold level of 6 times that of a control. In the case of patient 2, this threshold was 20 times and in the case of patient 3 this threshold was 100 times that of a normal control. When tested for contrast threshold levels, the three patients demonstrated a contrast threshold of between 14 (patient 1) and 100 times that of a normal control. In all three cases, both motion perception and contrast threshold detection (and hence the M pathway) was markedly compromised.

P pathway assessment

The CAD assessment result of a normal control is shown in figure 5. Figures 6, 7 and 8 show the results of each of the three patients, whose profound colour deficit at all

orthogonal directions lay beyond the phosphor limit of our detection system and has been shown at its limits. The high contrast acuity thresholds of patients 1,2 and 3 were 22, 40 and 70 times that of a normal control. Both these measurements indicate a damaged P ganglion cell pathway in all three patients.

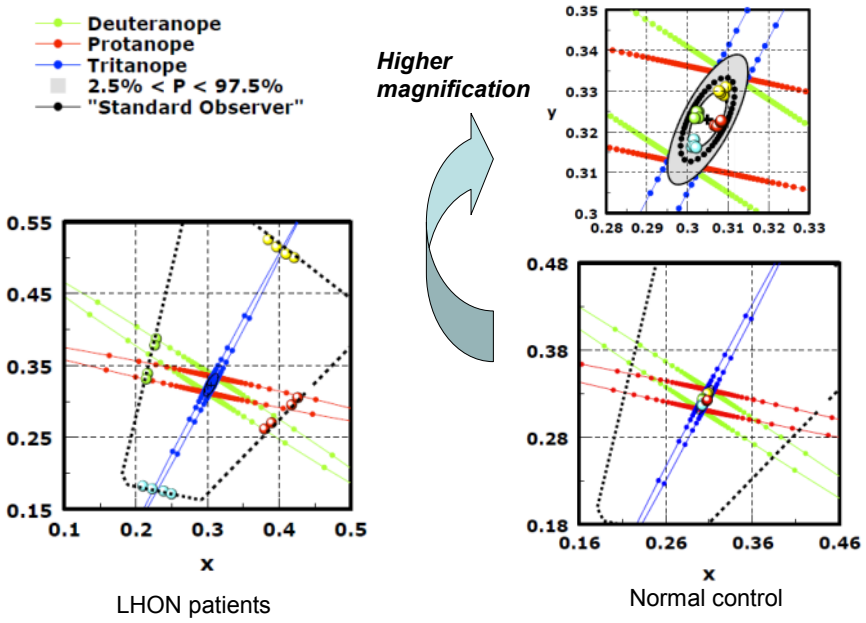

Figure 5.

Colour vision assessment (CAD) on chromaticity diagram

Pupillometry

The pupillometry traces obtained in response to the chromatic, grating and luminance stimuli in patients 1,2 and 3 are shown in figures 6,7 and 8. They are presented on an identical scale, with the traces shifted upwards or downwards for clarity.

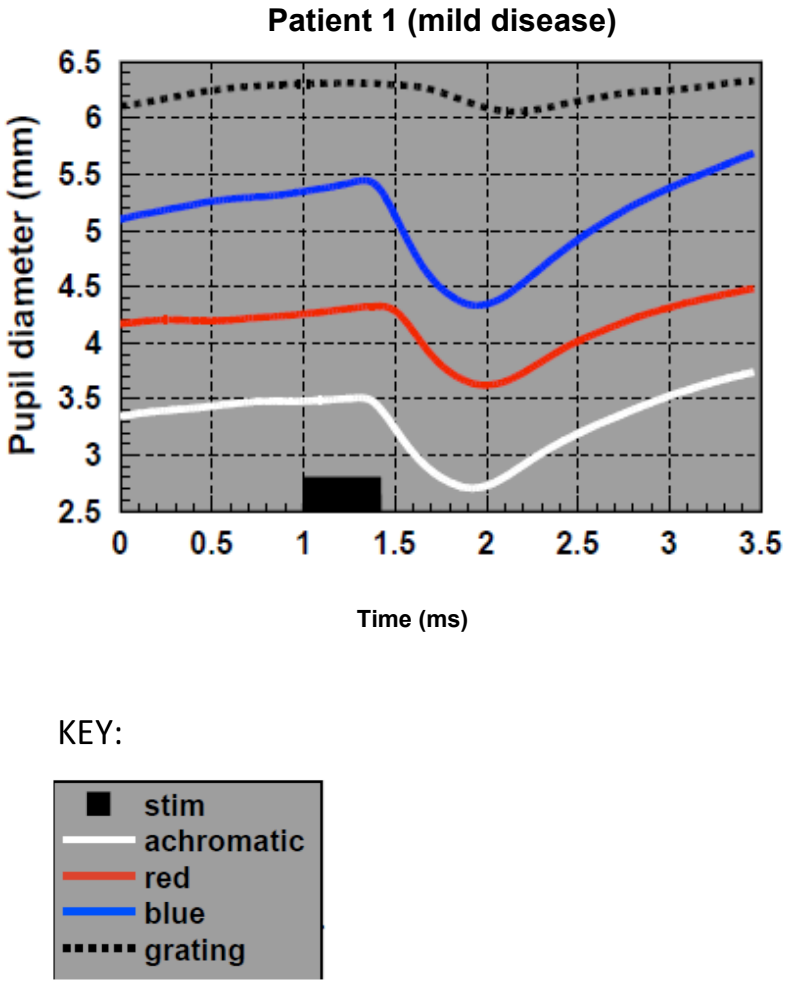

Figure 6

Patient 1 has mild disease and a clear response to all stimuli may be observed. The response to the grating stimulus is less pronounced than the response to the other stimulus modalities.

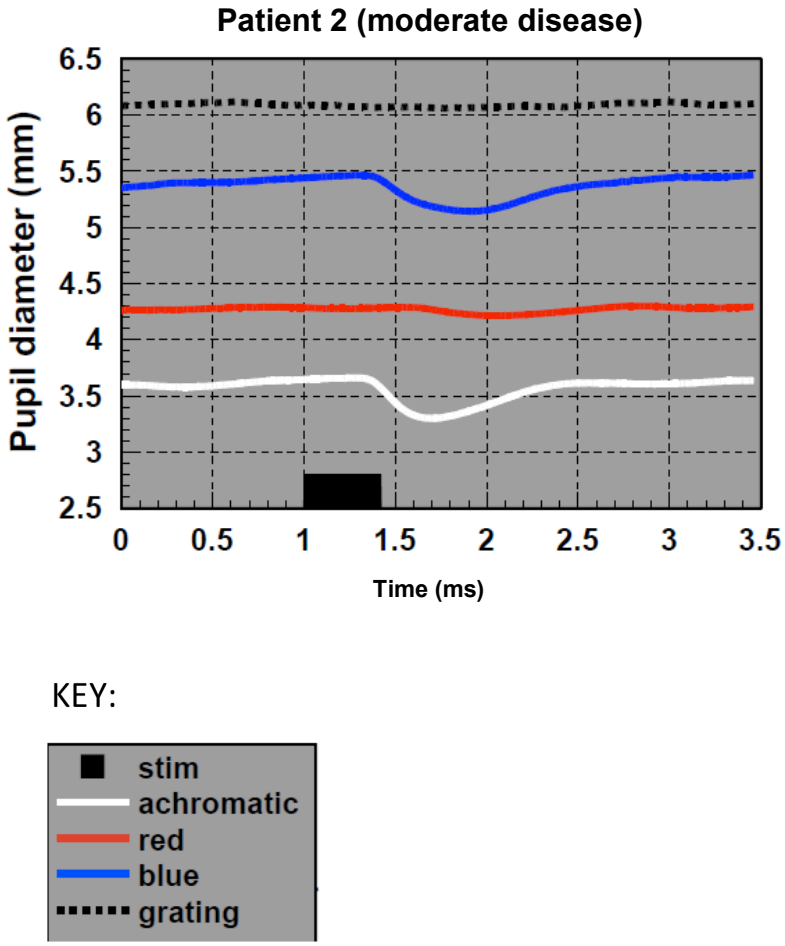

Figure 7

Patient 2 demonstrates a smaller response across all modalities with no response to the grating stimulus and a barely discernible response to the red chromatic stimulus.

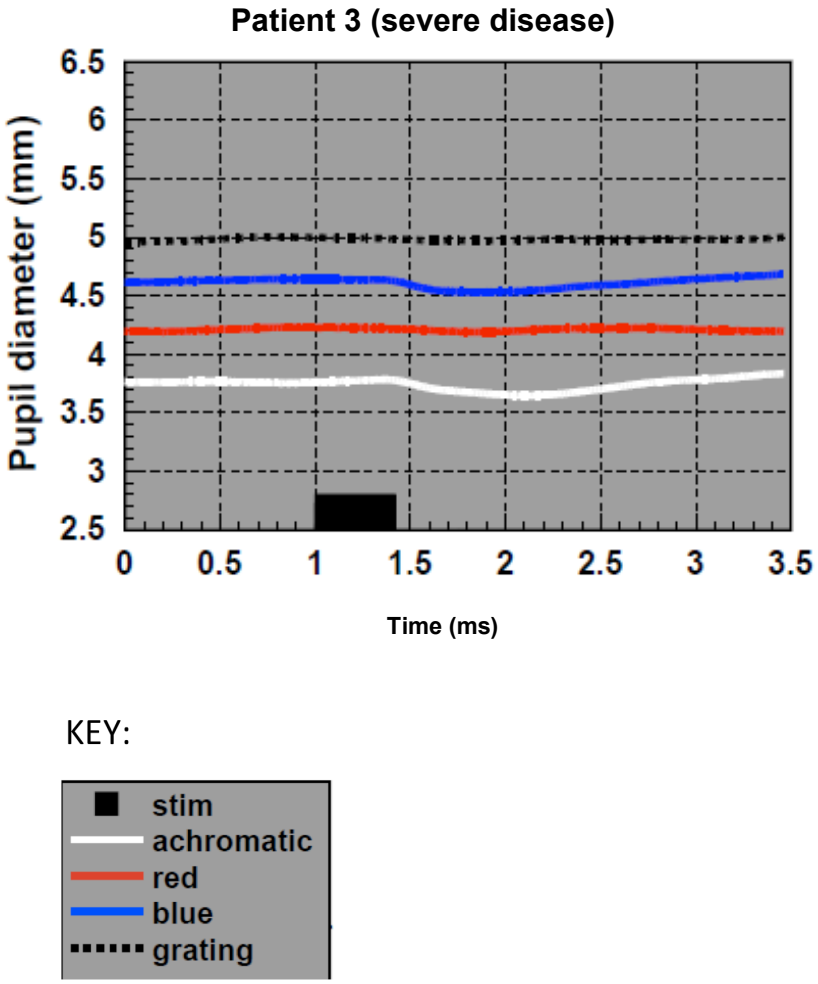

Figure 8

Patient 3 shows no response in the red chromatic and grating stimulus modalities and a minimal response to the grating and red chromatic stimuli.

Patient 1 shows a good response across all stimulus modalities. Patient 2 demonstrates smaller amplitudes of response across all modalities, with no response to the grating stimulus and a minimal, almost absent response to the red chromatic stimulus. Patient 3 does not show a response to the grating and red chromatic stimuli, and shows a minimal response to the achromatic and blue chromatic stimuli,

The three patients who display a worsening scale of disease severity appear to show that a pupillary response to an achromatic stimulus and a blue chromatic stimulus may be preserved in relatively severe disease when the response to grating and red chromatic stimuli may be lost. There may be a pupillovisual dissociation across the stimulus modalities at differing disease intensities. It may be most obvious in a patient with moderate disease and less obvious in a patient with either very early or very advanced disease.

The absolute amplitude of response of each of the control subjects, to the four stimuli (three stimulus modalities) are shown in figure 9 .

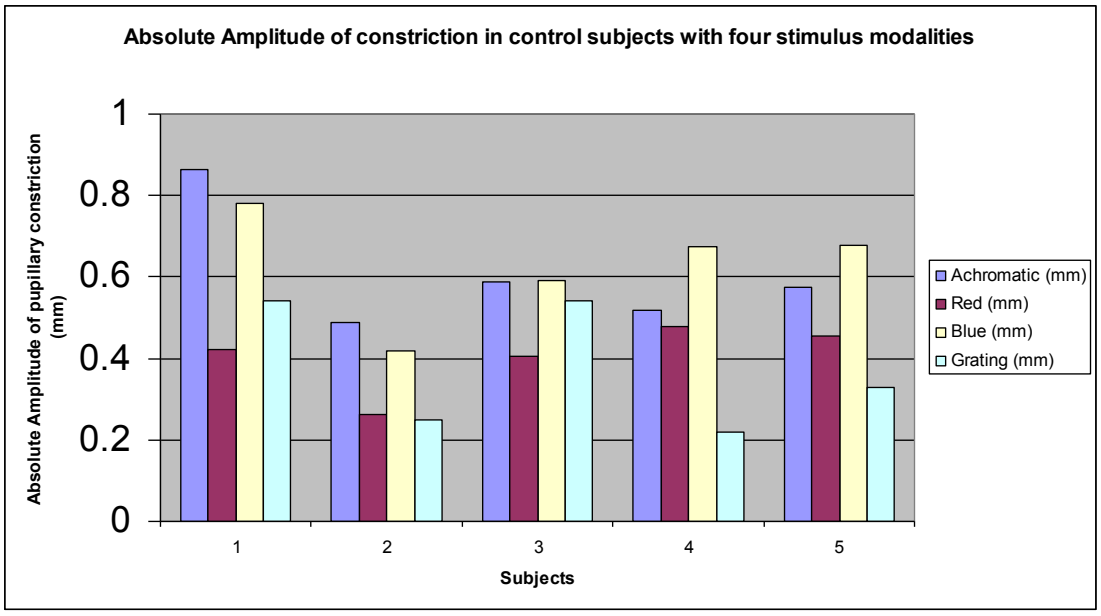

Figure 9

The mean absolute amplitude and standard deviation (s.d.) for each stimulus across all five controls are shown in table 2 below:

Stimulus	Mean	s.d.
Achromatic (mm)	0.6066	0.149288
Red (mm)	0.4046	0.085055
Blue (mm)	0.6282	0.135422
Grating (mm)	0.3758	0.156369

Table 2

The responses to the red chromatic stimulus and the grating stimulus were found to be slightly smaller in amplitude relative to the responses to the luminance stiumulus and the blue chromatic stimulus in all normals. The response to the grating and achromatic stimuli were the most variable (s.d. 0.156 and 0.149). The response to the red

chromatic stimulus had the smallest variance. All control subjects showed an absolute amplitude of response of more than or equal to 0.49mm and 0.42mm to the achromatic and the blue chromatic stimuli respectively. All control subjects had an absolute amplitude of response of more than or equal to 0.22mm and 0.26mm to the grating and the red chromatic stimulus respectively.

The absolute amplitude of response of each patient (1,2 & 3), to the four stimuli (three stimulus modalities) are shown in figure 10 below:

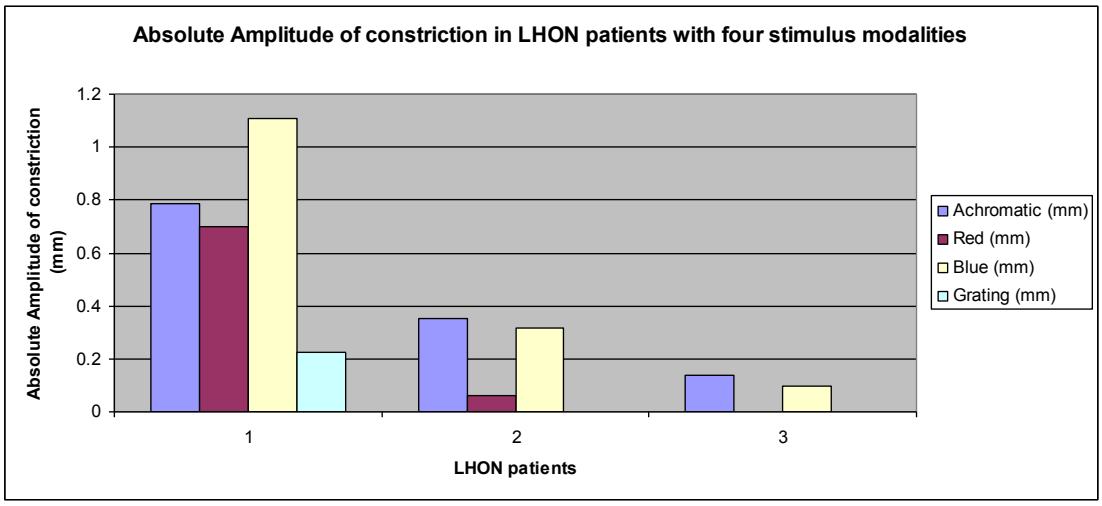

Figure 10

The results of patient 1 were within normal limits, despite a considerably compromised P pathway and a visual acuity of 6/36. Patients 2 and 3 displayed subnormal responses to all stimuli. The ratio of the absolute amplitude of constriction of the red chromatic stimulus to the blue chromatic stimulus (aR/aB) is seen to be far smaller in patients 1 and 2, when compared to each control. The aR/aB are shown for the 2 LHON patients with moderate and severe disease, and the 5 control subjects, in table 3 below.

LHON aR/aB	cntrl aR/aB
0.194968553	0.54230769
0	0.62440191
na	0.68813559
na	0.70962963
na	0.66961652

Table 3

The sample size is very small but it is clear that the LHON patients demonstrate smaller ratios than the normal controls. A similar comparison of the ratio of the absolute amplitude of constriction to the red chromatic stimulus and the achromatic stimulus (aR/aAC) shown in table 4 below.

LHON aR/aAC	cntrl aR/aAC
0.176136	0.489583
0	0.532653
na	0.691652
na	0.92471
na	0.790941

Table 4

The sample size limited statistical analysis, however, a trend for a difference may be observed.

A similar analysis was not undertaken with the ratio of the grating response amplitude because the pupillary response to the grating stimulus showed the greatest variation and neither patient 2 nor patient 3 demonstrated a grating response.

The presence of a higher ratio of the achromatic (luminance) signal and the blue chromatic signal, compared to the red chromatic signal in patients 2 and 3 demonstrates the presence of dissociation between the pupillary response to an achromatic luminance signal and a red chromatic signal in LHON patients with moderate to severe disease.

Discussion

This study firstly confirmed previous reports of the presence of pupillovisual dissociation in LHON by demonstrating a normal range of pupillary responses in Patient 1, whose other visual function tests, such as visual acuity, was significantly affected.

This study also demonstrates that the pupillary response to a red chromatic stimulus declines faster than the response to an achromatic (luminance) stimulus as well as a blue chromatic stimulus, at greater disease severity. Although the sample size in this study is markedly small (owing to the profoundly low incidence of LHON) the results of this study demonstrate that pupillovisual dissociation is not uniformly present across all stimulus modalities.

Although this study did not involve the specific testing of the IPRGC pathway, if we are to assume the relative preservation of the IPRGCs in LHON as demonstrated by La Morgia (2010) then this study would indicate that all signals culminating in a pupillary response do not traverse this pathway. Specifically, these results suggest achromatic signal coding and blue chromatic signal coding (Rod/S cone) is channelled through the IPRGC pathway and is hence relatively preserved, whereas red chromatic signal coding (S cone, M cone and L cone) and grating signal coding is processed through an extra-IPRGC route, perhaps through the P ganglion cell pathway which is severely compromised in patients with moderate or severe disease.

This study is made unreliable by the paucity of LHON cases, and a larger study would increase the accuracy and reliability of the findings. The proximity of the S cone spectral sensitivity to rod spectral sensitivity made it difficult to separate the role of the S cone pathway from the rod pathway, which caused S cone stimulation in both the red and blue chromatic stimuli. It is difficult to discern whether the S cone signal is carried along both the IPRGC and the M/P ganglion cell pathway. The two patients with moderate and severe disease were found to have differing mutations, suggesting that pupillo-visual dissociation is not limited to a particular mutation. The two patients with moderate and severe disease were of similar age, compared to the patient with a ‘normal’ range of pupillary performance. For an improved study, patients with moderate to severe disease from a range of ages would be recommended because pupillovisual dissociation may be manifest after a certain age in patients with LHON.

This study has demonstrated that a complex stimulus offers greater information regarding the pattern of damage in the optic nerve. The IPRGC pathway appears dissociated from the P- and M- ganglion cell pathways and in the next stage of study, an improved pupillometer with direct stimulation of the IPRGC channel as well as the chromatic and luminance channels will be used in the assessment of optic neuritis. Research into the nature, physiology and role of IPRGCs has progressed rapidly over the past few years. Current knowledge on IPRGCs is first reviewed.

INTRINSICALLY PHOTOSENSITIVE RETINAL GANGLION CELLS

The standard pupil light reflex has been shown in chapter one to be highly variable amongst normals. This variation amongst normals makes it difficult for absolute values to be assigned as 'normal' or 'pathological' and makes it difficult to draw comparisons between complex pathologies where the difference in the overall form of the standard transient or sustained PLR may not be pronounced. The study conducted examining pupillo-visual dissociation within 3 patients with LHON has indicated that exploring the 'intrinsically photosensitive ganglion cell (IPRGC) pathway may allow a greater complexity of information regarding the pupillary light response to be assimilated. The LHON patients demonstrated that the red chromatic (L,M,S cone) and grating components of the PLR may be carried along an extra-IPRGC pathway which is profoundly affected in LHON whereas the luminance pathway and blue chromatic pathway (rod + S cone) may be relatively spared perhaps due to an IPRGC route.

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Intrinsically photosensitive ganglion cells : classification

Two subtypes of IPRGCs, M1 and M2 are known to exist. An M1 cell has a small cell body and a large dendritic arbor with little branching. An M2 cell has a larger cell body and a more branched dendritic arbour. M1 and M2 cells in the mice have been shown to contain two isoforms of melanopsin in differing quantities (Pires et al, 2009). M1 cells express OPN4S and OPN4L isoforms whilst M2 cells express the OPN4L isoform only. Recent evidence suggests there may be further subclasses of IPRGCs. Immunostaining by Berson et al (2010) has demonstrated a weak presence of the photopigment melanopsin in ganglion cells separate from the M1 and M2

subtypes. Many of these cells are larger than M1 and M2 with a statistically different soma diameter. They cite unpublished observations of ON alpha-like cells in rodents which are capable of generating weak intrinsic light responses. There is therefore a possibility of the presence of further IPGC subtypes in the retina, each with distinct functional and anatomical characteristics, which are yet to be characterized.
IPRGCs comprise approximately 1% of all ganglion cells, and their axons primarily project to brain regions which are responsible for non-image forming vision, including the pretectum (for the pupillary light reflex), the suprachiasmitic nucleus and the intergeniculate leaflet. It has been recently shown that periods of darkness or daily cycles of dark and light periods may be necessary for the normal development of the Melanopsin system (Gonzalez-Menendez et al, 2010).

The exact role of IPRGCs and specifically of melanopsin, within the in-situ setting of the human retina, still remains partially unknown, and much recent work in the field of pupillometry has focussed on the anatomical, biochemical and physiological characteristics of IPRGCs.

Intrinsically photosensitive ganglion cells : phototransduction

Do et al (2009) examined the electrophysiological response of mice melanopsin to single photon photostimulation. The response to a single photon of light was shown to be far slower than the equivalent response in rods and slower still than that of cones (approximately 20 times and 100 times respectively). However the response itself was found to be far more powerful than the rival receptor classes. Do et al (2009) justified the relatively lower sensitivity of IPRGCs containing melanopsin compared to other receptor classes by calculating the likely density as being equivalent to 3 molecules

per μm² of IPGC membrane, which is 10000 times lower than the pigment density in rods and cones (Liebman et al, 1987). The low sensitivity of IPRGCs is acceptable for their role as rods and cones feed into these cells and drive their responses at lower light levels. The photon capture probability in IPRGCs was inferred to be 10-fold lower than rods and cones, however Do et al (2009) observed that a single absorbed photon is adequate for the IPGC to signal to the brain. The resting membrane potential of an IPGC is maintained very near the spike threshold for firing in darkness. It fires spontaneously at a low rate and a small depolarization increases the spike rate by several fold. The response has a slow decay, which prolongs this effect. The practical implications of these findings are considerable, although the relative implications on the different IPGC classes, which are morphologically different needs to be ascertained.

Intrinsically photosensitive ganglion cells : bistability

Melanopsin has been shown to be a phylogenetically separate class of photoreceptor from rods and cones, with regard to its bistability. Light stimulation simultaneously converts and regenerates 11-cis-retinal. Photon absorption at one wavelength initiates the phototransduction cascade of 11-cis-retinal, while photon absorption at a second wavelength photoisomerizes all-trans-retinal back to 11-cis-retinal (Pepe & Cugnoli, 1992). This bistability property is thought to confer the ability for prolonged pupilloconstriction in sustained bright light (Mure et al, 2007; Zhu et al, 2007).

The bistable nature of human melanopsin was recently demonstrated. Mure et al (2009) demonstrated that prior light exposure affected the post-stimulus persistence of pupillary constriction in a wavelength-dependent manner. This response was shown

to increase by 28% after exposure to a priming red light (620nm) and to decrease by 21% after exposure to short wavelength blue light (480nm). An equilibrium point (termed the isosbestic point) was discovered at a wavelength of 514.3nm (s.d.1.2) where prior photostimulation had no influence on the pupillary light reflex. Priming stimulation by longer wavelengths increased the response, and shorter wavelengths had the opposite effect. This point was described as being the isosbestic point where an equivalent rate of conversion between the 11-cis and all-trans isoforms was occurring in the bistable system. This isosbestic point was found to be almost identical in all subjects tested. Mure et al argue that the presence of this point is proof of the bistability of human melanopsin.

Intrinsically photosensitive ganglion cells : anatomy

Recent studies on the anatomical characteristics of IPRGCs have prompted an evaluation of the traditional model of retinal circuitry. It is known that changes in irradiance level are detected as 'ON' and 'OFF' inputs by the retinal photoreception circuitry.

It has long been known that each is generally channelled by a distinct pathway. The inner three-fifths of the Inner Plexiform Layer (IPL) of the retina have been known to form the 'ON' sublayer where 'ON 'bipolar cells synapse with 'ON' ganglion cells and 'ON' amacrine cells. The outer margin of the IPL is known to form the 'OFF' sublayer, where 'OFF' bipolar cells synapse with 'OFF' ganglion cells.

M1 cells have been shown, (along with dopaminergic amacrine cells (DA)) to 'break the rule' in the isolated organization of the 'ON' and 'OFF' channels. M1 cells'

dendrites stratify in the ‘OFF’ sublayer, despite receiving input from ‘ON’ bipolar cells (Wong et al, 2007). Their somas are located in the ganglion cell layer, so the dendritic processes must ascend through the ‘ON’ sublayer to reach their destination. One possible explanation lay in the possibility that proximal M1 cell dendrites may conceivably receive ‘ON’ input as they ascend from the ganglion cell layer, through the ‘ON’ sublayer.

