

**Ophthalmic Correlates of Myalgic Encephalomyelitis
(ME)/Chronic Fatigue Syndrome (CFS)**

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Abstract

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a chronic, debilitating disorder. With the exception of disabling fatigue, there are few definitive clinical features of the condition. As a consequence, patients often have difficulty gaining an appropriate diagnosis. As such, identifying distinct clinical features of ME/CFS is an important issue. One under researched area of ME/CFS-associated symptoms concerns problems related to vision. People with ME/CFS consistently report a range of symptoms related to the quality of their vision including pain in the eyes, hypersensitivity to light, difficulty focusing on images, slow eye movements, and difficulty tracking object movement. However, there has been little attempt to verify patients' self-reports using objective methods. The purpose of the experiments presented in this thesis was to determine the effects of ME/CFS on: (i) performance on a range of tests of visual sensitivity and (ii) the morphology of the retina. Compared to controls, the ME/CFS group exhibited reduced accommodation, larger pupil diameters, reduced colour discrimination and poorer contrast sensitivity towards lower spatial frequencies. Thinning in the photoreceptor layers of the retina (the Outer Segment & the Outer Nuclear layer) was also apparent. These findings support the claims of people with ME/CFS that they experience problems related to their vision and its function. They also represent a potential marker of ME/CFS that may aid in its diagnosis.

Declaration

I declare that all areas of research and the conducting of experiments were conducted by myself and this thesis is comprised of my own written work

Nadia S. Ahmed. September 2017

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First and foremost, I would like to extend my gratitude to all the participants who volunteered to take part in this study. I am aware that members of the ME/CFS group suffered fatigue for a number of days after the testing yet, in spite of this, still returned for a 2nd session. I am truly grateful for their participation.

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List of Abbreviations

AHP = Abnormal Head Posture

ANOVA = Analysis of Variance

ARMD = Age related macular degeneration

c/deg = cycles per degree

CCL11 = C-C Motif Chemokine Ligand 11

Cd =candelas

CDC = Center for Disease Control and Prevention

CF= Chronic Fatigue

CFS = Chronic Fatigue Syndrome

COST= Cone outer segment tips

CS =Contrast Sensitivity

CSF = Cerebro spinal fluid

CSF = Contrast Sensitivity Function

Cpd = Cycles per degree

CRT = Cathode Ray Tube

CXCL1 (GRO α) = chemokine (C-X-C motif) ligand-1

CXCL9 =Chemokine (C-X-C motif) ligand 9

CXCL10 (IP-10) = chemokine (C-X-C motif) ligand-10

D = Dioptre

dB = decibel

df = degrees of freedom

DSQ =DePaul Symptom Questionnaire

ETDRS = Early Treatment Diabetic Retinopathy Screening chart

EOM =Extraocular Muscles

EWN = Edinger Westphal Nuclei

FGF =Fibroblast Growth Factor

FM-H100 = Farnsworth Munsell Hue-100
GCC= Ganglion Cell Layer
G-CSF =Granulocyte-Colony stimulating Factor
GM-CSF =Granulocyte Macrophage -Colony stimulating Factor
H1N1 =Influenza A
ICC =International Consensus Criteria
ICVT = Ishihara Colour Vision Test
IFN- γ =Interferon Gamma
IL = Interleukin-
ILM= Internal Limiting Membrane
INL= Inner Nuclear Layer
I.O.O.N.T = Institute of Optometry Near Test Card
(IP-10) =Inducible Protein-10
IR =Inferior Rectus
ISL= Inner Segment Layer
LGN = Lateral Geniculate Nucleus
LE = Left Eye
LED = Light emitting diode
LIF =leukaemia inhibitory factor
LIO = Left Inferior Oblique
LIR =Left Inferior Rectus
LogCS =Log Contrast Sensitivity
LogMAR =Log Minimum Angle of Resolution
LLR =Left Lateral Rectus
LMR =Left Medial Rectus
LR =Lateral Rectus
LRL= Left Lateral Rectus
LSR =Left Superior Rectus
LSO = Left Superior Oblique

LUT = Look Up Tables

(MCP-1) (MCAF) = Monocyte chemoattractant protein-1 (monocyte chemotactic and activating factor)