Dumitrescu et al (2009) studied displaced M1 cells in the mouse retina, whose somas were located in the IPL and whose dendrites arborized in the ‘OFF’ sublayer without traversing the ‘ON’ sublayer. It was demonstrated that even in these cells, a subgroup of ‘ON’ cone bipolar cells makes ribbon synapses with M1 and DA cells in the ‘OFF’ sublayer, and some of this input is provided specifically by type 6 ‘ON’ cone bipolar. These findings were supported by fluorescent transgenic labelling of ‘ON’ bipolar cell projections to non-ectopic M1 cells and DA cells.

These results suggest there is an additional thin ‘ON’ sublayer in the most distal margin of, but not strictly segregated from the ‘OFF’ sublayer of the IPL.

Intrinsically photosensitive ganglion cells : role in sustained pupillary response

The pupil shows a typical response profile to a white light stimulus. There is first a rapid constriction, with the constriction velocity first increasing, peaking, and then decreasing until maximal constriction is reached transiently. This is known as the transient phase. Thereafter, the pupil partly redilates, or escapes, to a state of partial pupil constriction that represents the sustained phase of the pupil light reflex. After stimulus offset, there is a small constriction, followed by the gradual dilatation of the

pupil to reach the original baseline diameter (Kawasaki & Kardon, 2007). The transient response is thought to be driven by rods or cones (Young & Kimura, 2008). In-vivo characterization of the relative contribution of rods and melanopsin to sustained pupillary constriction has proven difficult in the past, owing to the input from the rod and cones into IPRGCs.

The contribution of the three receptor classes to the pupil's sustained response to prolonged steady-state stimuli has been recently investigated by McDougal and Gamlin (2010) . In their study, the three photoresponses driving the PLR did not show a linear summation at the level of IPRGCs; instead, the outer and inner signals competed in a 'winner takes all' capacity. At and above a specific threshold of activation, the melanopsin photoresponse acts to shunt the outer retinal signals reaching the IPRGCs and the melanopsin exclusively drives the pupil's response. L- and M-cone driven input was found to not contribute significantly to maintenance of pupillary constriction at any intensity of steady-state light stimuli. Their input diminished after the first 30 seconds. Rods were found to be the primary player for the human PLR for light intensities below the threshold of activation of the melanopsin photoresponse. After rapid adaptation over approximately 8 seconds, rods provided a tonic signal which maintained a sustained level of pupillary constriction in response to steady-state light stimulation. As light intensity increases or the spectral nature of the light is increasingly influenced by short wavelengths, the melanopsin photoresponse becomes the dominant photoreceptive influence on the human PLR in this setting. Kardon et al (2009) had earlier shown that the light intensity at which the intrinsic activation of melanopsin first begins to contribute to the sustained pupil response seems to vary among normal human eyes. In response to a blue light stimulus at

medium light intensities (10 cd/m^2), melanopsin was sometimes activated, and sometimes not.

Kimura and Young (2010) suggest cones do contribute to the sustained pupillary constriction phase via an L- and M-cone opponent process which encodes a chromatic stimulus and not irradiance alone.

Intrinsically photosensitive ganglion cells : clinical applications

The establishment of wavelength, intensity and duration preferences for the three mediators of the pupillary response to light has resulted in a novel application of the field of pupillometry to the diagnosis of retinal disease. Kardon et al (2009) tested patients with primary rod dysfunction (secondary to retinitis pigmentosa), achromatopsia and ganglion cell dysfunction (secondary to anterior ischaemic optic neuropathy) with different wavelengths, durations and intensities of light and found that each patient responded best to a certain stimulus 'specification'. Their responses raise the possibility of the use of 'pupil fingerprinting' to diagnose specific eye conditions in the future.

One disease in which the pupil response has been shown to be characteristic is in Leber's hereditary optic neuropathy (LHON) where the pupil response is out of proportion with the patient's visual ability, as deomstrated in chapter 3 of this thesis. La Morgia et al (2010) studied the retinae of post-mortem cases of LHON and found that the ratio of IPRGCs to total ganglion cells increased from 1% in healthy eyes to 49% in the most severe cases. They speculate on the possible reason for this, and whether the presence of the photopigment melanopsin may confer a protective shield

from light exposure which may be harmful in the setting of dysfunctional mitochondria (Osborne et al, 2008).

THE USE OF PUPILLOMETRY IN THE DIAGNOSIS OF THE UNDERLYING AETIOLOGY OF OPTIC NEURITIS BY STIMULATION OF THE IPRGC PATHWAY

Within the acute setting, where the swinging flashlight test is of the greatest relevance in clinically establishing the presence of optic nerve disease, the pupillary response is used as a reliable indicator of the presence or absence of the optic neuritis. This response is tested in the clinical setting with a white light shone transiently into one eye and then the other. If one eye is affected, a relative afferent pupillary response is observed.

After establishing that the pupillo-visual dissociation which has been observed amongst patients with LHON may be modality-dependent, my next step was to explore whether there is any component of modality-specific pupillovisual dissociation in patients with other forms of optic nerve disease such as optic neuritis caused by different aetiologies. The testing of Patient 1 within the LHON cohort produced results similar to those obtained from normal control subjects. In order to obtain meaningful results, it appears that testing patients with moderate to severe disease is warranted.

The LHON patients demonstrated that the luminance pathway which can be relatively spared may be carried along a non-P non-M ganglion cell pathway, most likely the IPRGC pathway. The chromatic and grating components of the PLR are compromised in LHON, in keeping with the extent of M and P ganglion cell damage.

An acute and chronic phase is known to occur in LHON (Yu-Wai-Man et al, 2011). Optic neuritis in the context of relapsing and remitting MS (RRMS) occurs as an acute episode, which spontaneously resolves in the majority of cases. In the optic neuritis treatment trial (ONTT) 95% of patients recovered to a visual acuity of 6/12 or better without treatment a year later. Although 79% of patients in the trial had begun to show signs of improvement within 3 weeks of onset and 14% of patients started to improve between 3 and 5 weeks after onset when no treatment was given (Beck et al, 1994), changes within the structure of the retina and optic nerve from the acute inflammation may remain for longer than this period of time. A recent OCT studies has demonstrated swelling of the retinal verve fibre layer (RNFL) in the acute phase (Costello et al, 2008) which subsides within 3-6 months when RNFL thinning becomes noticeable. This diffuse swelling of the retina during the acute episode makes it difficult to recognise the structural cause behind the visual deficit experienced in the acute phase and it is likely that the structure of both the outer retina and the optic nerve are affected. Ménage (1993) and Katz (1995) demonstrated that during the acute episode of optic neuritis, Köllner's rule (1912) was not obeyed and blue-yellow colour defects (suggesting outer retinal layer pathology) were more common. At six months after the acute episode, red-green colour defects became more common amongst patients, suggesting the presence of damage within the optic nerve, in keeping with Köllner's rule.

Following an episode of acute optic neuritis related to MS, there is dissociation between visual function tests of acuity and colour. Mullen and Plant (1986) reported chromatic sensitivity to be more severely impaired than luminance sensitivity in patients with a past history of optic neuritis. This finding was reported a recent study.

Wall (1990) tested 10 patients with a visual acuity of 6/6 or better following a resolved episode of optic neuritis. No significant difference was found between the involved and uninvolved eyes at low spatial frequency pattern contrast sensitivity grating detection values whereas Farnsworth-Munsell 100 Hue colour testing was found to be abnormal in the affected eyes. The author attributed this finding to P cells being more damaged than M cells. Flanagan and Markulev (2005) used random luminance noise masking to assess luminance contrast and colour thresholds in patients with a history of optic neuritis. A dissociation was again found between the two, L and M cone input were found to be more impaired than S cone input. The authors pointed out the existence of a dissociation between the M and P as well as between the P and K (koniocellular) ganglion pathways.

In 2007, Moro et al measured both visual and pupil responses in 14 patients with a history of unilateral optic neuritis between 3 and 60 weeks of the onset of the episode. They reported finding dissociation between visual acuity and chromatic threshold performance as well as between visual acuity and the pupillary response to light and colour. Patients with a visual acuity of 6/6 or better were found to have a greater deficit in the pupillary light response than in the response to colour, whereas patients with visual acuity measurements worse than 6/6 showed larger deficits in the pupillary response to colour. They concluded that the chromatic pupillary response was more affected than the luminance response in optic neuritis. The red-green and blue-yellow axes of colour vision were found to be equally affected during colour vision assessment, suggesting the absence of dissociation between P and K cells.

Although it is not known how IPRGCs are affected in optic neuritis, the above reports suggest some degree of dissociation in the ganglion cell pathways may occur. The majority of the above studies have been undertaken on patients suffering from optic neuritis secondary to MS. It is not known whether any form of ganglion cell dissociation occurs in optic neuritis secondary to Neuromyelitis optica (NMO). Although the disease process in NMO is still being unravelled, there is agreement on the role of the anti-Aquaporin 4 antibody in contributing to its pathogenesis. Astrocytic damage has been shown to be more common in NMO than in MS. Structural differences have recently been reported within the retina between patients with NMO and MS related optic neuritis. The retinae of patients with NMO related optic neuritis have been reported to show vascular attenuation and arteriolar narrowing as well as more severe RNFL thinning even after controlling for visual acuity (Green & Cree 2009; Ratchford et al, 2009). The site of RNFL thinning has been found to be different in OCT studies carried out in MS and in NMO patients. In patients with NMO related optic neuritis, thinning of the superior and inferior quadrants have been reported whereas win MS related optic neuritis, thinning has been found to occur in the temporal quadrant (Naismith et al, 2009). Such histological and pathological differences reported within the retina and optic nerve between the two diseases raise the possibility that ganglion cell involvement may not be identical in MS and NMO.

To my knowledge, there has been no comparison of pupillovisual dissociation occurring in the context of NMO and MS related optic neuritis. In addition, there has been no modality specific testing for the presence of pupillovisual dissociation in the two diseases.

The use of pupillometry in the diagnosis of subacute optic neuritis.

Introduction.

Since the discovery of the Aquaporin 4 autoantibody, there is increasing agreement worldwide on Neuromyelitis Optica (NMO) being a distinct entity from multiple sclerosis (MS). However, three areas remain where an overlap between the two disease conditions may be observed.

Disease severity

Although NMO related attacks have been reported to be more severe than MS related attacks , a subset of MS related relapses can be severe, requiring immunosuppressive therapy or plasma exchange for resolution which has been described as effective treatment for NMO (Trebst et al, 2009; Watanabe et al, 2007).

Disease pattern

It was initially believed that patients with NMO do not display brain abnormalities in MR imaging (Wingerchuk et al, 1999). However, it is now becoming increasingly apparent that patients with NMO may have a similar pattern of lesions on brain imaging to patients with MS. In a recent report, the overlap in the radiological appearance of the brain has been shown to be as high as 50% (Matsushita et al, 2010) and two recent studies describe the incidence of longitudinally extensive transverse myelitis lesions (which is accepted as a specific sign for NMO) in cases of MS (Wingerchuk et al, 2006; Bot et al, 2004; Verhey et al, 2010).

Disease pathology

In NMO, one primary target of attack is thought to be Aquaporin 4 water channels situated on astrocytic foot processes (Roemer et al, 2007). However, the extensive loss of Aquaporin 4 beyond the geographical boundaries of myelin loss has been reported in MS and conversely, the preservation of Aquaporin 4 has been reported in an autopsied case of NMO (Matsuoka et al, 2009; Kobayashi et al, 2009).

In the ONTT, 90% of cases of optic neuritis spontaneously recovered to a visual acuity of 6/12 or better a year after the episode (Beck&Cleary, 1993). However, in 3% of cases, the visual acuity was equal to or worse than 6/60 after five years of follow-up.

In this study, we assess patients with MS related optic neuritis who have had poor recovery and compare them to patients with NMO related optic neuritis. By testing separate categories of ganglion cells in an objective and non-invasive manner, we investigate whether the pattern of ganglion cell loss in MS related optic neuritis is identical to that seen in NMO related optic neuritis.

Aim

To assess if the pattern of ganglion cell loss in optic neuritis with poor recovery secondary to MS is different to that in NMO, as tested with pupillometry.

Methods

Patients

All patients attending the Neuro-ophthalomology clinics at Moorfields Eye Hospital, The National Hospital, Queen Square and St. Thomas' Hospital with acute isolated optic neuritis over a two year period (march 2009 until Mar 2011). During this period, 39 patients presented with MS related optic neuritis and 19 patients presented with NMO related optic neuritis. Out of the 39 patients with MS, 4 patients presented with a visual acuity at or worse than 6/60 on follow-up and 4 patients with NMO related optic neuritis with a visual acuity of 6/60 or worse on follow-up consented to partaking in this study. Additionally, 1 patient was recruited with non-MS non-NMO recurrent isolated optic neuritis. Lastly 4 patients were recruited with Leber's Hereditary Optic Neuropathy (LHON).

Testing for colour and motion detection

Motion detection testing

An achromatic stimulus composed of a 5x5 square array of same achromatic checks moving diagonally in a randomly selected direction at a constant speed of 4˚/s in a 15 x 15 square array which subtend 8.5^{o} x 8.5^{o} was presented in a uniform background using a Multiscan 500PS Trinitron monitor (Sony Corporation, Tokyo, Japan) using a frame rate of 75Hz. The background subtended an area of 30^{o} x 24^{o}, the background chromaticity in CIE co-ordinates was 0.305, 0.323 and the background luminance level was 24cd/m^2. The 15 x 15 achromatic square array that acted as random luminance modulation scintillated their luminance above and below the background

luminance level dynamically but the overall luminance of these checks were the same as the background. The threshold of detection of the moving target was measured using interleaved staircases with three random luminance noise levels, i.e., 6%, 12% and 24%. The observer was asked to press one of four buttons arranged as a square in a forced-choice procedure, to demonstrate the direction of movement of the square.

Measurement of the colour sensitivity

The CAD test was employed to measure the chromatic sensitivity. An isoluminant 5x5 square array of checks, of which 21 are coloured, moving diagonally in a randomly selected direction at a constant speed of 4˚/s in a 15 x 15 square array of achromatic checks and was presented in the centre of a 26cd/m^2 (0.305, 0.323) background field that subtends a 28^o x 23^o visual angle using a Multiscan 500PS Trinitron monitor (Sony Corporation, Tokyo, Japan) using a frame rate of 75Hz. The achromatic checks scintillated their luminance above and below the background randomly but the overall mean luminance was the same as the background. These checks acted as spatio-temporal luminance noise which minimized any luminance detection from the colour defined stimulus. The stimulus colour was defined by CIE co-ordinates and sixteen different directions in CIE space were employed. The threshold along each direction was measured using 16 interleaved staircases. The observer was asked to press one of four buttons arranged as a square in a forced-choice procedure, to demonstrate the direction of movement of the square.

Pupillometry.

Sei-ichi Tsujimura from Kagoshima University recently described a pupillometric setup which was used to study the contribution of melanopsin containing ganglion

cells on steady-state pupil responses (Tsujimura et al, 2010). A similar pupillometric setup was used in this experiment, which was set up by Professor Tsujimura at the Applied Vision research Centre, at City University in London.

Apparatus

The apparatus consisted of two parts: a stimulus display unit and a stimulus control unit. The display unit was formed of an integrating sphere within which light emitting diodes (LEDs) of four peak wavelengths (615 nm, 525 nm, 500 nm and 470 nm) were used as light sources, and an optical diffuser. The LEDs within the integrating sphere projected a circular test field of 17° visual angle on the optical diffuser. A personal computer and an interface board (NI-6733, National Instruments, USA) controlled the luminance output of the four LEDs through analogue pulse width modulation (PWM) units, which were controlled by a 16-bit digital/analogue converter. A black maltese cross (of 95% contrast level) was situated in the centre of the optical diffuser at 1.5° of visual angle to aid fixation.

Calculation of cone, IPRGC and rod fundamentals

The aim of this experiment was to target the ganglion cells receiving input from the three cone types, rods as well as IPRGCs.

The photopic luminous efficiency function describes the visual sensitivity of the human eye to light of different wavelengths under (light-adapted) photopic conditions. The Commission Internationale de ‘Eclairage (CIE) which has been responsible for the standardization of the measurement of light, described the photopic luminous efficiency function V (λ) in 1924 for a standard observer which is

normalised to a maximum value of 1.0 at a wavelength of 555nm. This function is approximately the sum of the sensitivities of the long wavelength (L) and medium wavelength (M) cones. The cone excitation space is a three dimensional representation of the sensitivity of all three (L, M and S) cone types at each wavelength within the visible spectrum.

The stimuli used in this experiment were based on a receptor-excitation space, which is an extension of the cone excitation space, with the inclusion of the spectral sensitivity of IPRGCs.

The L and M cone fundamentals were designed such that the sum of their excitation was approximately equal to the photopic luminous efficiency function V (λ). S cone excitation was designed on the basis of a fundamental with peak spectral sensitivity of 1.0. The IPRGC fundamental was created based on its spectral sensitivity curve which was based on a pigment template nomogram with a peak sensitivity of 482nm, as demonstrated by Dacey et al, in 2005. The peak axial optical density of a receptor type (Dpeak) determines the amount of incident light absorbed by the receptor. The 10° cone fundamentals proposed by Stockman et al. (1999) and Stockman & Sharpe (2000) were used to calculate the excitation of each variety of cone. In the case of M/L cones and S cones , the Dpeak values used by Stockman et al (1999) in a previous study were 0.38 and 0.30 respectively. There is no published measurement of the Dpeak value for IPRGCs, so a value of 0.5 was chosen. This resulted in a spectral sensitivity function for IPRGCs with a peak at 502nm.

These fundamentals were mapped onto four orthogonal axes in receptor-excitation space. Photopic luminance units (at 22cdm) were used, but it was assumed that S cones and IPRGCs do not affect the photopic luminosity function.

Testing

Each candidate was first background-adapted for 10 minutes. The candidate was asked to place his/her chin upon a chin rest situated 30cm distal to the optical diffuser. Testing was carried out monocularly. In the case of normal control subjects, the right eye was tested. In the case of patients, the eye with the pathology was tested. An infra-red camera was placed adjacent to the optical diffuser to obtain recordings.The camera captured images with a resolution time of 20ms.

The pupillary diameter was recorded from 0 to 6 seconds. A control (background) stimulus was presented between 0 and 6 seconds. The test stimulus was a sinusoidal stimulus presented between 1 and 3 seconds, peaking at 2 seconds (figure 1). The stimulus was presented 20 times and a final detrended trace was acquired.

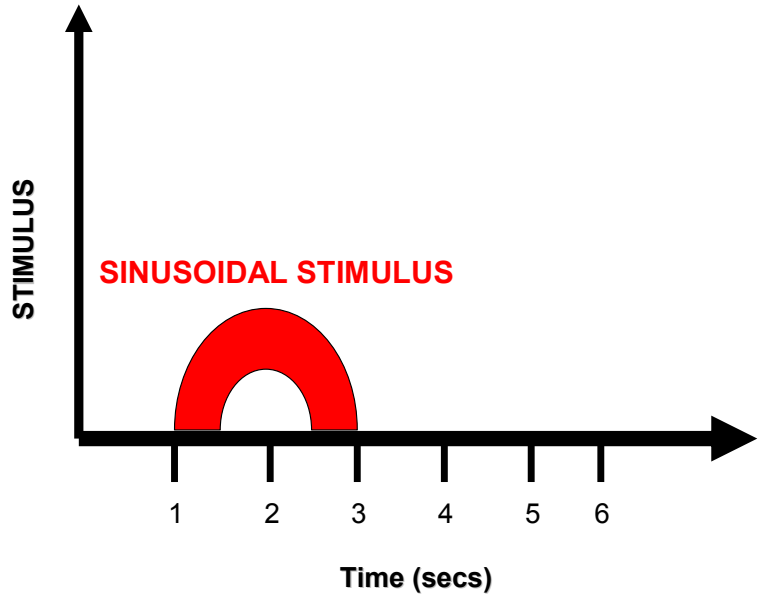

Figure 1

A ***silent-substitution technique*** was used in this experiment. Three conditions were tested. The substitution technique was approached by first establishing a baseline excitation level for each receptor channel in a prototype stimulus profile. Within each condition, the excitation level for each receptor channel was either held constant, or altered with a substitution, as follows:

1. **Luminance condition (L+M)**: only the luminance of the test stimuli were varied without any change in spectral composition or in IPRGC stimulation.

2. **IPRGC condition (IPRGC):** IPRGC excitation was increased without a change in the excitation of L, M and S cones, and hence no change in either

the luminance or the spectral composition of the stimulus (it was assumed that S cones and IPRGCs do not affect the photopic luminosity function).

3. **Chromatic condition (L-M):** the relative excitation of L and M cones was varied without a change in the overall luminance or in IPRGC excitation.

The relative receptor excitation for each stimulus condition is shown in figure 2.

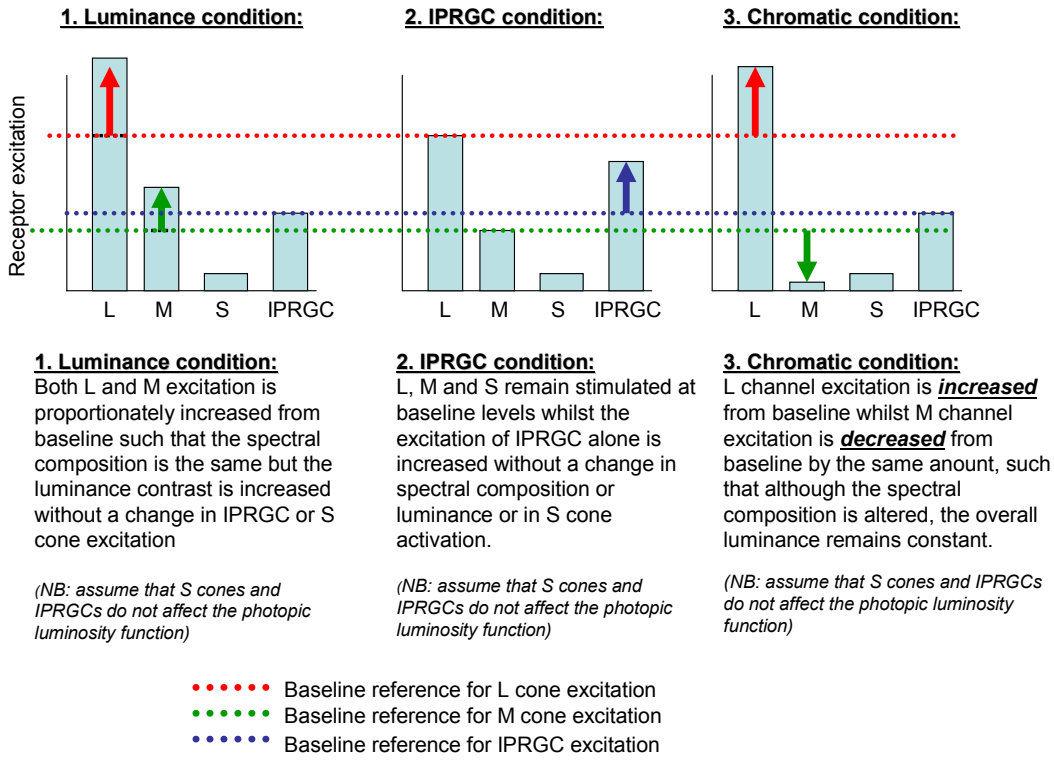

Figure 2

Calibration

Although it was the hypothetical aim to maintain a constant background luminance and to precisely control the excitation of each input channel, in practice, the readings of the apparatus during calibration offered slight deviations from the expected and observed stimulus specifications.

The luminance measurements and spectral measurements for the three conditions were acquired using a spectral radiometer and a Lichtmesstechnik (LMT) luminance meter. The background luminance and luminance contrast range for each stimulus condition is shown in Table 1 below.

L+M	Background luminance	438cd/m²
	Peak Luminance	560cd/m²
	Luminance contrast range	27.9%
IPRGC	Background luminance	456 cd/m²
	Peak Luminance	463 cd/m²
	Luminance contrast range	1.5%
L-M	Background luminance	440 cd/m²
	Peak Luminance	441 cd/m²
	Luminace contrast range	-0.7%

Table 1

The modulation amplitude of the stimuli (or the amplitude of the stimulus relative to the background) in each stimulus conditions was as follows:

- L+M = 25.7% (Expected = 40%)
- IPRGC = 56.4% (Expected = 60%)
- L-M = 19.4% (Expected = 15%)

This resulted in the following excitation level of each cone, IPRGC and rod, relative to the background (table two):

	L cone (%)	M cone (%)	S cone (%)	IPRGC (%)	Rod (%)
L+M	25.4	25.8	-0.1	-0.6	3.1
IPRGC	-0.5	-0.9	-3.7	56.4	47.4
L-M	-6.8	12.6	0.2	-0.4	1.8

Table 2

Data processing

The raw pupil trace results obtained after 20 repetitions are then 'detrended' using a MatLab© program (http://www.mathworks.co.uk/help/dsp/ref/detrend.html)

Results

Results in control subjects

Thirty-three control patients were first tested, of whom twelve were female. The mean age was 32 years (s.d. 8.8yrs). All control patients had an unaided visual acuity to near (at the distance required for stimulation) of 6/6 or better and normal colour vision as tested on an Ishihara chart. A good response along all three channels (L+M, L-M and IPRGC) was obtained in all subjects. All control subjects displayed a double response to the chromatic stimulus, one at stimulus-ON and the second at stimulus-OFF.

Baseline pupil diameter and absolute amplitude

The baseline pupil diameter ranged from 2.3mm to 6.85mm across all modalities. The mean baseline pupil diameter in the Luminance, Chromatic and IPRGC tests were 3.48mm (s.d. 3.9), 3.62mm (s.d.3.79) and 3.47mm (s.d. 3.85) respectively.

The absolute amplitudes of contraction in the Luminance, Chromatic and IPRGC modalities were 0.29mm s.d. (0.14), 0.25mm (s.d. 0.16) and 0.35mm (s.d. 0.15) respectively.

In chapter one of this thesis, the relationship between the constriction amplitude and diameter was visited and reference was made to the measurements of Usui and Stark in 1982 who demonstrated a quadratic relationship between the resting pupil dark diameter and the absolute amplitude of constriction to be in the form of y+-2.794*(x-

1.17)^2*(x+0.174)^2/((x-2.11)(x+1.11)*(x^2-1.01*x+0.436)). Maximum amplitude of constriction was achieved at an approximate pupillary diameter of 5mm.

In this experiment, the resting pupil diameter was not the dark diameter but the baseline diameter as the subject was adapted to a background luminance of between 438 and 446 cd/m²

The relationship of absolute amplitude of constriction and pupil diameter was also found to follow a quadratic relationship in the results of the normal controls. However, the result differed strikingly between the Luminance (y = -0.0171x^2 + 0.2031x - 0.1967) and Chromatic (y = -0.0232x^2 + 0.2414x - 0.2969) channels, which suggested a peak in constriction at a baseline diameter of 5.9mm and 5.2mm respectively, and the IPRGC (y = -0.0063x^2 + 0.1343x - 0.0524) channel where the peak could be extrapolated to lie at 10.7mm. The relationship in the case of the IPRGC was therefore more linear than quadratic. The relationship of absolute amplitude of constriction and pupil diameter in the three channels is shown in figures 3, 4 and 5 below.

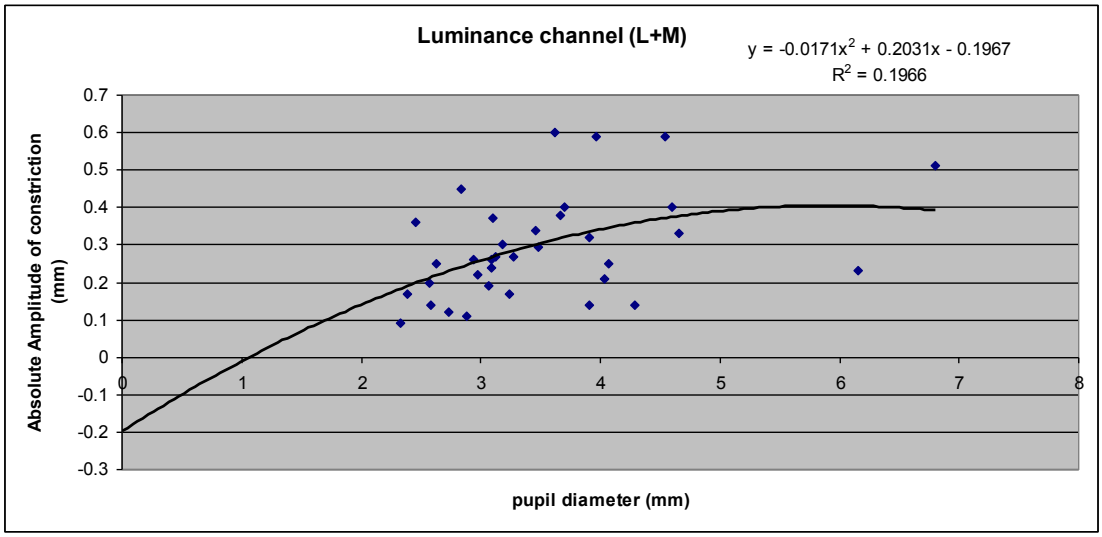

Figure 3

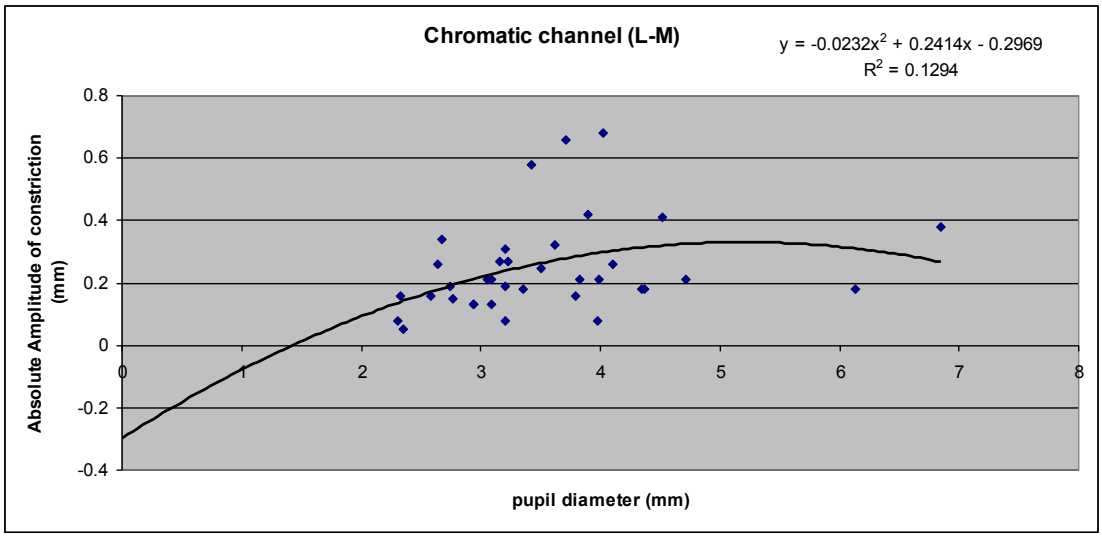

Figure 4

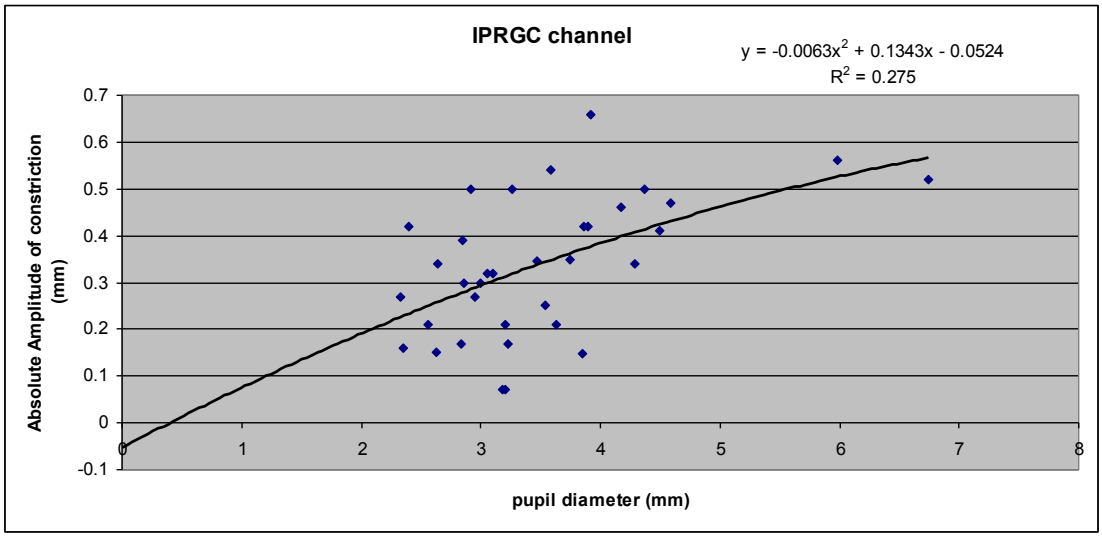

Figure 5

Pupillary response to the three modalities in control subjects

In 19 out of 33 controls (58%), the largest pupillary response was in response to the IPRGC channel, in 7 cases (21%) the luminance channel (L+M) and in the remainder of cases (21%) the chromatic channel. The mean relative amplitudes of constriction to the Luminance, Chromatic and IPRGC channels were 8.48% (s.d. 3.5), 7.04% (s.d. 4.1) and 9.94% (s.d. 3.9) respectively. These results are shown in figure 6.

Tukey Box plot showing Relative Amplitude of constriction (%) across the three stimulus channels

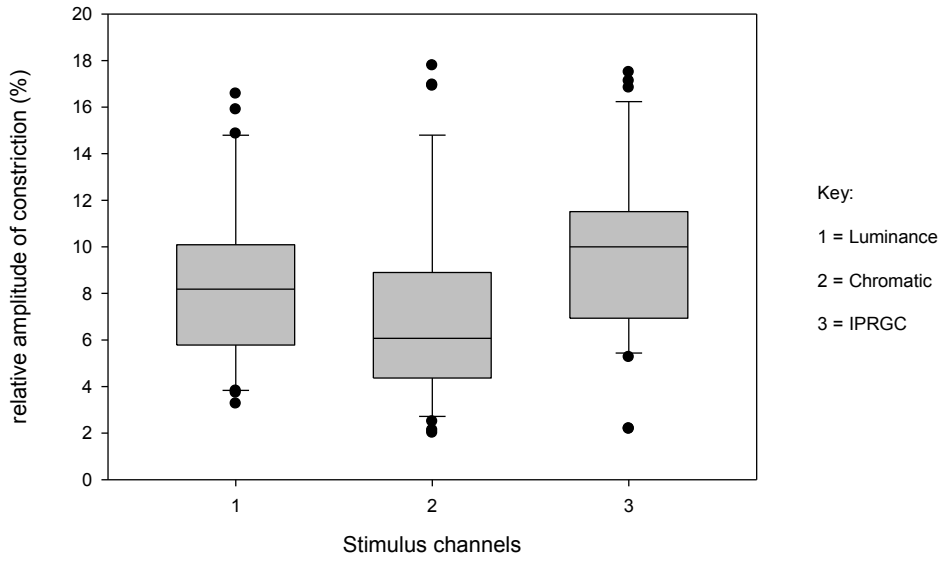

Figure 6

A comparison of the relative amplitude of constriction across all 33 control subjects and across the three groups was carried out with a Kruskal-Wallis One Way Analysis of Variance on Ranks, followed by Dunn's method of pairwise multiple comparison. The analysis suggested an absence of a statistically significant difference between the Luminance and IPRGC responses, but there was a significant difference between the IPRGC and the Chromatic responses (P=0.004).

As a result of these findings, analysis in patients will involve the use of relative amplitude instead of absolute amplitude measurements, as the amplitude:diameter relationship appears linear within the range of the pupil diameters in the majority of cases. The response of the IPRGC channel will be compared to the response of the

Luminance (L+M) channel, as a result of the absence of a significantly different response between these two in normal control subjects.

Results in patients

MS group

After analysing the results obtained in controls, all MS patients with some degree of visual impairment were recruited for pupillometric analysis. However at levels of visual acuity of 6/36 or better, where the patients demonstrated a strong pupillary response, the patients' results fell within the range obtained with control subjects. It was therefore decided that in order to observe a pronounced effect of the disease on the ganglion cells, it was necessary to recruit patients with significant damage to the optic nerve. A cut-off visual acuity of 6/60 over at least 20% of the central visual field was decided upon as the inclusion criterion.

Five patients met the criteria for study. All patients had a visual acuity of 6/60 or worse in the tested eye across the central 17 degrees of visual field. Each patient was unable to detect colour or motion in the relevant CAD and motion detection tests described above. Each patient had been relapse-free for at least six months.

The patients were aged from 30 to 57. The mean age of the patients was 46 years (s.d. 11). Two patients were male and 4 patients were of white Caucasian heritage. Each patient had been diagnosed with Multiple Sclerosis (MS) in accordance with the Mcdonald criteria (McDonald et al, 2001). In one patient (MS3) each relapse (cord, optic nerve) had been particularly aggressive and the patient had been placed on

Azathioprine treatment at a daily dose 150mg orally. In the other four cases, the disease had followed a 'typical' relapsing and remitting course. None of the other five patients had been placed on long-term therapy.

Patients MS1 and MS4 had a Snellen visual acuity of 6/60, patient MS5 was able to count fingers at 1m, patient MS2 could just perceive light (PL) and patient MS3 was unable to perceive any light (NPL).

Every patient had, in the course of their illness, experienced an episode of acute optic which was 'atypical' in that no recovery had occurred from the episode. All patients had experienced other episodes of optic neuritis both before and since the 'atypical' episode, which had followed a 'typical' course and had completely resolved. As a result of the 'atypical' episode, all patients demonstrated disc pallor, which correlated with their visual deficit (i.e. there was marked optic disc pallor in the case of patient 3 who had no perception of light and the pallor was significant, but less marked in the case of patients with 6/60 visual acuity).

In addition to clinical, serological and radiological tests which confirmed the presence of MS, testing was also carried out to assess for the presence of any coexisting condition, including Neuromyeltiis optica (NMO), collagen vascular disease, granulomatous disease, neoplastic lesions of the visual pathway and other autoimmune conditions. The results of all these tests were negative.

The results of patients MS1, MS2, MS3, MS4 and MS5 are shown in figures 7 to 11. In each case the traces for the Luminance and the Chromatic response have been

shifted upwards in order to demonstrate the features of each trace with clarity. None of the patients appear to demonstrate a definite pupillary Chromatic-ON response, whereas patients MS1,MS2 and MS5 appear to show a minimal Chromatic-OFF response. Only MS2 displays a very subtle Luminance response. In contrast, in all cases, there is a strong IPRGC response.

This is particularly striking in the case of patient 3 who has No Perception of Light and an atrophic optic disc. He appears to show a clear response to the IPRGC channel measuring 0.16mm in absolute amplitude and 3.1% in relative amplitude. This is comparable with the relative amplitude of response measured in the other patients (table 3).

	abs amp	rel amp %
MS1	0.07	3.1
MS2	0.27	4.2
MS3	0.16	3.1
MS4	0.1	1.6
MS5	0.2	6.9

Table 3

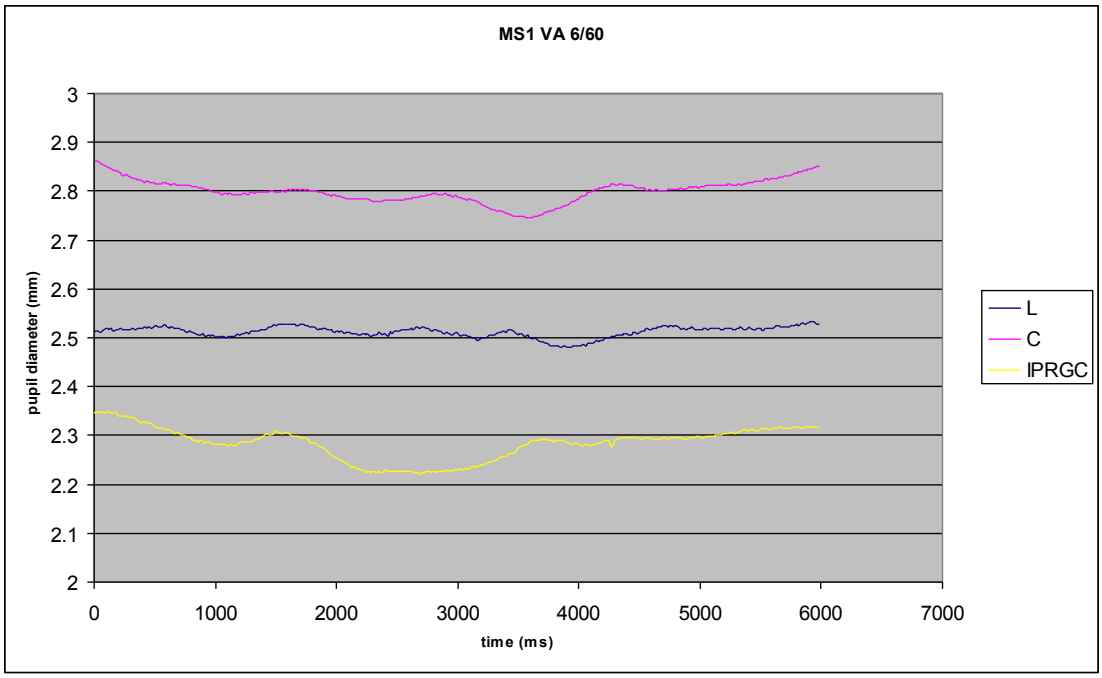

Figure 7

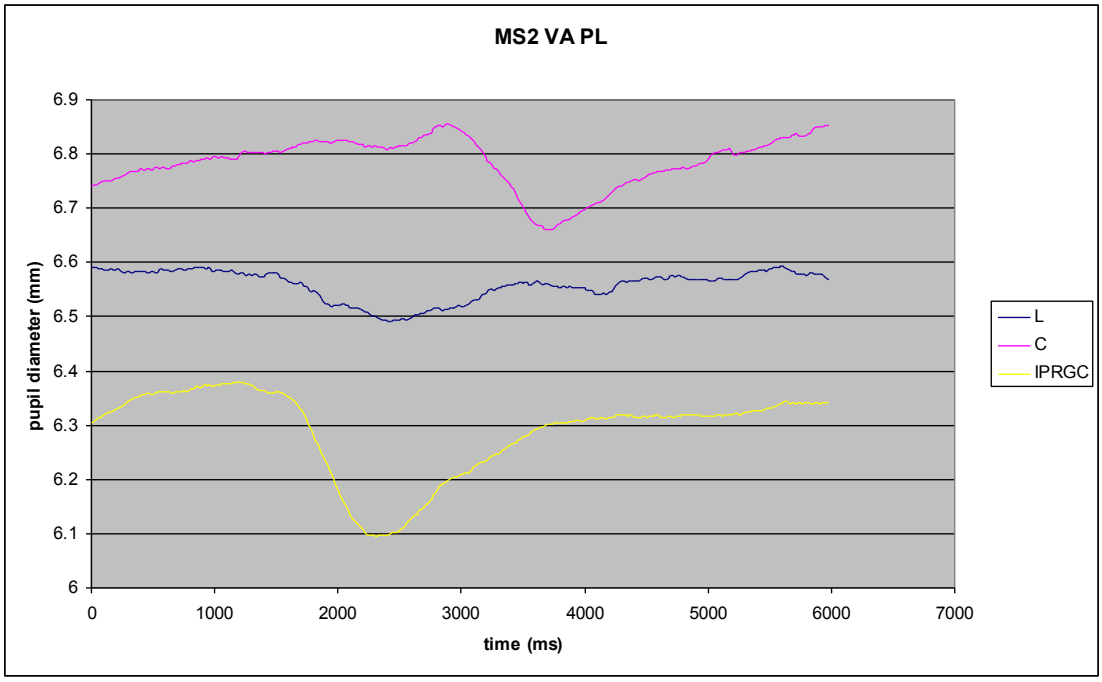

Figure 8

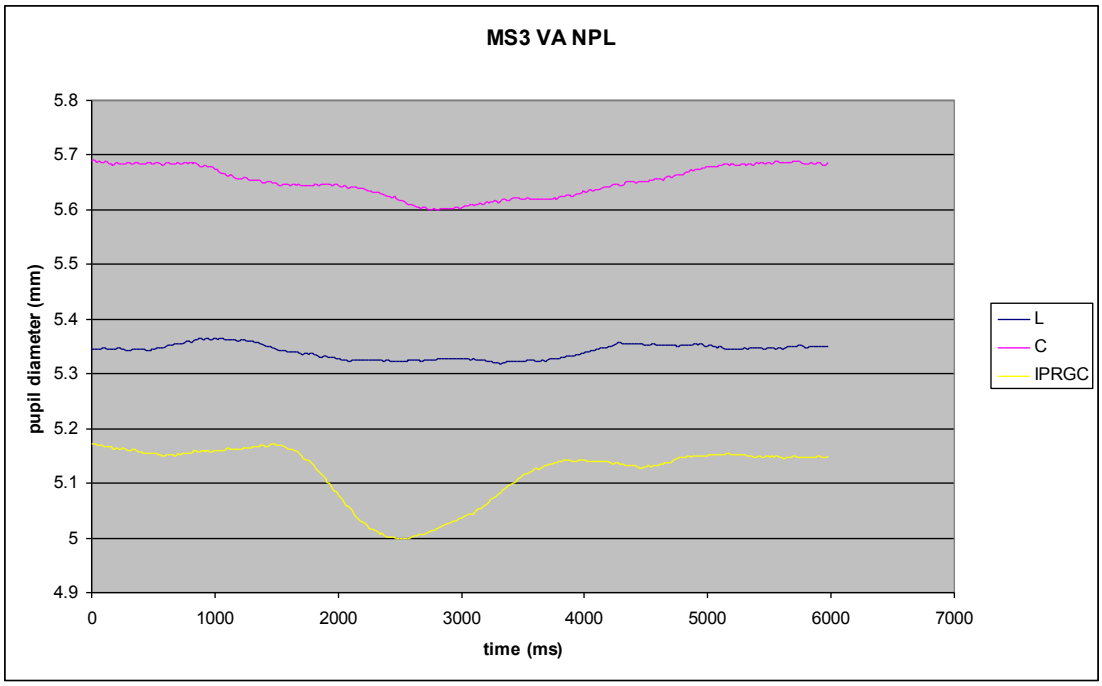

Figure 9

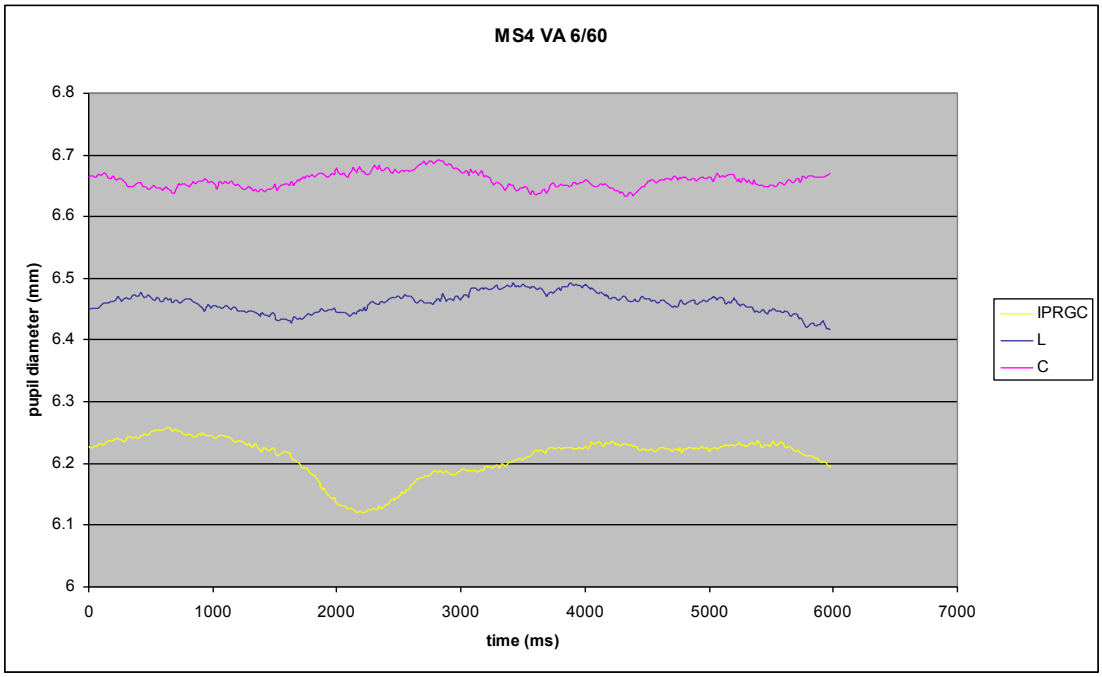

Figure 10

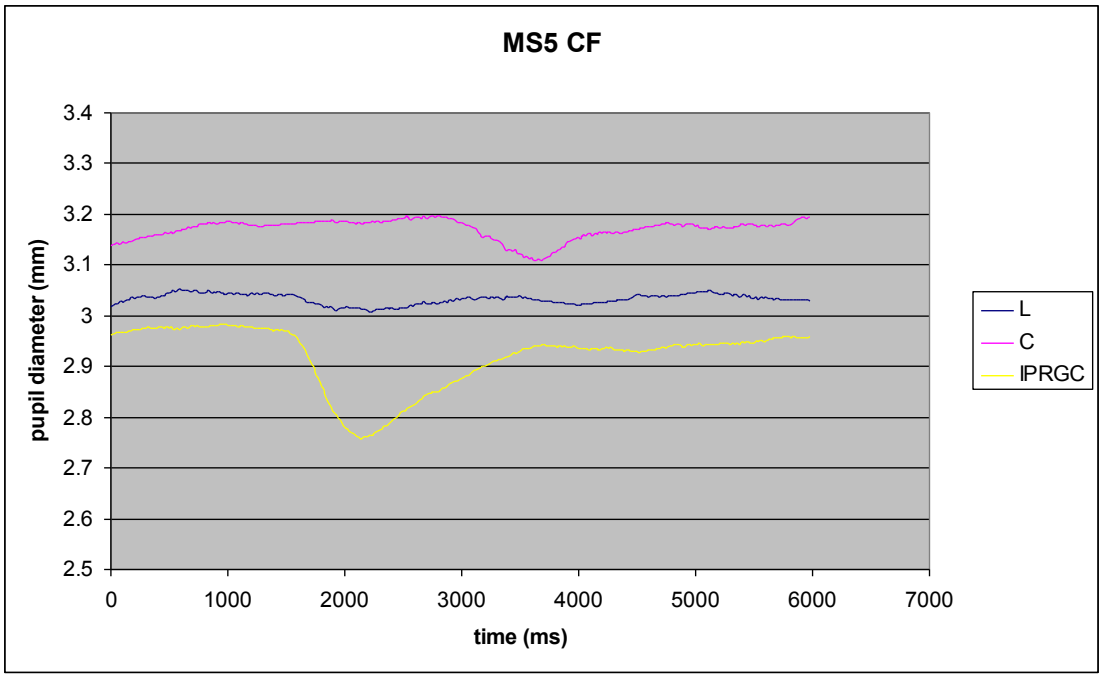

Figure 11

In the case of normal controls, no statistically significant difference in response was observable between the Luminance response and the IPRGC response.

The relative response amplitude of the Luminance and IPRGC pathways in the MS patients are shown in table 4 below:

	Luminance %	IPRGC %
MS1	0.00	3.1
MS2	0.01	4.2
MS3	0.00	3.1
MS4	0.00	1.6
MS5	0.00	6.9

Table 4

The relative response amplitudes to the luminance and IPRGC stimuli.

A Mann-Whitney Rank Sum Test analysis was performed on the above data and a statistically significant difference (P = 0.008) was found. There appears to be considerable dissociation between the IPRGC pathway and the Luminance pathway which is not present in normal controls.

In the case of MS patients with impaired vision following an episode of optic neuritis after which there is no recovery, there appears to be some IPRGC stimulus pathway preservation even when the vision is otherwise severely compromised, and the optic nerve function appears completely impaired. MS patients with severe impairment appear to demonstrate pupillovisual dissociation, however, unlike in the case of patients with LHON who demonstrated dissociation between the Chromatic and luminance channels, in the case of MS patients, this dissociation exists between the IPRGC and the Luminance/Chromatic channels. In both cases, the pupil response is relatively preserved compared to the remainder of visual function. In the case of LHON patients, this preservation is sometimes observable by the clinician using torchlight and has been widely reported (as discussed previously in this thesis). In cases of MS a specific IPRGC channel stimulus would be required to observe this dissociation. It is perhaps for this reason that the occurrence of dissociation has not previously been reported.

NMO patients

Four patients were recruited with a visual acuity of 6/60 or worse. None of the patients were of white Caucasian descent. Their mean age was 34year (s.d.0.6). Two

patients (NMO1 and NMO3) had a Snellen visual acuity of 6/60 and the remaining two patients were unable to perceive light (NPL). All patients were on chronic immunosuppression as a result of the diagnosis of NMO spectrum disease for at least one year. The last relapse in each patient had occurred at least six months ago.

All patients were unable to perform the CAD test or motion detection test described above. The results of patients NMO1, NMO2, NMO3 and NMO4 are shown in figures 12 to 15 below. In each case the traces for the Luminance and the Chromatic response have been shifted upwards in order to demonstrate the features of each trace with clarity.