M-cells = Magnocellular Cells

ME = Myalgic Encephalomyelitis

MG = Myasthenia Gravis

MIP-1 α = Macrophage inflammatory protein 1-alpha

MIP-1 β = Macrophage inflammatory protein 1-Beta

MR = Medial Rectus

n = number

NGF = Nerve growth factor

NICE = The National Institute for Health Care and Excellence

OCT = Optical Coherence Tomography

OMG = Ocular Myasthenia Gravis

ONH = Optic Nerve Head

ONL = Outer Nuclear Layer

OPL = Outer Plexiform Layer

OS = Outer Segment

p = probability

P-Cells = Parvocellular Cells

PDGF-BB = platelet-derived growth factor BB

PEM = Post Exertional Malaise

PIC = Pro-inflammatory cytokine

PLR = Pupillary Light Reflex

PMN = Polymorphonuclear

RPE = Retinal Pigment Epithelium

ppRNFL = Peripapillary Retinal Nerve Fibre Layer

PRN = Pupil Responses at Near

PVVAT = Precision Vision Visual Acuity Testing chart

R-G = Red- Green
RAF =Royal Air Force
RAPD =Relative Afferent Pupillary Defect
RANTES =Regulated on Activation, Normal T Cell Expressed and Secreted
RE = Right Eye
RGC = Retinal Ganglion Cell
RIO = Right Inferior Oblique
RIR =Right Inferior Rectus
RLR = Right Lateral Rectus
RMR =Right Medial Rectus
RNFL= Retinal Nerve Fibre Layer
RSO = Right Superior Oblique
RSR =Right Superior Rectus
SCF =Stem cell factor
SD = Standard deviation
SEM = Standard Error of Mean
SFL = Swinging Flashlight test
SR = Superior Rectus
SWIFT = Swinging Flashlight Test
t = Gosset's Student Distribution
TBUT = Tear Break Up Time
TES = Total Error Score
TGF- α =transforming growth factor alpha
TGF- β 1 =Transforming Growth Factor- β 1
TNF α =Tumor necrosis factor alpha
VEGF = vascular endothelial growth factor
VZV =Variella Zoster Virus
XOP = Exophoria

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1.0 Literature Review

1.1 General Introduction

Myalgic Encephalomyelitis (ME) or Chronic Fatigue Syndrome (CFS) is a persistent, recurrent, debilitating disorder of unknown aetiology affecting sufferers of all ages and ethnicities (Nacul et al., 2011). It affects over 250,000 people in the UK (Lorusso, 2009) and its prevalence is reported to be between 2.07- 2.64% in the UK, 0.2- 6.4% in Nigeria, 1.4- 4.8% in Iceland and between 1.23- 2.04% in Brazil (Johnston, Brenu, Staines, & Marshall-Gradisnik, 2013). It represents a substantial disease burden on sufferers, their families, the health service and the economy. Marked by debilitating fatigue, it is not well understood and its diagnosis is controversial (Horton-Salway, 2007; Underhill, 2015).

There are a variety of case definitions for ME/CFS (Christley, Duffy & Martin, 2012; Morris & Maes, 2013a) but the most widely used term for research is Chronic Fatigue Syndrome (CFS) initially conceived by Holmes in 1988 (Maes, Twisk, & Johnson, 2012) with the actual criteria eventually published in 1994 by Fukuda for the Center for Disease Control and Prevention (CDC) (Fukuda et al., 1994; Unger et al., 2016). This came to be known as Fukuda's criteria. The CDC definition is the most commonly used despite lack of clinical substantiation for reproducibility (Brurberg, Fønhus, Larun, Flottorp, & Malterud, 2014). In fact, all ME/CFS definitions are yet to be clinically verified for reproducibility (Brurberg et al., 2014). The lack of clinical verification could be directly responsible for the plethora of definitions as could the failure of any definition to be consensually and formally

operationalised (Haney et al., 2015; Jason, Evans, So, Scott & Brown, 2015a; Jason, Sunnquist, Brown & Reed, 2015b) and both factors contribute to criterion variance. It is a diagnosis of exclusion because there is currently no specific diagnostic test for ME/CFS (Griffith & Zarrouf, 2008; Nacul et al., 2011; Rowe et al., 2017). Diagnosing ME/CFS is therefore dependent upon categorising the symptoms reported by patients and differentiating them from symptoms of other fatigue-related illnesses (Fox & Sorenson, 2017; Griffith & Zarrouf, 2008; Haney et al., 2015). This however is problematic due to the number of symptoms overlapping with other illnesses (Aaron, Burke & Buchwald, 2000; Carruthers et al., 2011) and the reliance upon patients to report their symptoms accurately (Jason, Sunnquist, Brown, Furst et al., 2015c). The result of this is that ME/CFS is commonly misdiagnosed, often as depression (Griffith & Zarrouf, 2008), both conditions of which Maes, (2008) believes there to be a shared comorbidity.

In 2009, the National Institute of Clinical Excellence (NICE) published guidelines for ME/CFS adults and children. NICE recognised the delay in diagnosis/mis-diagnosis and endorsed tailor made management and/or treatment for each individual (Bayliss et al., 2014). NICE also made recommendations for healthcare professionals whereby consideration of potential diagnosis of ME/CFS should be given to cases if (i) they presented with unexplained fatigue that is associated with a specific point of onset or (ii) if the fatigue is recurrent or persistent and accompanied by a substantial reduction in activity level. NICE further recommended that post-exertional malaise (PEM) should persist after activity for a period of time at e.g. at least 24 hours and that there is a slow recovery. Further fatigue should be accompanied by at least one of the following symptoms: difficulty sleeping, non-inflammatory multi- sited

joint pain, headaches, unexplained lymph node pain, unexplained sore throat, cognitive dysfunction, flu-like symptoms, dizziness and nausea, mental exhaustion and palpitations without cardiac problems that become worse by physical exertion (NICE.org.uk, 2009).