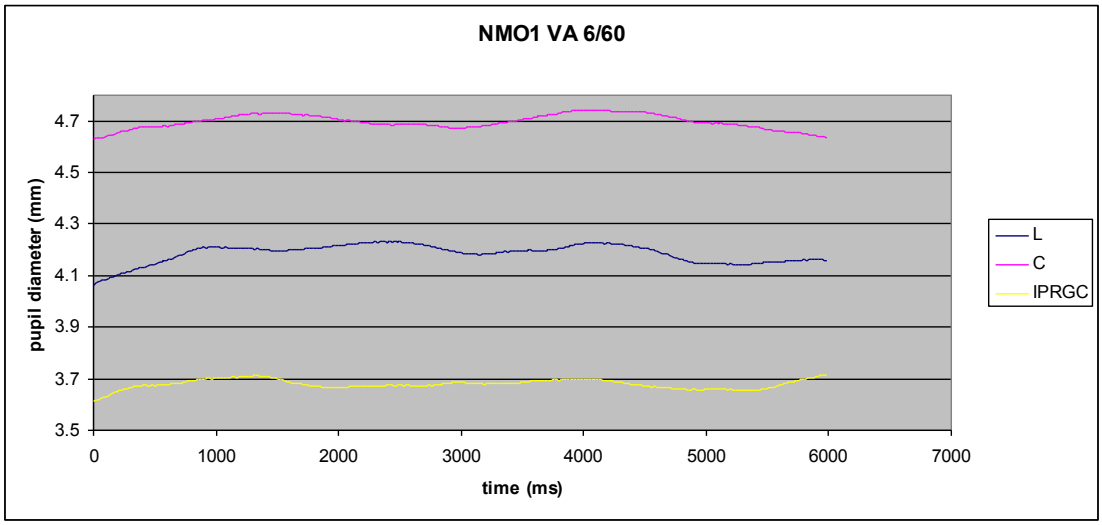

Figure 12

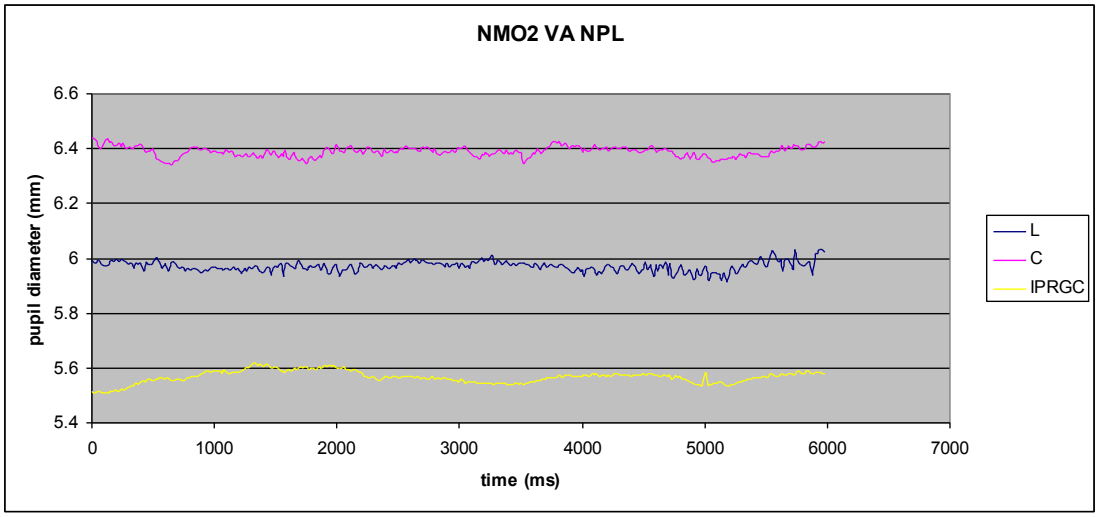

Figure 13

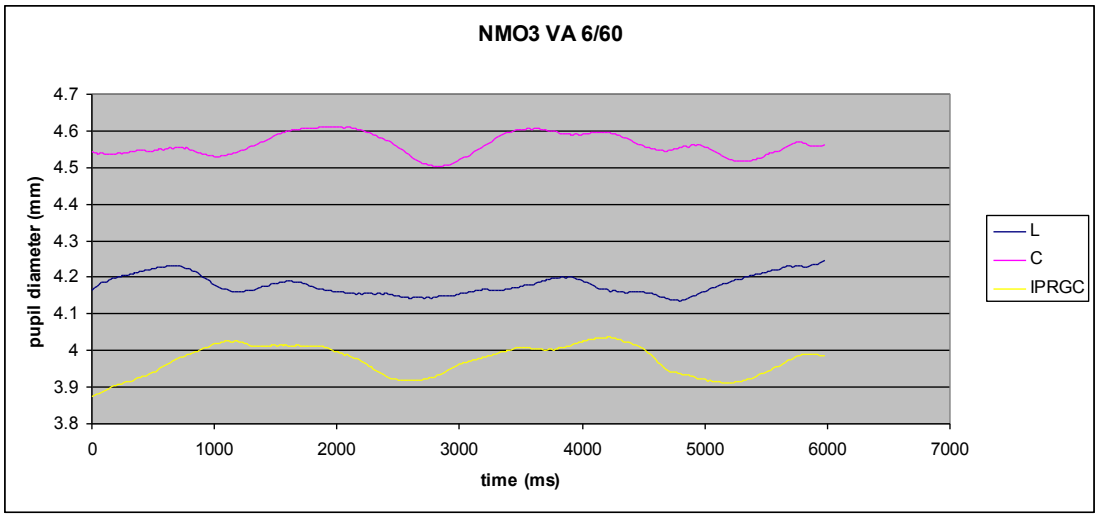

Figure 14

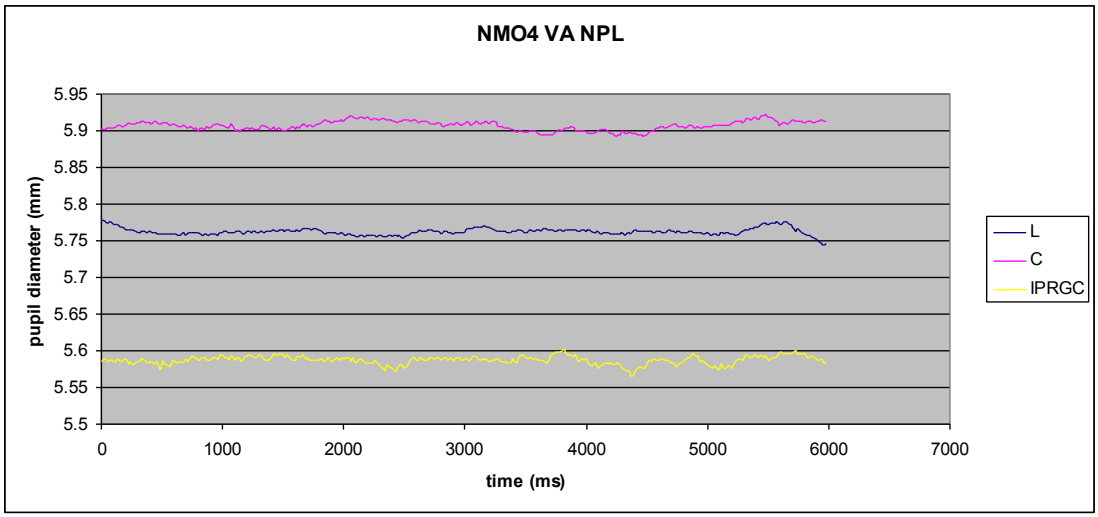

Figure 15

It was not possible to observe a pupillary response to any of the three stimulus channels in any of these patients. Pupillary unrest waves in patients NMO1 and NMO3 made the results difficult to interpret.

Despite these patients being matched for visual acuity with the four MS cases, unlike the MS cases, the IPRGC channel response does not appear to be preserved in any one of the patients.

Cases of non-MS non-NMO spectrum optic neuritis

The majority of cases of non-NMO and non-MS optic neuritis with impaired vision had a visual acuity which was better than 6/60. In these cases, the patients' responses fell within the range of control subjects. Only one patient with a history of non-NMO spectrum, non-MS optic neuritis with a visual acuity of 6/60 was recruited. The patient was a white Caucasian female aged 57 years. This patient had not recovered from the first attack of optic neuritis one year before and required chronic corticosteroid therapy at a dose above 40mg in order to prevent relapsing into further

episodes of optic neuritis. All other tests including radiological and serological examination had proved negative. This patient (CRION1) appeared to show a clear dissociation of the IPRGC channel response from the Luminance and Chromatic channel responses in a pattern resembling MS patients. Her results are shown in figure 16 below. The Luminance and Chromatic traces have been transposed upwards for clarity.

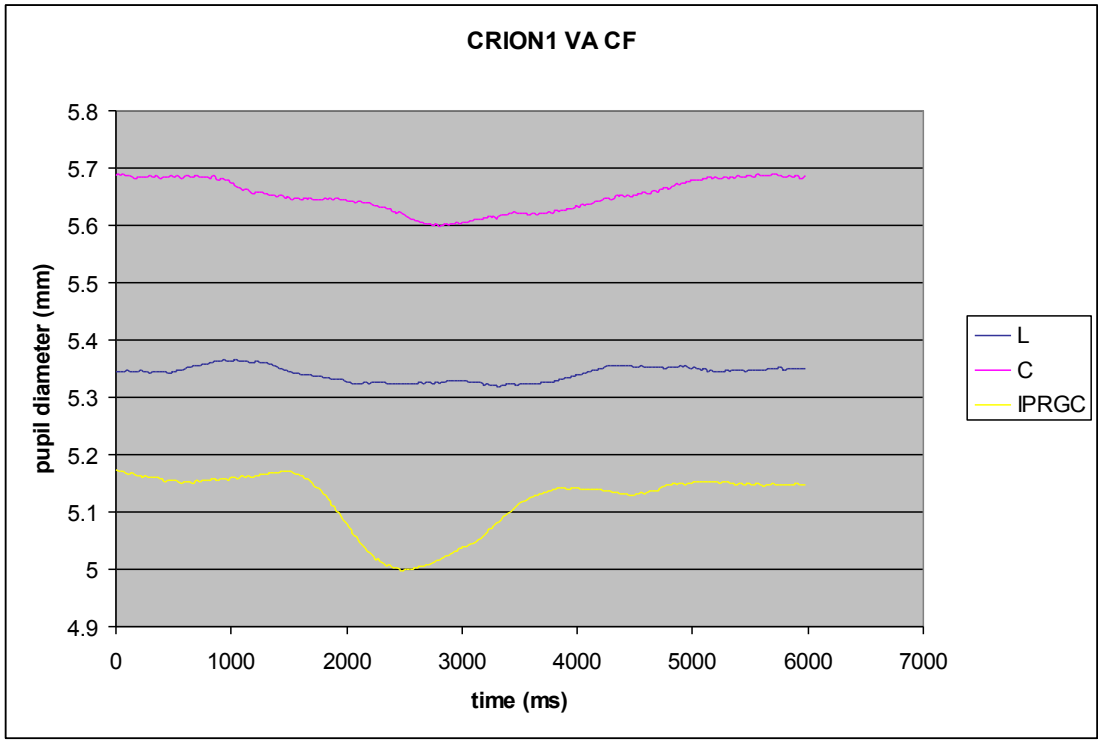

Figure 16

CRION1 showed absolute amplitude of constriction of 0.164 mm and a relative amplitude of constriction of 3.2%.

LHON cases

The setup used in this experiment was different to that used in the experiment described in chapter two of this thesis where the IPRGC pathway was not specifically stimulated. Four male LHON patients were recruited for this experiment. All were of white Caucasian descent. The patients ages ranged from 26 years to 58 years (mean 41, s.d. 14). All patients had a visual acuity at or worse than 6/60 over at least the central 17degrees of visual field. LHON2 had a visual acuity of 6/60 and LHON3 could count fingers at 1m (CF). The other two patients were able to perceive hand movements only in the tested eye. None of the four patients were able to perceive the colour red within the area tested. The CAD test and the motion detection test was not carried out in these patients owing to time constraints.

The pupillometry traces are shown in figures 17 to 20.

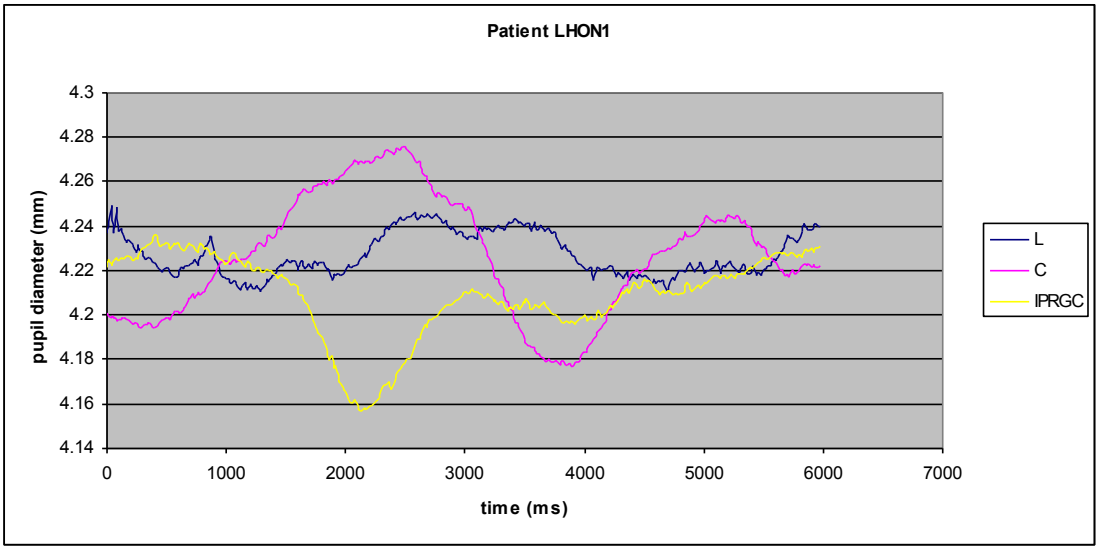

Figure 17

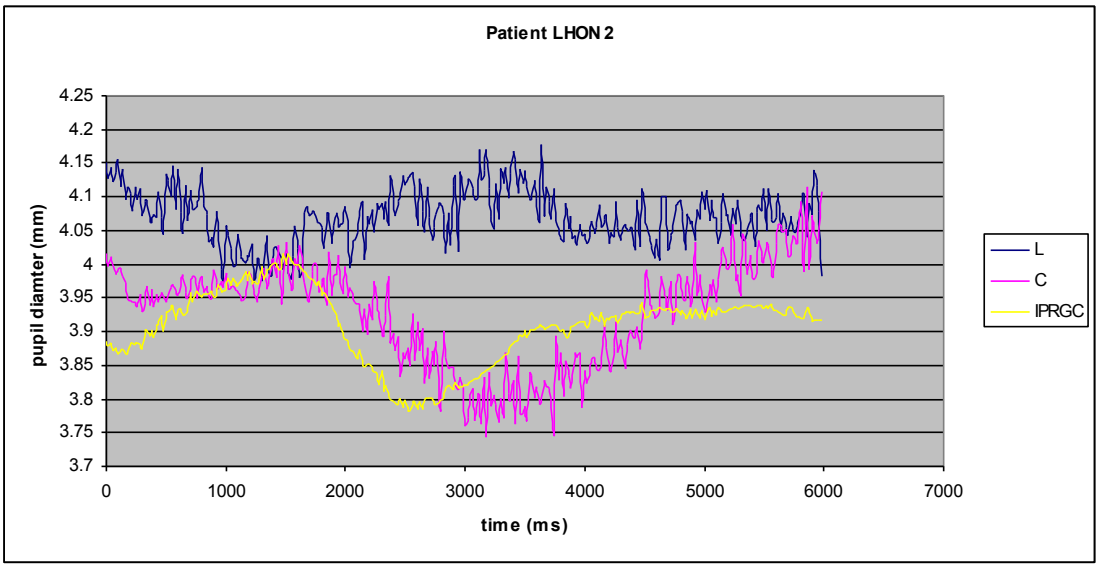

Figure 18

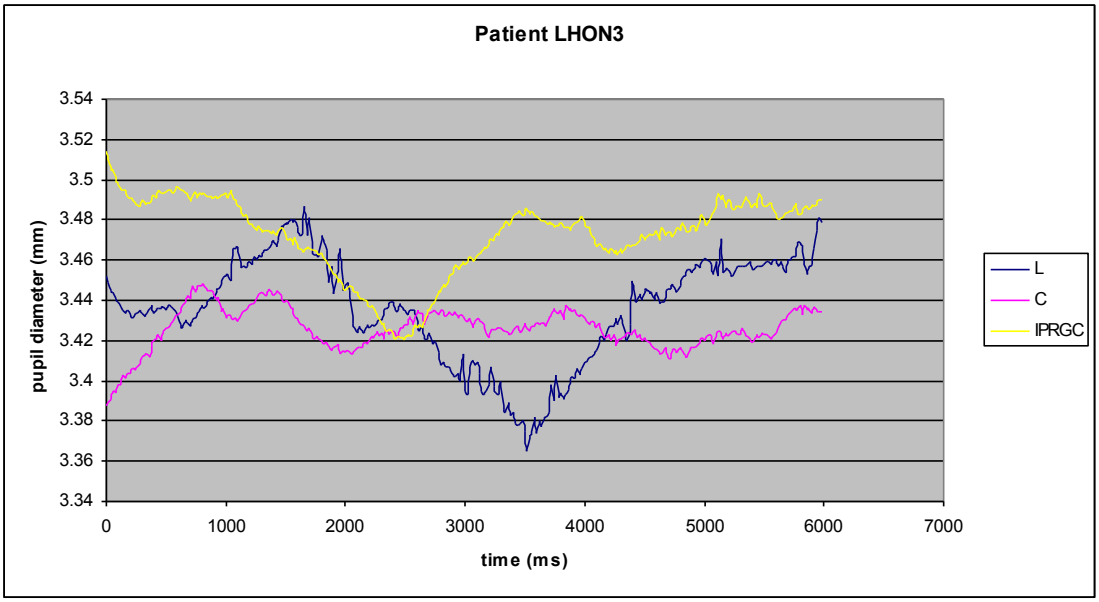

Figure 19

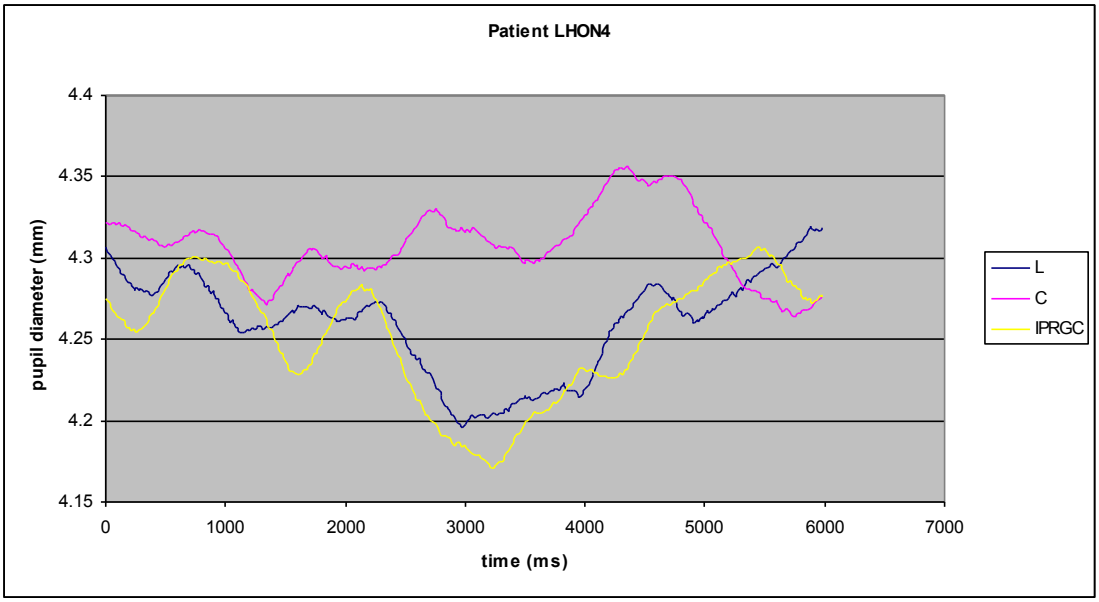

Figure 20

Patients LHON1 and LHON3 show a clear downward deflection of the pupil trace for the IPRGC channel between the times of 1000ms- 3000ms which is beyond the course of the trace at all other times. Patient LHON2 also shows this, although the trace is noisier. This was not seen with the traces of the other channels. The results of patient LHON4 were too noisy to draw meaningful conclusions.

In the three cases, distinct pupillo-visual dissociation was observed where the IPRGC pathway was relatively preserved, in comparison to the other channels. Calculation of the amplitude of response proves difficult in all cases because of the noisiness of the trace. However, using the criterion of 'downward deflection following a prolonged flat trace or an upward deflection', the absolute amplitudes for LHON1, LHON2 and LHON3 were measured at 0.06mm, 0.22mm and 0.05mm respectively, corresponding to relative amplitudes of 1.4%, 5.5% and 1.4%. The sample size is too small to assess whether or not these amplitudes are comparable to the MS patients who are matched for visual acuity.

Conclusions

This study suggests that in patients with a past episode of optic neuritis in whom there has been no recovery, the underlying aetiology of the optic neuritis may be inferred by carrying out pupllimometric testing on the three channels described here. In cases where there is no preservation of the IPRGC channel, a possible diagnosis of NMO may be considered.

The study has demonstrated that the pattern of ganglion cell type damage occurring secondary to NMO is different to that occurring secondary to MS in patients matched for visual deficit. In NMO spectrum disease, no ganglion cell group appears to be spared, whereas in MS, the IPRGC channel is relatively spared, similar to cases of LHON.

This study therefore supports the idea of NMO spectrum disease having a separate and distinct underlying pathogenesis compared to non-NMO demyelinating disease, such as MS.

Discussion

The test used in this experiment would have a clinical use in diagnosing the aetiology of a past isolated episode of optic neuritis from which recovery has been poor. This test would permit the distinction of NMO from non-NMO causes. The distinction of MS from LHON is less clear, as a degree of pupillovisual dissociation is apparent in both aetiologies, and the sizes of the IPRGC responses in the LHON group lay within the range obtained in the MS group.

In all cases of MS and NMO, P- and M-ganglion cell pathways were shown to be significantly impaired (resulting in an absence of colour or motion detection and a low visual acuity) to comparable levels. In all the MS cases, although the pupil chromatic onset-response and the luminance response were grossly impaired, the IPRGC response was relatively spared, confirming that these responses were mediated through P- and M- ganglion cell pathways which were shown to be impaired. Koniocellular ganglion cell (K-) pathway input was maintained at constant level through regulation of S cone stimulation and the photopic conditions prevented rod input into the K-ganglion cell pathway (Field et al, 2009).

The pupillovisual dissociation observed in the patient with corticosteroid dependent optic neuropathy suggested the geographical extent of damage within the optic nerve mirrored that seen in MS rather than in NMO, suggesting the optic neuritis may be an 'atypical' and corticosteroid dependent form of MS which has been reported previously (Zelnik et al, 1991).

The absence of a distinct luminance response in the LHON group was contradictory to the result obtained in the previous experiment. However, the equipment used in the previous experiment was not as precisely calibrated as in this experiment, and the luminance signal may have been propagated through a rod/s-cone/ IPRGC pathway; whereas in this experiment, there was no IPRGC or S cone stimulation by the Luminance stimulus, as a result of using the 'silent-substitution' technique. Dissociation of the chromatic onset pupil response from the IPRGC response

confirmed the finding in the previous experiment of differential pupillo-visual dissociation in LHON.

The relative sparing of the chromatic offset response when compared to the chromatic onset response in three out of five MS patients is a challenging result. It has been shown to be mediated via the M-ganglion cell pathway (Tsujimura et al, 2003), but the patients were unable to detect any motion and there was no pupillary response to the luminance stimulus. A previous report suggested the chromatic offset response may be mediated by the S cone/ K-ganglion cell pathway, but the testing parameters used in this study were different to those used in the report and S cone stimulation was maintained at constant levels during chromatic stimulation (Kimura & Young, 1999).

The data obtained from LHON patients was considerably noisy and may have masked subtle luminance and chromatic onset and offset responses. The entire experiment was limited by sample size. It is uncommon for MS patients to never recover to beyond 6/60 visual acuity from an attack of typical optic neuritis and in a previous report, only 4 out of 151 patients with optic neuritis observed over an eight year period recovered to an acuity of less than 6/60 or worse (Slamovitis et al, 1991; The optic neuritis study group, 1997). This limited the sample size. NMO is a rare condition, and relapses tend to be more severe than in MS (Collongues et al, 2010). It was only possible to recruit patients who were ambulant enough to visit our laboratory to carry out the experiments. LHON is also a rare condition. We took care to include patients with definitive diagnoses which excluded many patients with unresolved optic neuritis of unknown aetiology. In addition, we excluded all patients

with moderately impaired vision, as their responses fell within the boundaries of normal control responses as a result of inter-individual variability (as demonstrated in an earlier experiment).

This experiment setup was an improvement on the previous experimental setup with the use of the 'silent-substitution' technique to control for S cone stimulation which is difficult to separate from IPRGC stimulation due to its spectral proximity. However, it was not possible to separate the rod response from the melanopsin response in this setup, as calibration measurements indicated some rod stimulation occurring during the IPRGC channel program (see above). The aim of this study was to assess ganglion cell function and therefore whether IPRGC stimulation occurred via rods or through the direct stimulation of the melanopsin pigment was of little consequence.

In summary, pupillometric measurements hold immense potential in their diagnostic use in optic nerve disease such as optic neuritis. Optic neuritis often presents a diagnostic dilemma as both NMO and MS related optic neuritis may present in identical ways but require different approaches to management. Optic neuritis will now be reviewed, with a particular emphasis on the distinction between that caused by MS and that caused by NMO.

OPTIC NEURITIS

Optic neuritis is defined as an acute, non-infectious optic neuropathy (Volpe, 2001). Historically its definition was made more precise following the invention of the ophthalmoscope by Helmholtz in 1847. Optic neuritis as it is recognised today, was first accurately defined by von Graefe in German in 1860 and by Nettleship nearly a quarter of a century later in English when he described cases of optic neuritis as "…. not very common cases in which acute inflammation seems to take place in some small part of the course of the optic nerve." (Von Graefe, 1860; Nettleship, 1884).

Aetiological relationship with multiple sclerosis

The association of optic neuritis and multiple sclerosis has been recognised ever since optic neuritis was first defined. In 1893 Buzzard reported visual failure in the setting of disseminated sclerosis and Adie attempted to explore the relationship between the two further forty years later (1932) by suggesting multiple sclerosis and optic neuritis were always linked. In 1934 Lillie described 200 of 500 patients with multiple sclerosis who developed optic neuritis at some point during their illness and 75 of the 500 patients had presented with optic neuritis as their first symptom. Studies prior to the advent of magnetic resonance imaging (MRI) techniques described various proportions of patients who first presented with optic neuritis and then went on to develop multiple sclerosis as 71% (follow-up time 1-29 years), 57% (follow-up time 11.6 years) and 38% (follow-up time of 12.9 years). After the advent of MR imaging facilities, the relationship of the likelihood of developing MS was explored with respect to MRI findings. The Optic Neuritis Treatment Trial (ONTT) was the largest and most comprehensive study on optic neuritis carried out to date. The ONTT

showed that 51% of previously healthy patients with 'abnormal' MRI scans and 16% of previously healthy patients with 'normal' MRI scans developed MS within 5 years of presenting with isolated acute optic neuritis (Optic Neuritis Study group, 1997). As a result of the ONTT, an evidence-based guideline for the management of optic neuritis was established, and is followed to variable degrees worldwide (Lueck et al, 2008; Atkins et al, 2008; Calvetti et al, 2008).

Aetiological relationship with Neuromyelitis optica (NMO)

In 1870 Albutt reported optic disc pathology existing concurrently in several patients with myelitis. Further case reports followed until Devic summarised these cases in his 'Thése de Lyon' as the syndrome *neuromyelite optique aigue* which became known as Neuromyelitis Optica or Devic's disease (Devic 1894). Its aetiology was unknown at first. Some wondered at a viral aetiology, whereas others thought it a variant of multiple sclerosis.

NMO was initally thought of as being a monophasic disease and its distinction with multiple sclerosis appeared hazy (Devic 1894; Gault 1894; Beck 1927; Stansbury 1949; Scott 1952; Mandler et al, 1993). In 1999 Wingerchuk et al, described a case series of NMO patients in whom 23 had a monophasic course and 48 had a relapsing course. Features of NMO which differed from typical MS were shown to include leucocytosis within the cerebrospinal fluid (CSF), a long spinal cord lesion extending over 3 or more vertebral segments and a normal initial brain MRI.

The discovery of a serum autoantibody marker in NMO patients allowed a serological distinction of NMO from MS for the first time (Lennon et al, 2004). Further

investigations revealed the autoantibody marker to bind to the water channel Aquaporin 4 (Lennon et al, 2005). The anti-AQP4+ antibody assay was found to be 83.3% sensitive and 100% specific for the NMO IgG when compared with patients with conventional MS, patients with other neurological diseases and with healthy controls (Matsuoka et al, 2007).

Recent studies looking into the pathogenic impact of AQP4 antibodies have demonstrated that the direct injection of AQP4+ antibody containing immunoglobulin from patients with NMO into non-inflamed mouse brain, in the presence of human complement produced active NMO lesions (Sadun et al, 2010).

Other aetiologies of optic neuritis

Although its association with MS is well known, it has been long recognised that optic neuritis may occur in the absence of demyelinating disease. In 1926, Percival described cases of optic neuritis which did not progress to multiple sclerosis. Acute isolated optic neuritis is known to also occur secondary to direct or indirect infection, following vaccinations, or in the context of systemic collagen vascular or granulomatous disease (Hirayama et al, 2010; Rose et al, 2010; Erguyen et al, 2009; Cárdenas-Velázquez & Hernández-Molina 2010; Galetta et al, 1989) . In such cases, the optic neuritis often has an 'atypical' profile and course, such as bilaterality in the case of infection and corticosteroid dependence in the setting of granulomatous disease (Albussera et al, 2006; Graham et al, 1986).

The ONTT demonstrated that where the aetiology of the optic neuritis is unknown, many patients with acute isolated optic neuritis will develop multiple sclerosis in the

future (Optic Neuritis study group, 2008). There are however, also patients who have isolated optic neuritis in either a monophasic or recurrent pattern, who may either require prolonged corticosteroid immunosuppression or who may recover spontaneously.

This thesis is concerned with cases of optic neuritis in whom the aetiology is either MS, NMO or unknown. Cases of optic neuritis occurring secondary to known systemic disease of a non-demyelinating nature or secondary to infection will not be discussed further.

The histopathology of optic neuritis

In 1974 de Preux & Mair described the post mortem findings in the optic nerve of a 50 year old woman with visual impairment who had suffered from multiple sclerosis for fourteen years (De Preux & Mair, 1974). Light microscopy revealed some parts of the optic nerves showed complete demyelination whereas other parts were partially demyelinated. Examination under electron microscopy of the demyelinated parts of the optic nerve revealed naked axons containing swollen vacuolated mitochondria, which were surrounded by phagocytes with large vacuoles and numerous astrocytes and their processes. No oligodendrocytes were found. In the partially demyelinated regions of the optic nerve, numerous astrocytes were again seen, along with phagocytes. In these sections, oligodendrocytes were found to be present in near normal quantities.

The open biopsy of optic neuritis is indicated only after a completely negative metabolic, infectious and inflammatory work-up and an interval increase of the optic

nerve on magnetic resonance imaging along with failure of the patient to recover vision. This is why histopathological findings on biopsy specimens of acute isolated optic neuritis are uncommon. Rush et al (1982) reported a biopsy specimen of acute isolated optic neuritis of an atypical pattern where the patient's vision failed to recover, despite corticosteroid treatment. A biopsy of the patient's optic nerve demonstrated a perivascular lymphocytic infiltration with a few atypical astrocytes.

The only report comparing the histopathological findings in optic neuritis related to Devic's disease and MS was that by de Preux & Mair in 1974. Destruction of myelin was found to be more extensive in Devic's disease and patchy in multiple sclerosis. Axonal destruction was also found to be severe in Devic's disease and less so in MS. Phagocytes were found to be larger in size and number in Devic's disease. Oligodendrocytes were found to be absent in Devic's disease but were found amongst the myelinated fibres in MS.

The Variability of Optic Neuritis

For many years, the link of isolated acute optic neuritis with multiple sclerosis resulted in the perception of optic neuritis as a condition with a typical profile, caused by a single disease: that of multiple sclerosis. It was believed that the pathogenesis of the two was the same, and insight into the pathology behind multiple sclerosis would also provide information into the pathology of optic neuritis.

The Optic Neuritis Treatment Trial (ONTT) has been the largest and most comprehensive trial conducted on isolated acute optic neuritis. Its conclusions suggested the majority of cases of acute isolated ON followed a particular pattern,

which may be referred to as ‘typical’. Once this ‘typical’ pattern was recognised, numerous patterns of optic neuritis which were different to the ‘typical’ pattern, and hence may be referred to as ‘atypical’ were reported. Although some of these cases had been reported prior to the ONTT, it was only following the ONTT that the contrast with the ‘typical’ pattern became salient.

The assumption that all cases of MS-related ‘typical’ optic neuritis are likely to be caused by the same pathogenesis has been considerably challenged following recent histopathological investigations.

The variability of MS pathogenesis

Although MS has historically been considered to be an autoimmune disorder involving T-cell mediated destruction of myelin, the variable response to immunomodulatory treatment and the marked heterogeneity in the clinical and morphological features of MS, have challenged this simplistic model.

It is accepted that the presence of focal demyelination at multiple sites within the central nervous system where T cells and macrophages are found at the sites of demyelination is characteristic of MS. A closer inspection of the MS lesion, however, reveals inter-individual differences and conflicting reports have been published on its histopathological findings. Within an active MS lesion there is known to be an increase in macrophage activity (Brück et al, 1995). Oligodendrocytes are damaged by a range of assaults including immune generated attack and are required for remyelination following a relapse, during early stages of MS (Raine 1997). However, as regards the fate of oligodendrocytes following an active lesion, some studies have

reported an ultimate increase and others a decrease in their number (Raine 1997; Prineas et al, 1989; Brück 2005; Ozawa et al, 1994). A study on 113 patients with MS, involving the analysis of oligodendrocytes in 300 of their lesions revealed that there are two distinctly different patterns of oligodendrocytic cell pathology (Lucchinetti et al, 1999). In one group of patients, oligodendrocytes reappear within an inactive lesion, following initial reduction in number, and remyelination occurs. Their reappearance is thought to be a result of progenitor cell recruitment. In the other group, there is complete destruction of oligodendrocytes within active lesions and there is sparse or absent remyelination. Each patient showed either one or other pathology. Luchinetti et al concluded that the heterogeneity observed suggests that myelin, mature oligodendrocytes and possibly also progenitor cells of oligodendrocytes are differentially affected within different MS patients and different pathogenic mechanisms may be operating within individual MS patients. In a further study examining MS lesion pathology Lucchinetti (2001) demonstrated marked inter-individual differences in the histolopathology of the lesion.

Given the potential heterogeneity in the pathogenesis of optic neuritis cases occurring in the context of confirmed MS, it is of little wonder, that cases of isolated optic neuritis occurring without evidence of MS, may show a wider spectrum of variation in clinical presentation, course and prognosis.

The variability of the pathogenesis of NMO

Devic's reports on NMO described it as a monophasic illness consisting of a severe transverse myelitis and bilateral optic neuritis, occurring within a short space of time (Devic 1894). The spectrum of NMO was eventually expanded to include cases with

unilateral ON and less severe acute attacks as well as cases with recurrent attacks (Stansbury 1949; Scott 1952). An analysis of 71 patients diagnosed with NMO who presented to the Mayo Clinic, Rochester, Minnesota between 1950 and 1997 resulted in the conclusion by Wingerchuk et al (1999) that NMO may be relapsing or monophasic. Patients with a monophasic course present with rapidly sequential events and show moderate recovery. Patients following a relapsing course had a worse prognosis and each attack resulted in an increase in disablility. They additionally observed that patients with relapsing optic neuritis and myelitis were more likely to have NMO rather than MS. The definition since then expanded further, with the observation that the time interval between relapses in relapsing NMO may range from days to months (O'Riordan et al, 1996; Wingerchuk et al, 1999). Diagnostic criteria The detection of a serum autoantibody marker - the serum autoantibody to the Aquaporin 4 channel – in patients with NMO provided a serological definition for the disease (Lennon et al, 2004). Revised diagnostic criteria for NMO proposed by Wingerchuk et al in 2006 included both the presence of this antibody and the presence of an MR image of the brain which is not diagnostic of MS as additional criteria. A new description of 'Neuromyelitis spectrum disorders' was more recently discussed which included the wide spectrum of clinical presentations of the disease (Wingerchuk et al, 2007).

As the definition of NMO spectrum disease has expanded over time, observations on histopathological and immunological heterogeneity are increasingly made, and the distinction with MS becomes increasingly weaker.

Kira et al (2010) proposed the pathogenic mechanism of NMO based on anti-AQP4 autoimmunity. In keeping with this hypothesis, in a post-mortem analysis of NMO cases in Japan, Misu et al (2007) discovered extensive loss of Aquaporin 4 in 18 of 22 active NMO inflammatory lesions, where Myelin Basic Protein staining was relatively preserved. The opposite pattern of loss was not seen in any of the 22 active inflammatory lesions. The study of MS lesions, revealed an up -regulation of Aquaporin 4 in keeping with astrogliosis. Additionally, Misu et al also noticed perivascular immune complex deposition within NMO lesions, confirming a previous observation by Lucchinetti et al (2002). Roemer et al (2007) also noticed a striking loss of Aquaporin 4 in NMO lesions regardless of the site of CNS involvement, the stage of demyelinating activity and the extent of tissue necrosis; however, they noticed that there was also a reduction in Aquaporin 4 in acute cavitary MS lesions and in inactive longstanding MS plaques. AQP4 was diffusely increased in the periplaque white matter of early and late active lesions, and in both the periplaque white matter and lesion centre in early remyelinating lesions.

Matsuoka et al (2009) found extensive AQP4 loss in MS lesions, and in post-mortem cases of NMO spectrum disorder, some cases demonstrated preservation whilst other cases demonstrated loss of AQP4 even in acute lesions. Considerable intra-individual variability of AQP4 expression – with some lesions showing loss and others showing up-regulation – was observed. They concluded the mechanisms underlying AQp4 loss may be heterogenous.. There is considerable overlap in the clinical features on imaging of NMO and MS. Pittock et al (2006) found 10% of patients testing positive for the NMO IgG had brain lesions indistinguishable from those found in MS.

The role of the AQP4 autoantibody in the diagnosis of NMO spectrum disorder has been called into question by the heterogeneity shown across ethnic groups, of the expression of the autoantibody. In Lennon et al's original report on the NMO IgG antibody, 73% of white Caucasian patients tested positive for the antibody out of 102 patients with NMO spectrum disorder. However, immunofluorescence, flow cytometry and radioimmunoprecipitaion assays in an Italian patient population with NMO showed a positivity rate of between 30 and 47% (Fazio et al, 2009). In a study carried out in the French West Indies and Cuba (Cabrera-Gomez et al, 2009), the positivity rate was 33% whilst a Japanese group found 20 out of 22 patients with NMO to be carry the antibody (Nakashima et al, 2006).

9% of MS patients tested positive for the antibody in the original series by Lennon et al. Testing for the Aquaporin 4 antibody as opposed to the NMO-IgG, has still resulted in MS cases being found to have the antibody (Paul et al, 2007; Matsushita et al, 2009).

Many of the MS cases prevalent in Japan and the Far East has been noted to be different to MS cases found in the west, with more severe involvement of the optic nerves and the spinal cord and rapid progression. Their existence blurs the divide between NMO spectrum disorders and MS even further. 15-40% of cases of MS in Japan fit this phenotype and this type of MS found in Asia which is not commonly found in the west has been described as optic-spinal MS (OSMS) or Asian MS (Kira et al, 1996; Kira 2006) , whereas conventional MS is referred to as CMS. 63% of patients with optico-spinal MS in northern Japan and 27% in southern Japan were recently found to test positive for the anti-AQP4 antibody suggesting that the eastern

phenotype of MS may have more overlap with NMO (Nakashima et al, 2006; Matsuoka et al, 2007).

The presence of high titres of anti-AQP4 antibody does not prevent NMO patients from remaining in remission and seroconversion of NMO-IgG or anti AQP4 during the course of the disease has been known to take place (Matsuoka et al, 2007; Matsushita et al, 2009). These observations have led to the suggestion that the antibody may be produced as a secondary phenomonenon to primary tissue destruction, as may be seen in MS patients who may produce various autoantibodies (including to neuronal antigens) during the course of their disease (Mathey et al, 2007). Kira (2006) has proposed a hypothesis regarding the pathogenic mechanism behind MS and NMO, where NMO without overt autoimmune diseases or paraneoplastic conditions may represent one end of an MS spectrum.

In cases of optic neuritis associated with MS and NMO one expects considerable variation in pathogenesis, presentation and clinical features, as MS and NMO themselves show extensive heterogeneity at all levels. The clinical features of optic neuritis and their variation will now be discussed.

The clinical features of optic neuritis

Optic neuritis has an incidence of about 1 in 100000 per year in the UK (MacDonald et al, 2000) and mostly affects patients aged 20-49 years, with a higher predilection for women than men (Jin et al, 1998).

In the ONTT, the mean age of onset of optic neuritis was 32 years. Patients typically suffer from an acute to subacute visual loss which may progress over days to weeks and then remain stable, before improving spontaneously. The improvement is rapid at first then slows down, but may continue for up to one year after the onset of visual loss (Beck 1998). In 92% of patients, the visual loss is accompanied by static pain or pain on eye movement (majority) on presentation, and the pain has been shown to be more frequent if the inflammation involves the orbital section of the optic nerve (Optic Neuritis Study Group 1991; Fazzone et al, 2003).

Katz et al (1995) summarised the clinical features of optic neuritis patients enrolled in the ONTT. The maximal visual loss reported in optic neuritis is variable, with over 10% of patients preserving a good visual acuity. Patients may be found to have abnormal colour perception, reduced contrast sensitivity, visual field loss and a relative afferent pupillary defect (Cox et al, 1981). In addition, patients may complain of positive visual phenomenae such as light flashes in the form of phosphenes and photopsia upon moving the affected eye, and Uhthoff's phenomenon (Davis et al, 1976; Selhorst & Saul, 1995). Uhthoff's phenomenon is thought to be a result of demyelination. Severe demyelination has been shown to result in total failure of conduction through the nerve fibre with consequent conduction block. Less severe demyelination allows transmission of nerve impulses but the transmission is delayed due to a slowing of the conduction velocity through the demyelinated zone. A fast train of impulses cannot be conducted due to an increase in the refractory period following each impulse. The degree of demyelination influences the temperature at which conduction block occurs. Schauf and Davis (1974) demonstrated that the

reduction of the myelin sheath to a quaretr of its normal thickness can reduce the blocking temperature from 40 degrees Celsius to 20 degree Celsius.

Visual field defects may be variable and may include altitudinal loss, arcuate defects, nasal steps and defects respecting the vertical meridian in only the affected eye, however intracranial nerve lesions give rise to temporal visual field defects (Fazzone et al, 2003).

Most patients in the ONTT recovered visual function. 79% of patients had begun to show signs of improvement within 3 weeks of onset, 14% of patients started to improve between 3 and 5 weeks after onset, when no treatment was given. The initial rapid recovery experienced by most of these patients is thought to result from an improvement in the structural changes of inflammation such as oedema and swelling within the optic nerve, as this phase of improvement may parallel the subsidence of swelling of the optic disc. Visual evoked potential recording can be used to show that in the early days of recovery, the amplitude of the pattern response may greatly recover, but the latency remains prolonged, suggesting persistent demyelination (Mcdonald & Halliday ,1977).

Remyelination can continue for until two years following the ON episode, but the objective measurement of progressive latency shortening may not correlate with a subjective improvement in visual function (Brusa et al, 2001). It was discovered in the ONTT that objective methods of testing did not always correlate well with patients'subjective visual function. A questionnaire was completed by 382 (87%) of 438 patients in the ONTT after the 6 month study visit. 56% of the patients reported

their visit to ne “somehat worse” or “much worse” that it had been prior to the episode of optic neuritis, however only 20% of the total group showed an abnormal result on all the four tests of visual function, including visual acuity, contrast sensitivity, colour vision and visual field mean deviation (Cleary et al, 1997).

The Impact of the Optic Neuritis Treatment trial

The optic neuritis treatment trial (ONTT) is the largest and most conclusive trial to have been carried out on isolated optic neuritis occurring either without a known cause or in the presence of lesions suggestive of demyelination. It was the first trial to offer a large scale of information on the nature of this type of optic neuritis, its natural course, its prognosis and its association with multiple sclerosis (MS) over a period of 15 years (Optic neuritis study group, 2008). Its findings continue to hold true in the context of the majority of cases of ‘typical’ optic neuritis. Its legacy has been the creation of a protocol in the management of idiopathic isolated optic neuritis which is followed all over the world.

There have been many reports on idiopathic optic neuritis before the conduction of the ONTT, however, it is only ***after*** the ONTT clearly defined the course of the majority of cases of idiopathic optic neuritis that centres around the world began to identify cases which did not quite follow this pattern.

The clinical profile of patients within the ONTT

The cohort of patients on whom the ONTT was conducted demonstrated the following characteristics:

- 85% were of white Caucasian ancestry (59 out of 448 patients were of African ancestry). [Phillips et al, 1998]
- All were aged between 18 and 48 years
- 35% of patients had a visual acuity of 6/60 or worse (15.6% of patients had a visual acuity of Counting Fingers, Hand Movements, Light Perception or No Light Perception).

The clinical course and prognosis of patients from the ONTT

- Approximately 72% of patients with a visual acuity worse than 6/60 at presentation had recovered to a visual acuity of 6/6 or better at 5 years (Cole et al, 1998)
- There was no significant difference in visual prognosis between patients treated with intravenous corticosteroid therapy and patients given no treatment when visual function was measured after 5 years.
- After 5 years, only 3% of patients had residual visual loss of 6/60 or worse.

These observations amalgamated previous studies and allowed for a unified approach to optic neuritis in patients with the above profile.

Post ONTT developments

Following the ONTT, the 'typical' form of optic neuritis was appreciated and cases which did not follow the course outlined in the ONTT were recognised. Focus shifted to patients with ON of unknown cause who demonstrated the following profile,

response to treatment and course, on whom the ONTT does not offer reliable information:

1. Profile
    - Patients of African or Asian descent
    - Patients of an age group >48 years
    - Presence of bilateral optic neuritis which may be simultaneous or consecutive
    - Presence of optic perineuritis

2. Response to treatment
    - Cases where no recovery is observed until immunosuppressive treatment is started
    - Cases where the optic neuritis episode relapses upon the withdrawal of immunosuppressive therapy.
    - Cases which are resistant to therapy.

3. Course
    - Cases where optic neuritis recurs many times over a long period of time in the absence of pathology on repeated brain imaging or systemic investigation.

Profile

Case series and reports of optic neuritis occurring either in the absence of a known cause or in the context of demyelination within non-white Caucasian population groups, highlight differences with the profile of optic neuritis observed in the ONTT.

The ONTT did not find a significant difference between the visual acuities of the white Caucasian and non-white Caucasian patients at 6 months after onset (Beck et al. 1994). However, studies on patients of African descent have suggested their course of optic neuritis may be different. Phillips et al (1998) demonstrated that African American patients presented with significantly more frequent severe visual loss (at VA worse than 6/60) compared with white patients (93% vs 39%) and they failed to recover to a visual acuity of 6/12 significantly more frequently than their white counterparts after one year (39% vs 8%). Differences have also been reported in the optic neuritis profile of patients in Japan, where the incidence of pain and the presence of periventricular plaques on first imaging has been found to be lower, whereas the incidence of disc swelling has been found to be higher (Wakakura et al, 1999) than that reported by the ONTT.

The presence of bilateral optic neuritis has been reported in the context of both MS and Neuromyelitis Optica (NMO) (Burman et al, 2011; Marignier et al, 2008). There is as yet no established definition of bilateral optic neuritis in the context of non-demyelinating optic neuritis.

Idiopathic optic neuritis is also known to occur for the first time at an age greater than 48. However, it is not known how the majority of these cases present.

Response to treatment

It has long been known that optic neuritis associated with underlying collagen vascular disease, granulomatous disease or infection requires corticosteroid therapy.

The majority of cases in the ONTT recovered without treatment and the presence of treatment did not influence the prognosis at 5 years. However, we know there are patients who have been diagnosed with MS related ON in whom recovery from the episode of ON may be dependent on immunosuppression, where the ON is refractory even to intravenous corticosteroid therapy and intravenous immunoglobulin is required for improvement (Tselis et al, 2008). It has also been shown that a patient's ethnic background can influence corticosteroid dependence in MS (Zelnik et al, 1991).

Previously healthy patients who develop a corticosteroid dependent optic neuritis without solid evidence of underlying disease including demyelination has been reported prior to the ONTT and is being increasingly reported since. Kupersmith et al (1988) called this group 'Autoimmune optic neuropathy' on the grounds that the majority of patients reported had a raised ANA titre. Since the ONTT, one centre has suggested naming this syndrome of corticosteroid dependency in the absence of underlying disease as 'Chronic Relapsing Inflammatory Optic Neuropathy' or 'CRION' (Kidd et al, 2003).

Since the discovery of the antibody to Aquaporin 4 and its high specificity in the context of NMO, some centres have tested the possibility of this type of corticosteroid dependence in cases of idiopathic optic neuritis occurring as a result of NMO (Pérez-Díaz et al, 2007; Petzold et al, 2010). Although corticosteroid therapy followed by some form of immunosuppression is widely thought to be the best treatment regimen for NMO associated optic neuritis, this has never been proven in a randomized controlled trial. Many patients with recurrent bouts of ON who are eventually diagnosed with NMO may not display corticosteroid dependence initially (Matiello et al, 2008).

Course

The ONTT estimated the likelihood of the diagnosis of MS at 5 years following the onset of optic neuritis to be approximately 50%, where the risk was highly correlated to the presence of white matter lesions on brain imaging.

However, isolated optic neuritis may occur repeatedly over a long period of time without the emergence of evidence of an underlying aetiology (Arndt et al, 2008). These cases may continue as they are, or may develop into NMO or may develop into MS.

Clinical investigation of Optic Neuritis

The basic clinical workup for acute isolated optic neuritis includes a thorough clinical evaluation, including:

- Visual Acuity
- Colour perception assessment
- Pupillary assessment for the presence of a relative afferent pupillary defect
- Visual field assessment.
- Fundoscopy
- Baseline blood tests
- Case by case specific tests to exclude infectious, granulomatous, paraneoplastic, and collagen vascular disease aetiologies

MR scanning is advocated in the light of the findings of the ONTT for prognostic purposes, and in the light of phase 3 clinical trials confirming the benefits of Interferon beta 1a, interferon beta 1b and glatiramer acetate in delaying the onset of 'clinically defined MS' or CDMS in patients with clinically isolated syndromes including optic neuritis in the United States. (Comi et al, 2001; Kappos et al, 2006). However, in the UK, if patients with no previous or additional history follow a 'typical' clinical course, MR imaging may not always be attempted. Where MR imaging is attempted, particularly in atypical cases, CSF analysis may be performed.

Additional investigations

This thesis has demonstrated the limitations of the use of pupillometry in diagnosing the presence of optic nerve disease, owing to the inter-individual variability in the pupil's response to white light. It has however, been shown that the pupillometric measurement of the various pupil channels when stimulated separately may allow a clinician to differentiate between NMO and MS related optic neuritis. Owing to the diffuse swelling within the retinal nerve fibre during the acute phase of optic neuritis,

this distinction can only be made in the long-term. In addition, as mild deficits in the optic nerve result in a pupillometric performance that is within the boundaries of normal inter-individual pupil variation, the distinction between MS related and NMO related optic neuritis can only be made in vision-matched cases where there significant resulting visual loss, following the acute episode. As most cases of MS related optic neuritis show good recovery, this tool is therefore of limited use in the acute diagnosis of optic neuritis.

This thesis now examines three methods of distinguishing between acute optic neuritis caused by MS and that caused by NMO in the acute setting:

- Serological analysis for the presence of serum Glial Fibrillary Acidic Protein
- The role of the ethnic background of the patient
- The radiological appearance of the visual pathway

THE USE OF GLIAL FIBRILLARY ACIDIC PROTEIN MEASUREMENTS IN THE DIAGNOSIS OF NEUROMYELITIS OPTICA SPECTRUM OPTIC NEURITIS

Introduction

The previous chapters of this thesis have shown that pupillometry has a role in helping a clinician to distinguish Neuromyelitis Optica (NMO) related optic neuritis from Multiple Sclerosis (MS) related optic neuritis (ON) in cases where there is a poor visual outcome and long after the acute episode.

Recent research has focused on the detection of markers of astrocytic damage, and their relative expression in NMO and MS. Glial fibrillary acidic protein (GFAP) is a specific intermediate filament (IF) of the cytoskeleton of the astrocyte and is absent from oligodendrocytes and neurons. GFAP measured in cerebrospinal fluid (CSF) has been shown to be marginally increased in MS patients when compared with normal controls but substantially increased in NMO patients undergoing relapses (Fujihara 2011; Takano et al, 2010; Axelsson et al, 2011; Misu et al, 2009). It has until now not been shown if GFAP may be measured in the serum following an MS or NMO spectrum relapse, especially if the relapse involves a single lesion in the optic nerve. It is also not known if 'atypical' patterns of ON (such as recurrent or corticosteroid-dependent: RION and CRION) result in differing degrees of astrocytic damage.

This is the first study to investigate levels of *serum* GFAP in the context of acute isolated ON. In this study I assess whether serum measurements of GFAP may allow

the distinction between MS and NMO related ON. This study tackled three main questions.

1. Is it is possible to detect GFAP in the serum of patients with ON associated with MS and NMO spectrum disease and is there a difference in the serum level between these two groups? It has been shown previously that other biomakers (nitric oxide and neurofilaments) can be detected in serum following an episode of ON even though the volume of diseased tissue is relatively small and levels are likely to be lower than in CSF (Petzold et al, 2004)

2. Does isolated optic nerve disease in the absence of lesions elsewhere result in the release of sufficient levels of GFAP into the serum, to be detectable?

3. Do the levels of serum GFAP (and hence the degree of astrocytic damage) found in patients with 'atypical' presentations of ON, such as CRION and RION pattern ON, resemble MS or NMO, or are they distinct from both? A significant difference between CRION and MS patients would allow the former to be identified earlier and given urgent immunotherapy.

This study also investigates if the level of serum GFAP is related to the degree of recovery from the most recent attack of ON. It shows that it is possible to measure GFAP in the serum of optic nerve patients and that this measurement may allow clinicians to identify NMO from non-NMO related ON.

Methods

Objectives

1. To investigate if NMO Spectrum ON can be distinguished from MS associated ON by measuring serum GFAP following an episode of ON, even in the absence of extra-optic nerve lesions

2. To investigate if serum GFAP levels in patients with 'atypical' patterns of ON (such as CRION or RION pattern ON) resemble those found in patients with NMO spectrum ON.

Participants

Out of 150 patients consecutively presenting to our eye hospital over the period March 2009 until July 2010 with an episode of acute ON at Moorfields Eye Hospital or The National Hospital of Neurology and Neurosurgery, Queen Square, we were able to collect a serum sample (within 210 days of onset of the ON episode) from 12 patients who had presented with MS related ON and from 10 patients who had presented with NMO spectrum disease-related ON. MS was diagnosed based on the McDonald criteria (Polman et al, 2005). The NMO spectrum group were all positive for the Aquaporin 4 antibody, 6 patients presented with isolated ON only and 4 patients also had myelitis at some point in the past or concurrently and hence satisfied Wingerchuk's criteria for diagnosis (Wingerchuk et al, 2006). If an Aquaporin 4 antibody-negative patient experienced two or more attacks of ON affecting one or both eyes, without evidence of an underlying demyelinating or other disorder during the period of assessment, where corticosteroid therapy was required for each episode to resolve and where withdrawal of the corticosteroid therapy prompted a relapse,

resulting in the patient being maintained on long term immunosuppression, then the patient was given a label of CRION pattern ON. This pattern of ON has been previously described with differing acronyms (Kupersmith et al, 1988; Kidd et al, 2003). In the context of this study, the label of CRION pattern ON is being used not as a diagnosis, but as a category to describe syndromically an atypical, Aquaporin 4 antibody-negative, corticosteroid-dependent pattern of ON displayed by some patients in whom the diagnosis is unknown. These patients may have a form of granulomatous disease (such as a highly localised form of Neurosarcoidosis) or an unrecognised autoimmune disease which has evaded detection during standard and specialised clinical testing. Patients who suffered from a single or from recurrent episodes of isolated ON in the absence of any other sign of underlying disease on clinical, radiological and serological testing were categorised as 'SION' or RION cases respectively. Eight patients were labeled as CRION. In a fourth group, 8 patients had experienced at least two episodes of ON. Five of these patients were treated with a short course of steroid therapy at their own request, and did not relapse upon its withdrawal. The patients displayed no evidence of demyelination elsewhere. As they displayed a recurrent pattern of ON in the absence of evidence of other pathology, we labeled this group as RION (Arndt et al, 2003) All patients except for those with MS related ON were tested for the Aquaporin 4 antibody. All patients with other existing neurological/connective tissue/ vasculitic/ ophthalmic disease were excluded from the study.

The demographic data of the participants are given in Table 1.

	ON Subtype			
	MS related ON	NMO spectrum	CRION pattern ON	RION pattern ON
White Caucasian ethnic background	6	2	3	6
Not 'white Caucasian' ethnic background	6	8	7	2
F:M	8:3	7:3	9:1	5:3
Median Age in years	32	34	44	42

Table 1

The age, gender and ethnic background of patients across all categories are shown. A higher female:male ratio is observed in all groups. The majority of patients with NMO spectrum and CRION pattern ON do not have a 'white Caucasian' ethnic background.

Patient Groups

MS group

The MS group was made up of 12 patients. In 4 of these, the ON episode was the first episode of MS which was diagnosed subsequently. Four of the 8 patients in whom the

diagnosis was already confirmed at the point of sample collection had experienced previous non-optic nerve relapses. None of the participants were on long term immunomodulatory therapy.

NMO spectrum group

This group was formed of 10 patients. All patients were seropositive for the antibody to Aquaporin 4. Of these, 6 patients had suffered from ON only, without any evidence of myelitis; these patients will be referred to as 'AQP4+ON'. The remaining 4 patients satisfied Wingerchuk's criteria (Wingerchuk et al, 2006)

RION group

This group consisted of 8 patients. Two of the patients presented with their first attack during the time of the study. None of the participants were on long term treatment of any kind.

CRION group

This group included 10 patients, all of whom had been diagnosed prior to the current episode of ON, and all of whom were on some form of immunosuppression at the time of the attack.

Serum Analysis for GFAP level.

Serum samples were collected in polypropylene tubes centrifuged (2,000 g for 10 minutes) and stored immediately at -80ºC in 1.5–2 mL Eppendorf tubes (polypropylene) until analysis. Serum GFAP was measured in duplicates with the analyst being blinded to all other information using an in-house developed ELISA. The analytical accuracy of the assay was found to be 5.8% (interassay coefficient of variation).

Aquaporin 4

All patients were tested for Serum aquaporin 4 antibodies (AQP4) aside from those with a diagnosis of MS associated ON. Testing was carried out at the Wetherall Institute of Molecular Medicine, University of Oxford by a method using the fluorescence immunoprecipitation assay (FIPA) technique described by Waters et al (2008).

Measurement of visual recovery

All patients were clinically assessed using a Snellen chart. We defined visual recovery in terms of the number of lines of improvement using a Snellen chart, from the point of the worst visual acuity during the acute episode to the visual acuity measured at a follow up assessment after recovery. In the case of bilateral ON, we used the reading from the eye with the greater degree of recovery. For comparison of the visual acuity during the peak of visual loss during an episode of ON, we converted the Snellen reading to a LogMar measurement for ease of comparison of visual acuities across the groups.

Statistical methods

SigmaPlot© and SigmaStat© statistical software were used to perform statistical analysis on the data. Tukey Box Plots were used to graphically represent the data showing either the 5^{th}, 25^{th}, 50^{th}, 75^{th} and 95^{th} percentiles, or the 25^{th}, 50^{th}, and 75^{th} percentiles. The unpaired student t-test was used for the comparison of two samples if the samples followed a normal distribution and had similar variances. Where this was not the case, the Mann–Whitney Rank Sum test was used instead. The Kruskal-Wallis One Way Analysis of Variance was used to compare multiple groups. Multivariable linear regression analysis was used to assess the influence of more than one independent factors on a dependent factor. A p-value of <0.05 was regarded as significant.

Results

The serum GFAP levels in pg/mL measured in patients with NMO spectrum disease, MS-related ON, and CRION and RION syndromes are represented in Figure 1. The median values of serum GFAP demonstrate a trend in the following order: NMO > CRION > RION > MS.

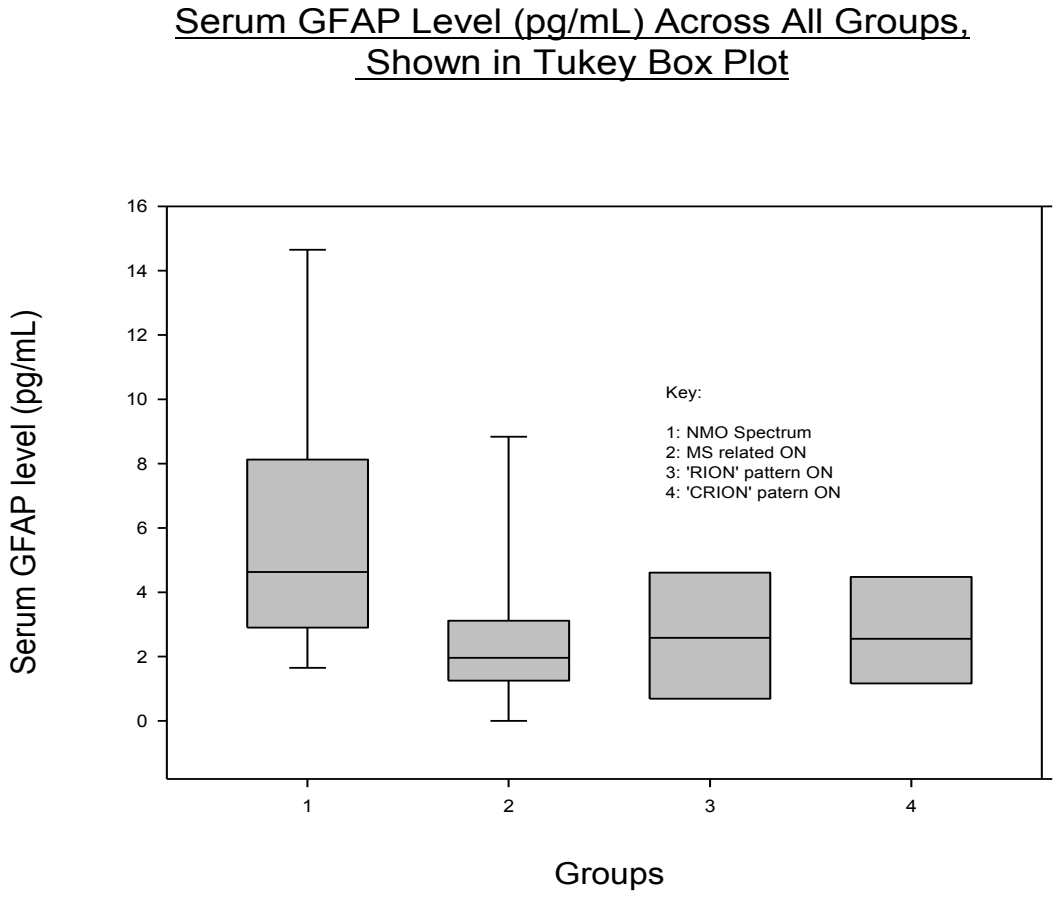

Figure 1
Tukey box plot showing the median, 25th percentile and 75th percentile of serum GFAP level measurements in each group. The 5th and 95th percentile of groups 1 and 2 are also shown.

The serum GFAP level was measured after variable time intervals (until 210 days) following the acute ON episode onset. Figure 2 demonstrates the time interval (in days) after which the serum GFAP was measured in all patient groups, and the serum GFAP level.

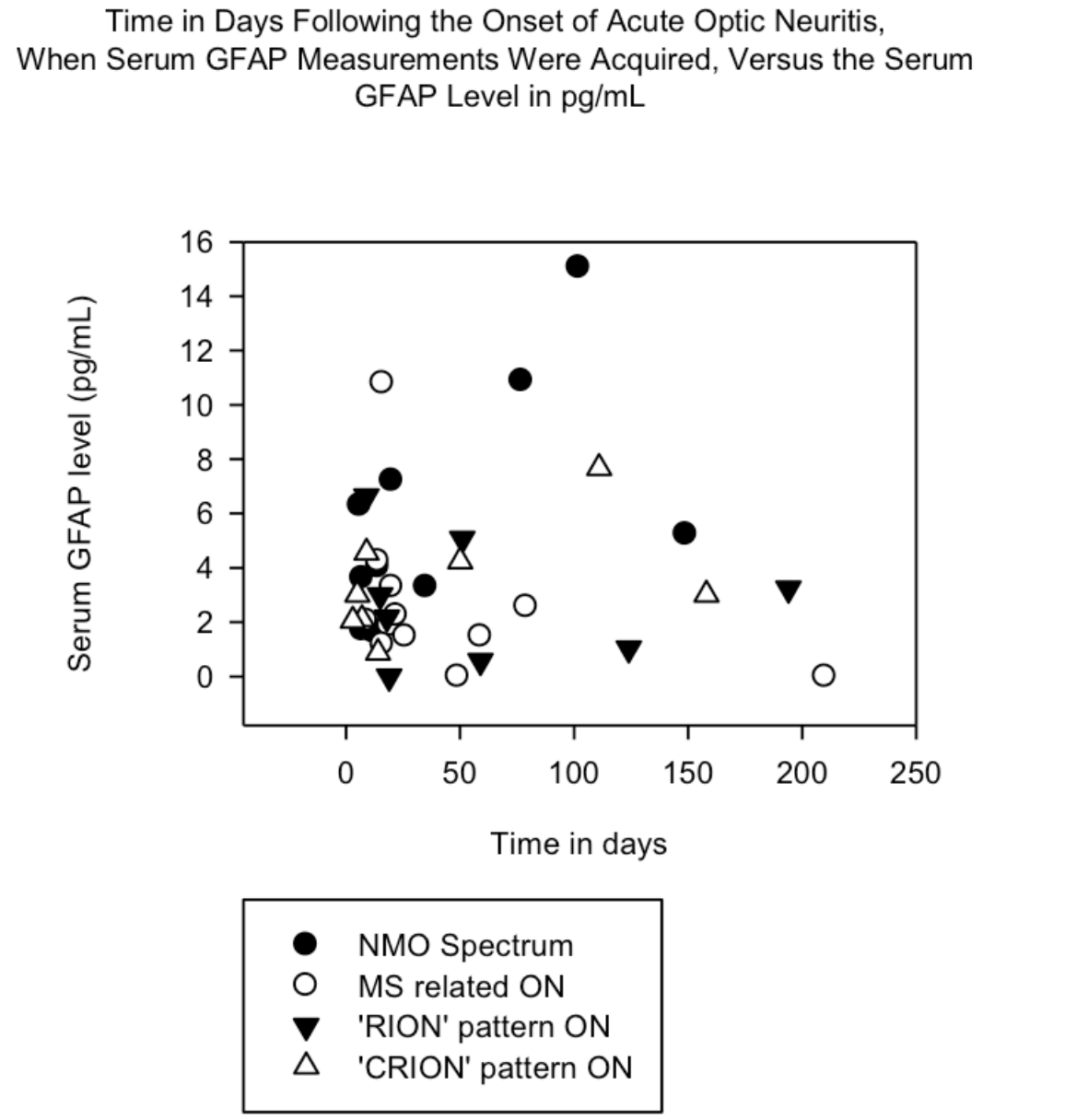

Figure 2
Although many patients' serum was tested within 50 days of onset, their serum sample was collected up to 210 days following the onset of acute optic neuritis.

1) Serum GFAP levels in NMO vs MS and in NMO vs all AQP4 negative cases.

A comparison of the NMO Spectrum group with the MS group is made in Table 2.

	NMO spectrum (pg/mL) n=10	MS related ON (pg/mL) n=12	RION pattern ON (pg/mL) n=8	CRION' pattern ON (pg/mL) n=10	All patients with non-NMO spectrum ON n=30
Median	4.63	1.96	2.58	2.56	2.14
25%	3.30	1.33	0.80	1.46	1.11
75%	7.21	2.94	4.16	4.39	3.77
Skewness	1.29	2.42	0.62	1.08	1.60
Kurtosis	1.30	6.99	-0.45	1.12	3.16
99% C.I.	4.36	2.56	2.82	2.83	

Table 2

Descriptive statistics of serum GFAP measurements (pg/mL) in patients across all categories are shown. In each case the mean value was greater than the median value, confirming the distribution was not normal. The mean and standard deviation values are therefore not shown. The fifth column shows the merged data of all 'non-NMO spectrum' patients. Both mean and median values for the serum GFAP level were highest in the NMO spectrum category and lowest in the MS related ON category. The results from patients with 'atypical' patterns of ON (RION and CRION pattern) fell halfway between the two groups. Patients in the NMO spectrum group showed the highest variance. All values are stated to within 2 decimal places.

The Mann-Whitney Rank Sum Test confirmed a statistically significant difference (P=0.02) between the median value of the NMO spectrum group (4.63pg/mL) and the median value of the MS group (1.96 pg/mL). 75% of readings within the NMO spectrum group fell above 3.3pg/mL whereas 75% of readings within the MS group fell below 2.94pg/mL .

The NMO Spectrum group is compared with all other patients (MS, RION syndrome and CRION syndrome combined together) in Table 2. After a significant Kruskal-Wallis result, the Mann-Whitney Rank Sum Test confirmed a statistically significant difference (P=0.01) between the median value of the NMO spectrum group (4.63pg/mL) and the median value of the three other groups combined together (2.14pg/mL). 75% of readings within the NMO spectrum group fell above 3.30pg/mL whereas 75% of readings within the three other groups, when combined, fell below 3.77pg/mL.

The Mann-Whitney Rank Sum Test did not show a statistically significant difference between the median values of the MS and CRION groups (P=0.44), nor between the median values of the NMO and CRION groups (P=0.17).

Serum GFAP is statistically significantly higher in NMO spectrum patients than in patients with MS, as well as all Aquaporin 4 antibody negative patients combined together.

2) Serum GFAP levels in AQP4 positive patients with isolated ON vs. all AQP4 negative cases.

The question arises as to whether it is the occurrence of extra-optic nerve disease – such as myelitis – that is the factor determining the higher GFAP levels in NMO.

The serum GFAP levels (in pg/mL) measured in patients without extra-optic nerve disease (AQP4+ON, CRION syndrome and RION syndrome) are shown in Figure 3.

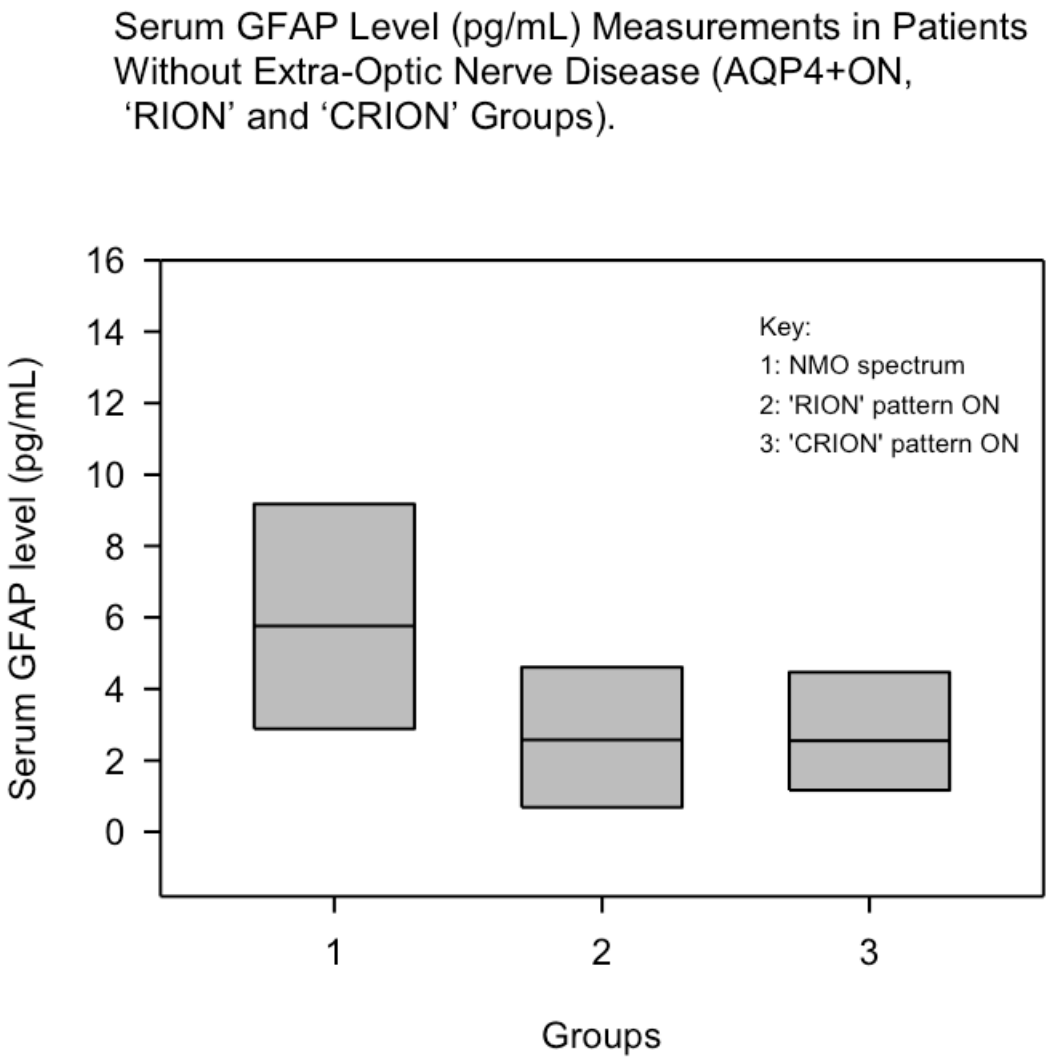

Figure 3
Tukey Box plot showing the median, 25th percentile and 75th percentile of serum GFAP level measurements in each group.

The descriptive statistics for the comparison of AQP4+ON patients with all antibody negative patients who do not have extra-optic nerve disease (RION syndrome and CRION syndrome) are shown in Table 3.

	NMO	RION	CRION	R+C
Median	5.76	2.58	2.56	2.58
25%	3.30	0.80	1.46	0.95
75%	7.21	4.16	4.39	4.39
Skewness	1.45	0.62	1.08	0.75
Kurtosis	2.72	-0.45	1.12	-0.09
99% C.I.	7.70	2.82	2.83	

Table 3

Descriptive statistics of serum GFAP measurements (pg/mL) in patients without extra-optic nerve disease are shown. AQP4+ON patients showed the highest levels of serum GFAp as well as the highest variance. Both the median and mean values for serum GFAP level are higher in AQP4+ON patients compared to the values in the NMO spectrum group as a whole (4.63 pg/mL, 5.897 pg/mL). All values are stated to within 2 decimal places

Use of the t-test confirmed a statistically significant difference (P=0.03) between the mean value of the AQP4+ON group (6.46pg/mL) and the mean value of the RION and CRION syndrome groups combined (2.94pg/mL). The 99% confidence interval of the AQP4+ON group was 7.70pg/mL whereas those of the RION and CRION groups were 2.82pg/mL and 2.83pg/mL respectively

Serum GFAP is statistically significantly higher in Aquaporin 4 antibody positive patients without extra-optic nerve disease than in all Aquaporin 4 antibody negative patients combined together.

3) Serum GFAP levels and visual outcome.

This analysis was carried out with 9 NMO spectrum patients, 9 MS patients and 7 RION patients on whom we had accurate data on visual acuity. The worst visual acuity during the most recent episode of acute ON (baseline visual acuity, measured as a LogMar conversion of the Snellen reading) was not significantly different between the groups. A Kruskal-Wallis Analysis showed there was no statistically significant difference between the groups (P=0.30).

Multiple linear regression analysis did not demonstrate a significant effect of the number of lines (on a Snellen chart) of recovery following ON and the serum GFAP level measured, taking into account the number of days following the ON episode, when the serum measurement was undertaken in all patients studied.

Discussion

This study has shown that Glial pathology in NMO related ON is reflected in elevated serum GFAP levels independently of whether or not there is extra-optic nerve disease. The level of serum GFAP during an attack of ON has no prognostic value.

Our results suggest it may be possible to separate patients with NMO spectrum disease from those with MS when they present with an episode of acute ON, on the basis of their group medians. A larger number of cases would be required in order to

determine the sensitivity and specificity of the test. The significant difference in the level of serum GFAP found in this study nonetheless holds promise for the use of serum GFAP to predict whether a previously healthy patient presenting for the first time is likely to require a non-ONTT based treatment protocol.

The differences in the level of serum GFAP between patients in the groups we have studied were less dramatic than those reported in a recent study examining levels of GFAP in the cerebrospinal fluid of patients with NMO and MS, where the overall levels of cerebrospinal fluid GFAP measured during a relapse were reported to be 2 476.6 ± 8,815.0 ng/mL in NMO and 0.8± 0.4 ng/mL in MS (Takano et al, 2010). However, a relapse in the form of ON was found to result in markedly lower levels of cerebrospinal GFAP (median 6.1; range 1.1–56.1) than myelitis (median 593.9; range 1.2– 47,843.3 ng/mL).

All our patients experienced a relapse in the form of ON. Additionally, measurement of GFAP in the serum instead of the cerebrospinal fluid is likely to result in a lower measurement following passage across the blood-brain-barrier and may explain the difference in the levels reported between the two studies. Takano et al (2010) carried out cerebrospinal fluid measurements in almost half of all patients within a week of onset and all patients within 25 days of onset and demonstrated a sharp decline in GFAP level (a factor of 100 within 25 days) over this time. In contrast, serum analysis in our study was carried out in fewer than half of all patients within 14 days and in all patients up to 210 days following onset. This may be an additional reason behind the difference in GFAP levels detected in the two studies.

We did not have sufficient numbers of patients to make a comparison between patients presenting for the first time with what is later diagnosed as NMO spectrum disease, versus patients presenting for the first time with what later becomes MSON.

The observation that isolated astrocytic damage in the optic nerves is sufficient to release GFAP at concentrations quantifiable from the serum, and that these are highest in NMO spectrum ON further supports a role for serum GFAP in the diagnosis of NMO spectrum ON at first presentation.

The absence of a statistically significant difference between the serum GFAP values in the CRION pattern ON group and either the MS or NMO spectrum groups may be due to the small sample size and consequent low power, or may be a consequence of immunosuppression. Most patients with CRION were immunosuppressed prior to the most recent episode of ON and this immunosuppression may have protected against the full extent of inflammation occurring during the attack, and limited astrocytic damage.

As our testing methods for the autoantibody to Aquaporin 4 have been shown to be 76% sensitive and 100% specific, there exists a possibility that the RION and CRION cohorts within this study also contain NMO spectrum patients which may have reduced the differences in serum GFAP levels between the groups (Waters et al, 2008). A previous study has described cases of RION pattern ON developing into NMO spectrum disease over time (Matiello et al, 2008). Our study provides some evidence that the astrocytic damage occurring within the RION pattern of ON is less extensive than that occurring in AQP4+ON, and that patients with a RION pattern of

ON may instead have a similar extent of astrocytic damage to MS associated ON. Although all patients labeled as having a RION pattern of ON in our study showed no demyelination on imaging, the overlap between a RION pattern of ON and MS has been described (Burman et al, 2010).

Patients from the AQP4+ON, MSON and RION groups showed no evidence of a difference in degrees of visual loss during the acute episode of ON (P=0.30). In all three groups, no association of the level of visual recovery following the episode was found with the serum GFAP level. Although this may be the result of testing small sample sizes and the wide temporal window within which sampling was carried out (210 days), an additional reason may be that visual outcome reflects neuronal loss, not astrocytic damage. Serum GFAP may be released as a result of astrocytic damage without resulting neuronal loss, for which other neuron-specific markers such as neurofilaments have been shown to be better biomarkers (Petzold et al, 2004).

We were unable to measure the level of serum GFAP over time within individual patients and this is a significant limitation of our study. The rate of decay of serum GFAP level following astrocyte damage is not known.

Serum GFAP levels need to be measured in patients presenting with acute isolated ON for the first time, with no known medical history, in order to accurately determine the role of GFAP in the diagnosis of ON.

The number of participants was small and a larger cohort would be required to reach further conclusions. This would require a longer time period of observation in a long-term prospective study

THE ROLE OF ETHNIC BACKGROUND IN THE DIAGNOSIS OF OPTIC NEURITIS

Multiple sclerosis (MS) and disorders resembling MS show marked differences in prevalence in certain ethnic groups and in different parts of the world. Classical MS has been shown to preferentially affect individuals from a white Caucasian background and patients with MS who are of African American descent have been shown to respond differently to treatment (Bhigjee et al, 2007; Zelnik et al, 1991). Many patients suffering from MS from East Asia appear to suffer from a particular sub-type of the condition, which has been referred to as opticospinal MS (Kira 2011)

There are few population studies of the less common demyelinating disorder, Neuromyelitis Optica (NMO), from which comparison may be made of the relative incidence of the disorder within white Caucasian and African heritage backgrounds. The prevalence of this disorder within a northern European predominantly white Caucasian population has recently been estimated at 4.4 per 100000 (95% CI 3.1–5.7) (Asgari et al, 2011). An African Caribbean population based study estimated a prevalence of 2.5 per 10000 and an annual incidence of 0.1 per 100000, whereas another population based study from Cuba reported a prevalence of 0.52 per 100000 and an annual incidence of 0.05 per 100000, where there appeared no difference in rate between white, black, mixed or non-white self-reported racial groups (Asgari et al, 2011; Cabre et al, 2009).

Optic neuritis is a manifestation of both MS and NMO. The time between an initial attack of acute isolated optic neuritis and a subsequent relapse eventually culminating

in a diagnosis of MS or NMO may be long, up to several years (Wingerchuk et al, 2007). Magnetic resonance imaging has a role in predicting the likelihood that a patient with isolated optic neuritis may develop MS and the discovery of the serum autoantibody to the Aquaporin 4 water channel has aided the early diagnosis of NMO (Optic Neuritis Study Group, 1997; Lennon et al, 2004; Wingerchuk et al, 2006). However both of these investigations are currently subject to false negative results. An early diagnosis of NMO is essential because the rapid initiation of immunosuppressive therapy may be critical whereas this is not the case for optic neuritis associated with MS where corticosteroid therapy is not mandatory (Nakamura et al, 2010).

To our knowledge, there has been no study to date on the relative incidence of optic neuritis caused by MS and that caused by NMO within different ethnic groups.

London has a large multi-ethnic population and our Neuro-Ophthalmology clinic has provided a unique opportunity to see cases of optic neuritis in patients of white Caucasian, of African and of Asian ancestry in significant numbers. These patients inhabit the same climate and latitude and mostly have similar diets and lifestyles. We have minimised variability in reporting and the potential influence of other unknown environmental factors which have been suspected to confound previous observations on the role of ethnicity in the incidence of disease.

In this study we first compare the ethnicity profile of all patients attending our Neuro-ophthalmology clinic with acute isolated optic neuritis with no previously known underlying cause (and who did not develop a collagen-

vascular/granulomatous/infectious/autoimmune/neoplastic illness to account for the optic neuritis) over a period of time, with that of the population of London. We then compare the ethnicity profile of patients within diagnostic categories of optic neuritis (such as Multiple Sclerosis and Neuromyelitis Optica spectrum) with the ethnicity profile of all patients attending our Neuro-ophthalmology clinic to assess whether a particular ethnic background is predominant within each diagnostic category of optic neuritis.

Aim

To investigate the presence of an ethnicity bias within patients presenting with optic neuritis in London and hence to establish if a patient's ethnic background is a factor to consider when assessing the need for the urgent administration of corticosteroid therapy.

Methods

All patients presenting to Moorfields Eye Hospital with acute isolated optic neuritis (who did not develop a collagen-vascular/granulomatous/infectious/autoimmune/neoplastic illness to account for the optic neuritis) between March 2009 until March 2011 were recruited during this time period.

Diagnostic categories

At the end of the period of monitoring, all patients were placed under one of the following categories:

1. Multiple Sclerosis (MS)
2. Neuromyelitis Optica (NMO)
3. Single Isolated Optic Neuritis (SION)
4. Recurrent Isolated Optic Neuritis (RION)
5. Corticosteroid dependent recurrent isolated optic neuritis (CRION)

The diagnosis of optic neuritis associated with MS (MSON) was made using recently revised McDonald criteria (Polman et al, 2011) All patients who did not demonstrate evidence of MS as the cause of the optic neuritis were tested for the Aquaporin 4 autoantibody. All patients with optic neuritis in the setting of antibody positivity, regardless of whether the remainder of the Wingerchuk criteria for the diagnosis of NMO were fulfilled, were diagnosed with 'NMO spectrum optic neuritis' (labelled AQP4+) as suggested by Wingerchuk in 2007. If an Aquaporin 4 antibody-negative patient experienced two or more attacks of optic neuritis affecting one or both eyes, without evidence of an underlying demyelinating or other disorder during the period of assessment, where corticosteroid therapy was required for each episode to resolve and where withdrawal of the corticosteroid therapy prompted a relapse, resulting in the patient being maintained on long term immunosuppression, then the patient was given a label of 'CRION' pattern optic neuritis. This pattern of optic neuritis has been previously described with differing acronyms (kupersmith et al, 1989; Kidd et al, 2003). In the context of this study, the label of 'CRION' pattern optic neuritis is being used not as a diagnosis, but as a category to describe syndromically an atypical, Aquaporin 4 antibody-negative, corticosteroid-dependent pattern of optic neuritis displayed by some patients in whom the diagnosis is unknown. These patients may have a form of granulomatous disease (such as a highly localised form of

Neurosarcoidosis) or an unrecognised autoimmune disease which has evaded detection during standard and specialised clinical testing. Patients who suffered from a single or from recurrent episodes of isolated optic neuritis in the absence of any other sign of underlying disease on clinical, radiological and serological testing were categorised as 'SION' or 'RION' cases respectively.

Patients

All patients presenting to our Neuro-ophthalmology clinic consecutively between March 2009 and March 2011 with acute optic neuritis in whom a non-demyelinating cause such as a granulomatous, collagen-vascular, autoimmune, infectious or paraneoplastic disorder was ruled out as per standard and specialised clinical testing, including imaging and serology were included.

Inclusion criteria:

- acute optic neuritis : unilateral or bilateral
- Acute presentation between March 2009 and March 2011
- Age above 18 at presentation
- Ability to communicate in English (with or without interpreter) in order to sign consent form.

Exclusion criteria:

- Granulomatous/collagen-vascular/autoimmune/infectious/paraneoplastic disorder
- No Magnetic Resonance Imaging of brain/spine

- Abnormal clinical/serological/radiological testing suggestive of pathology other than MS/NMO or isolated optic neuritis.
- Concurrent neurological or ophthalmological disease

Ethnic background classification

Patients were classified into ethnic groups based on self-reporting. Patients were offered four categories and asked to choose the category which best described their ethnic background. The categories offered to each patient were ‘white Caucasian’, ‘African or African-Caribbean’, ‘Asian’ or ‘other’. Patients from the Middle-East, South Asia and east Asia, were classified under the category of ‘Asian’. If a patient was unsure of his or her ethnic background and his or her parents were not of identical descent, the patient was classified under 'other'.

Aquaporin 4

All patients who did not demonstrate evidence of MS as the cause of the optic neuritis were tested for the Aquaporin 4 autoantibody. Serum analysis for the Aquaporin 4 autoantibody was carried out at the Wetherall Institute of Molecular Medicine, University of Oxford by a method using the fluorescence immunoprecipitation assay (FIPA) technique described elsewhere (Waters et al, 2008). Samples analysed between 2007 and 2009 were tested at the Mayo Clinic College of Medicine, Rochester, Minnesota using the immunofluorescence technique described by Lennon et al in 2004.

Statistics

For the most part, the sample size in this study was small. It may be argued that the trends observed were more valid than the results of statistical testing. Fisher's exact test and the Mann-Whitney U test were both used for statistical analysis. Where raw numbers were analysed, the Mann-Whitney test was used and where percentage ratios were compared, the Fisher's exact test was used. It may be argued that The Fisher's exact test would have been preferable throughout this study.

Results.

The total number of patients presenting over the time period studied and meeting the criteria detailed above are shown in table 1.

	Total in time period:	
	All presentations	1st presentation within time period
MS	58	37
NMO spectrum	23	2
SION	27	27
CRION	14	1
RION	26	12
Total	148	79

Table 1

1. All new cases

All new cases presenting with a diagnostic or syndromic classification of MS, NMO spectrum, SION, CRION, RION had an ethnicity profile that was similar to the

ethnicity profile of London as shown below in tables 2 and 3 ("London: Resident population estimates by ethnic group". Office for National Statistics Neighbourhood Statistics.retrieved 2004): (The ethnic backgrounds are abbreviated as follows: c=white Caucasian, ac = African or African Caribbean, as = Asian, other = Other).

	new presentations	% ethnic background
c	52	65
as	13	16
ac	14	17
other	0	0
Total	79	100

Table 2

	number in London	%
c	5214261	69
as	1133535	15
ac	831259	11
other	377845	5
Total	7556900	100

Table 3

The Mann Whitney U test was used to confirm the results were not statistically different (P=0.886).

2. All patients presenting for the first time vs. MS group

The ethnicity profile of the MS group is shown in table 4.

	MS	% ethnic background
c	38	65
as	9	15
ac	10	17
other	1	1
Total	58	100

Table 4

The Mann Whitney U test was used to confirm the results were not statistically different (P=0.886).

3. NMO spectrum group vs. MS group.

The ethnicity profile of all patients presenting with NMO spectrum disease is shown in table 5.

	NMO	% ethnic background
c	3	13
as	6	27
ac	12	55
other	1	5
Total	22	100

Table 5

A comparison of the percentage of patients of African or African Caribbean extraction between the NMO spectrum group and the MS group using the Fisher Exact test reveals a statistically significant difference (P=0.002). Similarly, comparison of the percentage of patients of white Caucasian descent between the NMO spectrum group and the MS group using the Fisher Exact test reveals a statistically significant difference (P<0.001).

There appeared to be a trend in migration after 18 years from different latitude amongst patients of African and African Caribbean as well as Asian backgrounds within the NMO spectrum group when compared to the MS group (tables 6 and 7). All African and African Caribbean patients within the MS group (n=10) were either born in the UK (n=9) or emigrated to the UK from the Caribbean by the age of 2 years (n=1). In contrast, of the 12 patients of African and African Caribbean background within the NMO spectrum group, half (n=6) were born in Africa (specifically in Ghana or Nigeria) and most of these patients (n=5) had emigrated to the UK at or after the age of 21. Amongst Asian patients within the MS group (n=9) 5 patients were born abroad and three of these patients had emigrated to the UK by the age of 15. Two patients had emigrated after the age of 29. Of all Asian patients with NMO spectrum disease (n=6), almost all (n=5) were born in warmer latitudes and had emigrated to the UK at or after the age of 19.

NMO	% born in UK or emigrated before 15	% emigrated after 15
ac	58.33	41.67
as	16.67	83.33

Table 6

MS	% born in UK or emigrated before 15	% emigrated after 15
ac	100.00	0.00
as	77.78	22.22

Table 7

There appears to be a statistically significant difference (according to Fisher's exact test) in the percentage of patients from an African and African Caribbean background who emigrated to the UK after the age of 15 years between the MS and NMO spectrum groups with a higher proportion ($P<0.001$) within the NMO spectrum group.

There also appears to be a statistically significant difference in the percentage of patients from an Asian background that emigrated to the UK after the age of 15 years between the MS and NMO spectrum groups with a higher proportion ($P<0.001$) within the NMO spectrum group:

A comparison of the level of visual disability (table 8) experienced by patients of African or African Caribbean extraction during the acute episode of optic neuritis in NMO spectrum disorder and MS reveals a visual acuity of 6/60 or worse in seven patients out of eight (88%) with MS (in whom the visual acuity during the episode was recorded) and a visual acuity of 6/60 or worse in nine patients out of twelve

(66%) with NMO spectrum disease. Only 1 patient out of 9 (in whom a follow-up visual acuity was recorded) did not recover to a visual acuity beyond 6/60 in the MS group (11%). Six out of 9 patients recovered to a visual acuity of 6/9 or better (66%), and one recovered to an acuity of 6/18.

The visual acuity during the episode of acute optic neuritis was recorded in 27 white Caucasian patients with MS. Thirteen of these patients (48%) experienced a visual acuity of 6/60 or worse during the acute episode. Four out of 25 patients (in whom a follow up visual acuity was recorded) did not recover to a visual acuity beyond 6/60 in the MS group (16%). Eighteen patients recovered to a visual acuity of 6/9 or better (72%). The visual acuity during the acute episode was only recorded in 2 out of 4 white Caucasian patients with NMO and in both of these cases the visual acuity was 6/60 or worse.

African/African-Caribbean	MS	NMO spectrum
% VA at onset 6/60 or worse	88	66
%VA follow-up 6/60 or worse	11	30
%VA at follow-up 6/9 or better	66	27
white Caucasian	MS	NMO spectrum
% VA at onset 6/60 or worse	48	100
%VA follow-up 6/60 or worse	16	0
%VA at follow-up 6/9 or better	72	0

Table 8

The sample size (the visual acuity during the acute episode was only recorded in 2 out of 4 white Caucasian patients with NMO) limits statistical analysis. There appears to be a trend for patients of African or African Caribbean background to manifest a more severe episode of optic neuritis in the context of MS than their white Caucasian counterparts. Six out of 8 patients from an African or African Caribbean background were administered a corticosteroid agent during the acute episode of optic neuritis caused by MS. It is not known if the visual outcome was influenced by this treatment.

4. MS group and RION group

The ethnicity profile of the RION group is demonstrated in table 9.

	RION	% ethnic background
c	22	85
as	2	8
ac	0	0
other	2	8
Total	26	100

Table 9

Although the sample size is small, a trend for a greater proportion of white Caucasian patients to be affected is apparent. This is confirmed with Fisher's exact test when comparing patients with MS and RION pattern optic neuritis where there appears to be a significant difference in the proportion of patients of African or African Caribbean extraction between the two groups (P=0.028).

5. SION group vs. MS group

The ethnicity profile of the SION group is shown in table 10.

	SION	% ethnic background
c	15	56
as	5	19
ac	7	26
other	0	0
Total	27	100

Table 10

There is no statistically significant difference between the ethnicity distribution within the SION group and that within the MS group (P=0.49) using the Mann-Whitney Rank Sum Test.

6. CRION group vs. MS group

The ethnicity profile of the CRION group is shown in table 11.

	CRION	% ethnic background
c	6	46
as	3	23
ac	4	30
other	0	0
Total	13	100

Table 11

There is no statistically significant difference (using Fisher's exact test) between the percentage of white Caucasian patients within the CRION group and that within the MS group (P=0.22), neither is there a statistically significant difference between the percentage of African or African Caribbean patients in the CRION and NMO groups (P=0.29).

7. Relative risk of atypical corticosteroid-requiring optic neuritis

Of all previously healthy patients presenting for the first time with acute isolated optic neuritis during the time period of observation, 2 out of 14 patients of African or African Caribbean heritage developed NMO spectrum disease (table 12). One out of 52 patients of white Caucasian background developed a corticosteroid dependent optic neuropathy (CRION). In all of these cases, urgent corticosteroid therapy would have been warranted.

	steroid-requiring cases	**not steroid-requiring cases**
ac	2	12
c	1	51

Table 12

Based on these numbers, the relative risk of an African or African Caribbean patient requiring corticosteroid therapy at presentation is 7.4 (C.I.= 0.2-24.7)

Conclusions

1. The ethnicity profile of all patients presenting for the first time with acute isolated optic neuritis was not statistically different from that of all patients presenting with MS related optic neuritis during the period of observation.

2. The ethnicity profile of all patients presenting for the first time with acute isolated optic neuritis over the period of observation was not statistically different from that of the population of London at this time.
3. There is a significantly higher proportion of patients from an African or African Caribbean background and a lower proportion of patients from a white Caucasian background within the NMO spectrum group, compared to the MS group.
4. The NMO spectrum group contains a higher proportion of patients from ethnic minority groups who emigrated to the UK at an age greater than 15, compared to the MS group.
5. A greater proportion of African or African Caribbean patients with MS related optic neuritis experience greater loss of vision (≥6/60 Snellen acuity) than white Caucasian patients. However, the visual outcome in the two ethnic groups is similar.
6. The RION group contains a higher proportion of white Caucasian patients and a lower proportion of African or African Caribbean patients than the MS group.
7. African or African-Caribbean patients with MS show a trend for more severe visual loss (88%) during an episode of acute optic neuritis than their white Caucasian counterparts (44%). However, there is a trend for the visual outcome in both groups to be similar.

Discussion

This report is the first of its kind from northern Europe comparing the ethnicity distribution of NMO spectrum (AQP4+ group) optic neuritis with MS related optic neuritis. We have shown that patients with acute isolated optic neuritis from African or African Caribbean backgrounds are over 7 times more likely than patients of white Caucasian backgrounds to have an 'atypical' pattern of optic neuritis where corticosteroid therapy may be required. This study also demonstrates that patients with an optic neuritis in the context of NMO spectrum disease are more likely to be from an African or African Caribbean background. The possibility that specific groups within a population may have a genetic basis for an increased or reduced susceptibility to NMO was recently reported by Deschamps et al (2011).

The question of age at migration and its influence on MS has been explored (Compston & Confavreux, 2006). A cutoff age of 15 years at migration has been shown to correlate with the risk of development of MS (McLeod et al, 2011). The difference in age at migration between NMO spectrum disease and MS has not been previously reported. It is not possible to conclude at this stage if the observation of a greater incidence of NMO spectrum disease amongst the African Caribbean population in the UK and (and to a lesser extent among the Asian population) and the increased frequency of emigration after the age of 15 to the UK within these patients are somehow related. These observations need to be explored further. It is of interest that all African or African Caribbean patients with NMO spectrum disease who were born abroad originated from one of two countries in Africa.

NMO spectrum disease is known to cause more severe relapses than MS (Wingerchuk et al, 2007). However, this study has shown that this observation does not hold true amongst patients from African or African Caribbean backgrounds. A greater proportion of patients from this background manifest a visual acuity of 6/60 or worse during optic neuritis caused by MS (88%), than during that caused by NMO spectrum disease (66%), though this difference is statistically insignificant. A small sample size of NMO spectrum cases limited the statistical analysis.

This study challenges the findings of Cabrera-Gómez *et al.* who did not find any ethnicity differences in the prevalence of NMO (2009). The difference in the ethnic backgrounds of patients with AQP4+ optic neuritis and patients with 'CRION' pattern optic neuritis make it much less likely that 'CRION' patients will be diagnosed with NMO spectrum disease in the future. This finding is compatible with the recent demonstration from this group that AQP4 antibody positivity is rare in the 'CRION' phenotype (Petzold et al, 2010)

This study highlights the importance of following African or African Caribbean patients more closely with a lower threshold for the initiation of therapy. This is of particular relevance in England, where census records from 1981, 1991 and 2001 have demonstrated a 30% followed by a 40% decadal rise in the African or African Caribbean population of England, mirroring a 40% followed by a 49% decadal increase in the ethnic minority population of England (Reese & Butt, 2004). Although the ONTT has been the most comprehensive trial on optic neuritis to date, only 15% of patients within the trial were of non-white Caucasian ancestry and the trial did not separate its patients according to ethnicity, before assessing their clinical profile,

response to treatment and prognosis. It has previously been shown that a patient's ethnic background can influence corticosteroid dependence in Multiple Sclerosis and that a patient's ethnic background can also relate to the clinical profile and prognosis of optic neuritis (Kira 2011; Phillips et al, 1998). The ONTT was also carried out in an era preceding the discovery of the Aquaporin 4 autoantibody.

At present, most eye clinics in the United Kingdom follow the Optic Neuritis Treatment trial (ONTT) protocol for acute isolated optic neuritis. The ONTT concluded that offering both no treatment and a three day course of intravenous corticosteroid therapy followed by an eleven day oral taper resulted in the same prognosis (Beck & Cleary, 1993) In accordance with this, corticosteroid treatment is often not offered for isolated acute optic neuritis of unknown aetiology in most parts of the UK (Ghosh et al, 2002) Although the efficacy of a particular treatment regimen for NMO has not yet been proven in a randomized control trial, the early initiation of intravenous steroid therapy is thought to be beneficial and following the ONTT protocol can result in a delay in treating NMO patients. The MS treatment regimens are inappropriate for other disorders which may present with optic neuritis, such as NMO and in many situations, the decision regarding treatment of a new presentation of optic neuritis has to be made some time before the results of serological tests and neuro-imaging are available (Shimizu et al, 2010). The results of this study show that ethnic background is an important factor to be taken into consideration when making this judgment.

Although the numbers of patients used in this report are relatively small for an epidemiologic study, this report shows that a large, population-based

epidemiological study on the relative incidence and prevalence of NMO spectrum optic neuritis in various ethnic groups within the population would be advised, in order to adequately treat a multi-ethnic urban population in Britain.

THE USE OF MAGNETIC RESONANCE IMAGING TO DISTINGUISH MS FROM NMO RELATED OPTIC NEURITIS

Introduction

The previous chapters of this thesis have discussed the roles of pupillometry, serological testing for GFAP and patients' ethnic backgrounds in being able to distinguish Neuromyelitis Optica (NMO) related optic neuritis from Multiple Sclerosis (MS) related optic neuritis.

Twenty percent of patients with MS in western Europe present with optic neuritis as their first relapse (Mcdonald & Compston, 2006). NMO has been recently found to be more common amongst the Caucasian population of northern Europe than previously believed (Asgari et al, 2011). Patients with NMO may experience a long temporal delay after acute isolated optic neuritis before another relapse occurs (Wingerchuk et al, 2007). In the past this episode was labelled a clinically isolated syndrome (CIS). Today, seropositivity for AQP4-Ab allows the episode to be identified as forming part of a Neuromyelitis optica spectrum disorder.

MR imaging has a role in the diagnosis of NMO. The presence of transverse myelitis spanning three or more vertebral segments of the spinal cord and involving the central grey matter has been shown to have high specificity for NMO. This has resulted in Longitudinally Extensive Spinal Cord Lesions (LESCL) forming part of the diagnostic criteria for NMO (Wingerchuk et al, 2006).

However, patients with NMO may experience a long temporal delay after acute isolated optic neuritis before a relapse in the form of transverse myelitis occurs (Wingerchuk et al, 2007). In such cases an episode of optic neuritis caused by NMO may be indistinguishable from optic neuritis caused by MS. The only abnormality detected on MR imaging may be the presence of inflammation within the visual pathways. A comparison of brain MR imaging may be of limited use as a recent study indicates there may be up to a 50% similarity in brain MR imaging between patients who are seropositive for AQP4-Ab and those with typical MS (Matsushita et al, 2010).

The Optic Neuritis Treatment Trial (ONTT) shifted the focus of MRI in ON away from the visual pathways and towards the brain when its usefulness in predicting the progression to MS based on brain lesions was demonstrated (Optic Neuritis Study Group, 1997). To date, studies on the appearance of the visual pathways in acute optic neuritis using standard MRI have focussed on the lesion site, lesion length and lesion volume (Miller et al, 1988; Hickman et al, 2004).

Since the discovery of AQP4-Ab, no comparison has been made of the appearance on imaging of the visual pathway during an episode of acute optic neuritis in the context of MS and NMOSD. It is unknown whether lesions in the visual pathway are more extensive in NMOSD than in MS, in parallel to LESCL found on spinal imaging. In this pilot study, we compared the MRI appearance of the anterior visual pathway in acute optic neuritis in NMOSD with MS. We investigate differences in the site of inflammation. Instead of assessing the absolute extent of the inflammatory lesion, we use a novel 2-dimensional approach to quantify the extent of the lesion.

Methods

Study Design

This was a retrospective pilot study.

Patients

The MRI results of twenty-seven patients were studied. Fifteen patients had confirmed MS and twelve patients had confirmed NMOSD.

Inclusion Criteria

All patients presented over a three-year period with acute isolated optic neuritis and were scanned using a 1.5 Tesla or 3.0 Tesla scanner during the acute phase.

Exclusion criteria

Patients with co-existing neurological or systemic illness causing other visual pathway or brain lesions were excluded.

Diagnostic Criteria

A diagnostic label of NMO spectrum disorder (NMOSD) was given to patients who fulfilled Wingerchuk's criteria for (NMOSD) (Wingerchuk et al, 2007). MS was diagnosed according to the recently revised McDonald criteria (Polman et al, 2012). All MS patients were seronegative for AQP4- Ab.

Aquaporin 4 testing

All patients were tested for serum AQP4-Ab. Testing was carried out at the Wetherall

Institute of Molecular Medicine, University of Oxford by a method using the fluorescence immunoprecipitation assay (FIPA) technique described elsewhere (Waters et al, 2008).

Imaging

All patients underwent MR imaging of the anterior visual pathway, using standardized clinical protocols, performed on 1.5-Tesla and 3.0-Tesla scanners with 5mm thick slices. In all cases, the MRIs included Coronal T2-weighted fat suppressed and T1-weighted imaging of the anterior visual pathway in addition to structural imaging of the brain and/or spine. Post-Gadolinium imaging was not undertaken in the majority of cases. MRI images were assessed independently by two neuroradiologists (ID & MR), who were blinded to the patients' history and diagnosis. A consensus decision was reached in case of disagreement.

Protocol for the presence of inflammation

Post-gadolinium imaging has been reported as being the gold standard for the detection of inflammation in the visual pathway (Youl et al, 1991). However, gadolinium was not used in the majority of cases, in accordance with local hospital protocol. The increase in the thickness or 'cross-sectional area' (CSA) of the affected part of the visual pathway occurring during acute optic neuritis and its relation to time has been described and was used as an absolute marker for inflammation (Hickman et al, 2004). The presence of a T2-weighted signal hyperintensity alone was not used as an absolute marker as its persistence following the resolution of acute optic neuritis has been reported (Youl et al, 1996). The presence of T2-weighted signal hyperintensity was used to support the presence of inflammation.

Image analysis

T2-weighted fat-suppressed and corresponding T1-weighted sequences were used to

assess CSA. The visual pathway was divided into ten segments: intraorbital, intracanalicular and intracranial segments of the left and right optic nerves, the left and right halves of the optic chiasm and the left and right optic tracts (Figure 1). Each segment was objectively assessed for CSA and T2-weighted signal intensity. The presence of both features was taken to indicate acute inflammation.

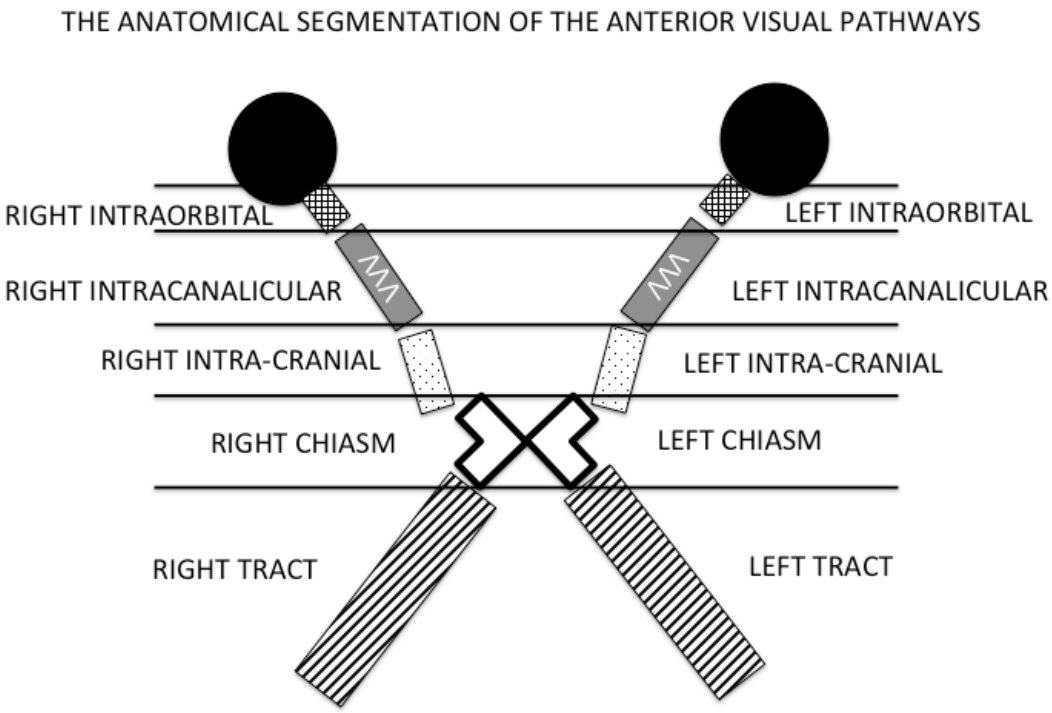

Figure 1

The segmental representation of the visual pathway. The visual pathway was divided into ten segments: intraorbital, intracanalicular and intracranial segments of the left and right optic nerves, the left and right halves of the optic chiasm and the left and right optic tracts.

Lesion Extent

A 2-dimensional approach was taken to quantify the extent of the lesion. Instead of

absolute linear measurements, the number of anatomical segments affected by an increase in CSA at any point on the segment was noted in each case. A score of +1 was given for each affected segment, such that a patient with the involvement of all segments would be given a score of 10 (Figure 2). A segment was not required to be thickened along its entire length, in order for it to be given a score of +1. The entire anatomical extent of the lesion did not need to be continuous along the extent of positive scoring.

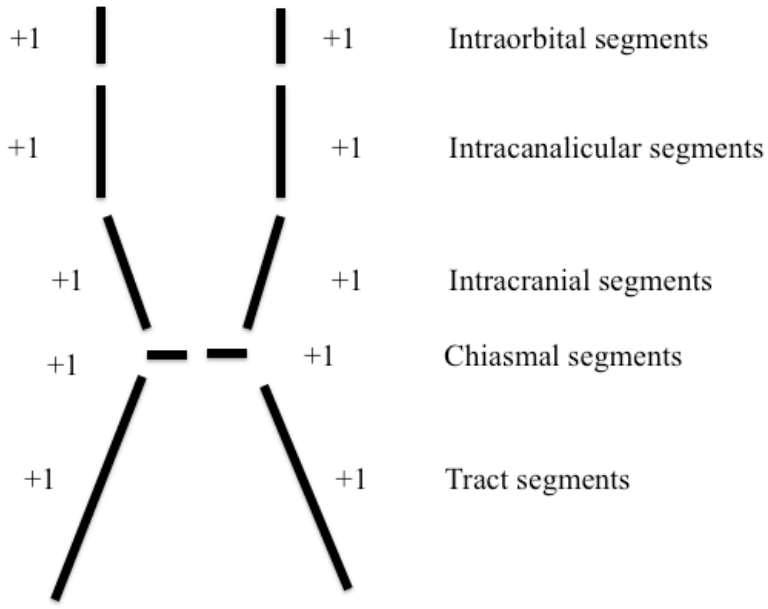

Figure 2

A linear, schematic representation of the visual pathway (cf. Figure 1). A score of +1 is given for each segment on each side affected by an increase in CSA at any point on the segment, such that a patient with the involvement of all segments would be given a score of 10.

Lesion Site

The pattern of segments affected was noted.

Statistical Analysis

Lesion Extent scores were compared between MS and NMOSD groups using the Mann-Whitney rank sum test. A P-value of 5% was used to define statistical significance. The relative risk (RR) of higher scoring was calculated for the two groups.

The involvement of each segment was compared between the two groups using the two-tailed Fisher's exact test. A P-value of 5% was used to define statistical significance. The relative risk (RR) of the involvement of each segment for the two groups was calculated.

Results

Twelve patients were diagnosed with NMO spectrum disease and fifteen patients with MS. The demographic features of all patients recruited in the study are shown in Table 1. Figures 3 and 4 are schematic illustrations or 'maps' demonstrating the pattern of visual pathway involvement in the NMOSD and MS groups respectively. A linear representation of the affected segments of the visual pathway (with regard to Figure 2) is illustrated in the case of each patient.

	Female to male ratio	Mean age (s.d.)	% of white Caucasian heritage
Patients with NMOSD optic neuritis (n=12)	10:2	39 (12)	17%
Patients with MS related optic neuritis (n=15)	11:4	34 (8)	69%

Table 1

Demographics for the patients included in this study. Age is expressed as mean with standard deviation in parentheses.

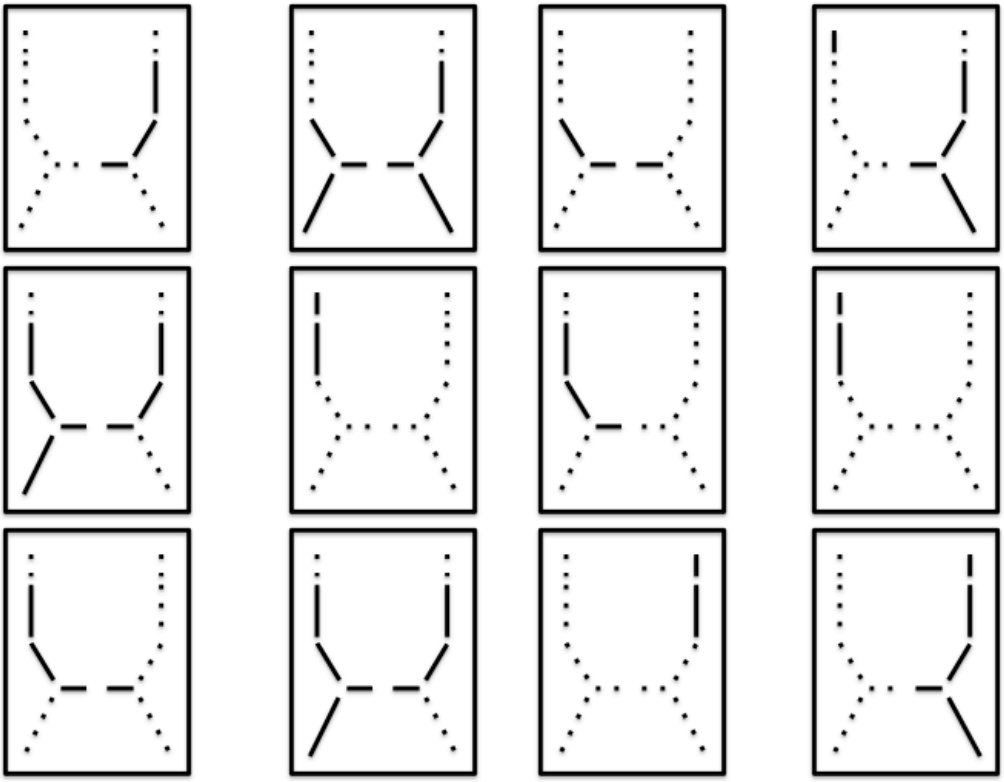

Figure 3

Schematic illustration demonstrating the pattern of visual pathway involvement in the NMOSD group (n=12). A schematic representation of the visual pathway in each patient is shown within each of the twelve boxes (cf. Figure 2). Each affected segment is represented by a solid line and unaffected segments are represented by a dotted line. A 2-dimensional 'map' of visual pathway disease is hence created for each patient.

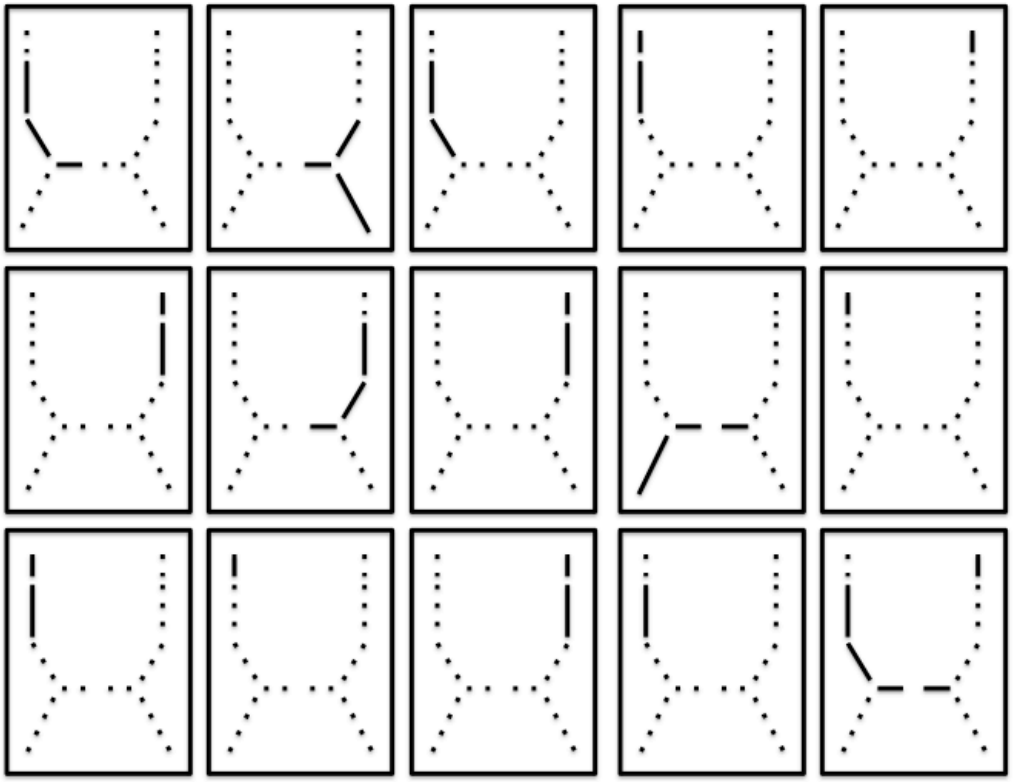

Figure 4

Schematic illustration demonstrating the pattern of visual pathway involvement in the MS group (n=15). A schematic representation of the visual pathway in each patient is shown within each of the fifteen boxes (cf. Figure 2). Each affected segment is represented by a solid line and unaffected segments are represented by a dotted line. A 2-dimensional 'map' of visual pathway disease is hence created for each patient.

Lesion Extent

Figure 5 shows the lesion extent scores in patients with NMOSD and MS associated optic neuritis. Patients with MS demonstrated a mean score of 2.2 (range 1-5) compared with a mean score of 4.0 (range 2-7) in NMOSD. The difference between the means was statistically significant (P=0.007). The relative risk of having a lesion extent score ≥4 in NMOSD versus MS was 7.5 (95% CI: 0.33 - 17.3). A score of greater than 6 was seen only in patients with NMOSD.

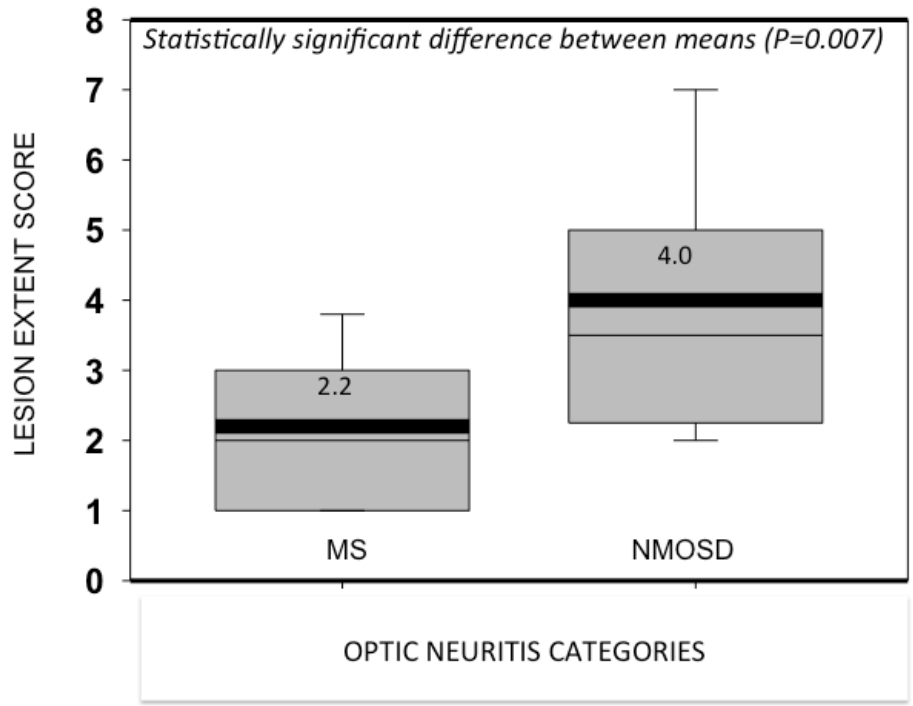

Figure 5

Box and whisker plot showing distribution of lesion 'extent scores' in patients with NMOSD and MS associated optic neuritis. Mean values and 5% and 95% percentiles are shown. The difference between the means was statistically significant (P=0.007).

Lesion site

Table 2 shows the frequency of involvement of each site and the relative risk for NMOSD over MS at each site across the patients within each group. A trend for anterior involvement was seen in MS patients. The relative risk (RR) of segment involvement within the NMOSD group increased with a more posterior location (RR for Optic Tract involvement = 3.13 versus RR for Intracanalicular involvement = 1.25). The number of NMOSD patients with chiasmal involvement was significantly greater than the number of MS patients (P=0.021). Both MS (n=2) and NMOSD (n=5) patients displayed bilateral chiasmal involvement.

Location	NMO (*n* =12)	MS (*n* = 15)	*P*-value	Relative risk*
Intra-orbital	5 (42%)	**10 (67%)**	0.258	0.63 (0.38 – 1.74)
Intra-canalicular	**10 (83%)**	**10 (67%)**	0.408	1.25 (0.71 – 1.71)
Intra-cranial	**9 (75%)**	5 (33%)	0.054	**2.25 (0.65 – 3.12)**
Chiasm	**9 (75%)**	4 (27%)	**0.021***	2.81 (0.64 – 3.86))
Optic Tract	5 (42%)	2 (13%)	0.185	3.13 (0.38 – 7.02)

The P-value is shown as calculated by the 2-tailed Fisher's exact test.
* 95% Confidence Intervals in parentheses.

Table 2

The frequency of involvement of each site and the relative risk for NMOSD over MS at each affected site across the patients within each group. The P-value is shown as calculated by the 2-tailed Fisher's exact test. * 95% CI in parentheses. A trend for anterior involvement is seen in MS patients.

Conclusions

This study demonstrates that a novel 2-dimensional 1.5 Tesla based scoring system based on 'Lesion extent' may aid in the diagnosis of NMOSD optic neuritis. A lesion extent score of ≥4 may be of use as a rapid and simple test of exclusion in the setting of an acute neuro-ophthalmology clinic. This is a simple diagnostic sign, which can be demonstrated with standard, unenhanced, clinical MR imaging protocols at 1.5 Tesla.

The results of this pilot study suggest that visual pathway inflammation in optic neuritis secondary to NMOSD may mirror the LESCL described in NMO spinal lesions (Matsushita et al, 2010).

While lesion distribution was not demonstrably different between NMO and MS patients, trends for a predilection for more posterior segments in NMOSD patients and for more anterior segments in MS, was found. The latter is consistent with a previous report (Zou et al, 1999). A significantly higher number of NMOSD patients suffered from chiasmal involvement, which was also present in MS patients.

This study is limited by small numbers and by the lack of use of intravenous gadolinium. The use of the presence of increased cross sectional area as the criterion for assessing the presence of inflammation along the visual pathway may have excluded patients with prior nerve atrophy, in whom no swelling is manifest. As this was a pilot study, there was no stated protocol to dictate the time interval between the onset of the episode and the time of scanning, or of the examination of visual parameters at the time of scanning. This resulted in a variable time interval between the onset of visual loss and the scan taking place, and also prevented patients from being matched for vision. Although a trend for more extensive visual pathway inflammation was observed in NMOSD, the degree of inflammation may have been underestimated as corticosteroid therapy was sometimes initiated on patients with

NMOSD before the scan could take place. This trend may therefore appear statistically significant if all patients are scanned prior to the initiation of therapy.

In conclusion, the results of this study suggest that a scoring system based on the findings of MR imaging of the visual pathways, may help to identify the underlying aetiology in acute isolated optic neuritis. Cases of optic neuritis where there is involvement of the chiasm and/or where the lesion is widespread resulting in a 'score' of >4, may warrant a deviation from the standard ONTT based hospital protocols for the management of optic neuritis (where no treatment is offered) (Ghosh et al, 2002).

At present, patients presenting with acute optic neuritis are not usually scanned according to management protocols. Following positive results from recent Phase 3 trials studying the effect of early therapeutic intervention following a clinically isolated syndrome (CIS), there is increased incentive for MR scanning of all patients presenting with acute optic neuritis as a CIS (Comi et al, 2001; Kappos et al, 2006). The results of this study demonstrate that the images acquired can also be used to assess the risk of NMO spectrum disease without the need for intravenous gadolinium administration and at no extra cost.

CONCLUSIONS

This thesis has explored differences in the manifestation of optic neuritis caused by Multiple Sclerosis (MS) and that caused by Neuromyelitis Optica (NMO) and has evaluated four ways in which the two aetiologies may be identified from one another. None of the methods discussed in this thesis offers a high specificity and sensitivity if used alone, however each method may be used in a specific context, or in synchrony with others to improve the diagnosis of optic neuritis, and afford appropriate management.

Pupillometry

This work has demonstrated that pupillographic measurements in the normal population are highly variable and hence subtle changes in an individual patient's pupillographic results may prove difficult to recognise. As a result, pupillometric recordings in the context of optic neuritis are limited in its use as a tool to measure for the presence of a mild attack of optic neuritis.

Instead, pupillometry holds great potential in its use as a diagnostic tool in patients who have never recovered from a past episode of optic neuritis with severe visual loss, where the cause of the episode, in the absence of other pathology detected on standard and specialised clinical testing, remains unknown. Although serological testing for the Aquaporin 4 antibody may be used to diagnose NMO, recent reports suggest serum levels of the antibody may be reduced during remission (Jarius et al, 2008), rendering them more difficult to detect. In Aquaporin 4 antibody seronegative cases of isolated optic neuritis with poor recovery, the absence of pupillovisual

dissociation in the setup described, would indicate that a higher suspicion for NMO spectrum disease as an underlying disease aetiology is maintained. Similarly, in cases where patients are seen for the first time with a past history of a poor outcome from an episode of optic neuritis, where no cause is found, the presence of pupillovisual dissociation using the setup described would increase the chance of MS as an underlying aetiology.

The pupillometry findings described in this thesis suggest that the pathogenesis of NMO spectrum optic neuritis may differ from the pathogenesis of MS related optic neuritis in that selective ganglion cells are targeted in the latter. This finding, if reciprocated in a larger study, has the potential to add to the debate of whether or not NMO and MS are distinct disease conditions.

The use of the serum marker Glial Fibrillary Acidic Protein (GFAP), ethnicity and MR imaging in the diagnosis of optic neuritis

In chapter 7 of this thesis it was found that serum GFAP may be used to assess the likelihood of Neuromyelitis Optica when a previously healthy patient presents with an episode of acute isolated optic neuritis. Although a high level of serum GFAP may be encountered in MS related optic neuritis, raised serum levels point to an increased likelihood of Neuromyelitis Optica. In chapter 8, it was demonstrated that there is an increased risk of NMO in optic neuritis patients of African and African Caribbean heritage, in whom a high rate of migration to the UK after the age of 15 was found. In chapter 9, the role of MR imaging in the likelihood of NMO determination was investigated, with a unique scoring system which was found to have a high sensitivity

for NMO. A greater lesion extent was found in NMO spectrum patients, compared to MS patients.

All three of these methods may be simultaneously utilised in an acute case of isolated optic neuritis to assess the likelihood of NMO spectrum disease, before the results of Aquaporin 4 serological testing becomes available (usually with a time delay of several weeks in the U.K.). If a patient is found to have a serum GFAP level of above 4.36pg/mL and is of African or African Caribbean ancestry, then the risk of NMO spectrum disease is likely to be elevated. If MR imaging then reveals a lesion extent score of 4 or more and there is intracranial, chiasmal and tract involvement, then the likelihood of the presence of NMO spectrum disease becomes particularly high and a treatment regimen for NMO spectrum disease involving the urgent administration of corticosteroid therapy would be strongly advised. All three approaches, when used in synchrony would assist clinicians in recognising patients with NMO spectrum optic neuritis in whom the appropriate treatment can be initiated without delay.

The question of steroid therapy

Until the middle of the last century, corticosteroid treatment was not routinely given for demyelinating optic neuritis (No authors listed,British Medical Journal. 1968). Corticosteroid therapy started to be explored and advocated over the next two decades, however the observation that cases often spontaneously improve always posed a conundrum (Hepler 1976; Spoor & Rockwell, 1988). The largest study to date, questioning the role of corticosteroid therapy in isolated acute optic neuritis was carried out as part of the Optic Neuritis Treatment trial (ONTT) where conservative

management was deemed as effective as intravenous corticosteroid therapy (Beck 1992).

The discovery of the Aquaporin 4 autoantibody allowed single isolated episodes of acute optic neuritis to be recognised as forming part of the NMO spectrum of disorders. This has created a challenge for the clinician as the management of acute optic neuritis secondary to NMO has not been investigated in a randomized controlled study. Most centres in Western Europe follow the ONT protocol as a default protocol for the management of acute isolated optic neuritis. However, a recent study has suggested the early and urgent administration of corticosteroid therapy prevents nerve fibre loss in NMO related optic neuritis (Nakamura et al, 2010). Discerning the aetiology of the optic neuritis episode and distinguishing between NMO from MS is of great importance to the clinician, and the methods explored in this thesis may be of use in this regard.

Anecdotally, the patient cohort studied in this thesis has shown some interesting and yet conflicting results with regard to disease aetiology and corticosteroid therapy. Patient MS2 discussed in chapter 5 of this thesis has typical relapsing and remitting MS. He first presented with an episode of acute isolated optic neuritis in 1996 where he experienced a loss of vision to ‘perception of light only’ and did not receive any treatment. His visual acuity never recovered. His MRI scan demonstrated the presence of demyelination and he had no further relapses until a subsequent episode of acute isolated optic neuritis involving his other eye in January 2010. Again no steroid therapy was offered, and his recovery was complete. This pattern was also seen in the cases of a patient (SG) who had experienced an episode of optic neuritis in 2007

where his vision was reduced to 'perception of light only' which recovered completely without treatment. His second episode three years later left him with a visual acuity of 6/36. It is not known in both cases if corticosteroid therapy is likely to have offered a better visual outcome. In the case of NMO patients, one patient (SH) first experienced an episode of optic neuritis in March 2009 which resolved completely without treatment. In the case of the second episode (occurring after the identification of Aquaporin 4 antibody seropositivity) intravenous steroid therapy was immediately initiated and it will not be known if this episode was likely to have resolved spontaneously. In the case of patients NMO3 and NMO4 from chapter 5 of this thesis, both did not recover from their first episode of acute optic neuritis, after which seropositivity to the Aquaporin 4 antibody was identified. Steroid therapy was administered for their subsequent episodes and they recovered completely. There are other patients with NMO spectrum disorder in whom plasma exchange is required in order for an episode of optic neuritis to resume.

Patients from both MS and NMO groups are at risk of poor recovery from an isolated episode of optic neuritis and at present it is not possible to predict whether a particular episode of optic neuritis will result in poor recovery. It is also not known if the administration of corticosteroid therapy in the cases of poor recovery improves the prognosis. Patients such as MS2 in this study demonstrate that a poor outcome from one episode does not necessarily result in a poor outcome from a subsequent episode.

Future work

In addition to continuing to find phenotypic indicators of the presence of NMO during an attack of acute isolated optic neuritis and continuing each of the studies outlined in

this thesis with a larger number of subjects, further study should perhaps also include the investigation of methods which enable the identification of episodes of optic neuritis resulting in a poor prognosis in all disease types (regardless of whether the cause is NMO spectrum disorder or MS). The ultimate aim of distinguishing between NMO and MS as the aetiology behind an episode of optic neuritis is to determine the appropriate management in each case. At present, the best acute management protocol for NMO spectrum optic neuritis is unknown and a large randomised control study rivalling the ONTT is warranted to establish this. A study of this scope could also be used to identify the risk factors for a poor prognosis from acute isolated optic neuritis regardless of aetiology.

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APPENDIX

The experiments described in chapter 7 involving patient MS3 have been repeated since the submission of this thesis. Upon repetition, it was not possible to observe a preservation of the IPRGC response. It may therefore be possible that the IPRGC response was observable due to unrelated factors. It is not possible to rule out the role of light scatter affecting the healthy eye.