ME/CFS has an unquestionable impact on lives and families. The road to diagnosis is typically a time-consuming, difficult and frustrating process. Unknown aetiology and numerous definitions appear to have a contributory effect on low diagnosis rates, slower referral processes and lack of confidence in Health Care Professionals and Clinicians (Bayliss et al., 2014; Bsted & Marshall, 2015; Bowen, Pheby, Charlett, & McNulty, 2005; Hannon et al., 2012). Even when referrals are made, the lack of a reliable set of symptoms and/or biomarkers means that excluding other diseases is costly and time consuming (Bansal, 2016; Leonard, Benton, Valentine, Johnson & Torres-Harding 2008). In the place of a biomarker, there is no agreed proposed battery of tests that has been put in place in priority of cost-effectiveness for instance. Even when a diagnosis is made, evidence suggests that referrals to specialist units are falling below NICE recommendations (McDermott et al., 2014).

1.2. ME/CFS Diagnosis

The first set of clinical features for Myalgic Encephalomyelitis (ME) were compiled after an outbreak in the staff of Royal Free Hospital London in 1955 (Simpson, Bennett, & Holland, 1997; Wojcik, Armstrong, & Kanaan, 2011). However, the term 'ME' was not created until 1988, whereupon it was used to describe the condition as multi-systemic, neurological and musculo-skeletal disorder initiated by a virus which is chronic in nature (Clark et al., 2016; Haney et al.,

2015; Hutchinson, Maltby, Badham & Jason, 2013; Jason, et al., 2015b; Maes et al., 2008; Maes, Twisk, Kubera & Ringel, 2012). Although historically, the outward symptoms of 'ME' have been documented, both its aetiology and disease process remain unknown (Knudsen et al., 2012; Rutherford, Manning & Newton, 2016; Underhill, 2015). This is problematic from a clinical perspective because the classification of ME/CFS's status of unknown aetiology (Underhill, 2015) means that diagnosis by exclusion is still relied upon which has led to confusion amongst the medical professions (Nacul et al., 2011; Van Hoof Clin, 2009). Furthermore, the fact that a medical professional can pick any definition also adds to the confusion. For instance, Fukuda's criteria requires debilitating and unexplained fatigue for 6 months which should be accompanied by at least 4 or 8 other symptoms such as PEM, impaired memory, concentration, muscle and joint pain, un-refreshing sleep many of which are flu-like (Johnston et al., 2013; Reeves et al., 2005). Fukuda's criteria however is itself still the subject of scrutiny as there is no empirical substantiation for the 8 symptoms (Brurberg et al., 2014; Reeves et al., 2005). There is a disproportionate emphasis on symptoms such as fatigue and disability, as opposed to underlying pathophysiology and functional impairments both of which can lead to reduced sensitivity and specificity in the diagnosing process (Morris & Maes, 2013a; Reeves et al., 2003). Indeed, many patients report feeling undermined that the definition of CFS is quite so symptom-specific such that debate and upset has been caused amongst sufferers diagnosed with CFS as opposed to ME (Jason, et al 2015b; Reeves et al., 2005). In light of the debate, a Consensus Panel comprising of professionals from 13 different countries compiled another definition in 2011. These professionals declared no affiliation to any group and no conflict of interest by confirming they were not recipients of sponsorship (Carruthers et al., 2011). This team were all co-authors of previous criteria but they achieved 100% consensus that PEM

should be the emphasis and that PEM alone is a hallmark for ME/CFS (Carruthers et al., 2011; Maes et al., 2012) hence the term fatigue was removed (Johnston et al., 2014). This came to be known as the International Consensus Criteria (ICC) (Carruthers et al., 2011). The progress made with the ICC was potentially encouraging within the research field however there was again a failure to enforce this and make it an obligatory single working definition (Brown, Jason, Evans, & Flores, 2013; Nacul et al., 2011). It also lacked external critique (Morris & Maes, 2013a).

The creation of yet another definition appears to have encouraged a pattern of retrospective research i.e. researchers often conduct studies comparing groups of patients diagnosed with ME/CFS in line with the Fukuda's criteria against groups of ME/CFS diagnosed in line with the ICC definition. The following examples show how sub-groups are almost being created to fit in line with the definitions. One such study selected a few symptoms and ranked symptoms according to their sensitivity and specificity and found a few core symptoms such as un-refreshed sleep, fatigue, PEM and neurocognitive symptoms as having both good sensitivity and specificity (Twisk & Arnoldus, 2012). The same study sub-classified concentration and neurocognitive symptoms into 3 distinctions i.e. ME, CFS and CF and they concluded that ME could be classified as sufferers experiencing PEM > 24 hours.

It remains the case that no international consensus on a precise working case definition has been achieved. It is not helped that some definitions require a presenting core symptom and others do not and that there is still no actual requirement of inclusion of any core symptom (Jason et al., 2015a). Each time research is

undertaken within the ME/CFS community questions are likely to be posed e.g. Have the patients been diagnosed against the Fukuda's Criteria or against the ICC definition or any other definition? Do the patients from the same disease or a variant of the same disease? Should the participants be tested against the same variables or similar variables? Could the participants actually be suffering from an entirely separate disease altogether rendering them ineligible for participation? In the absence of a known cause, the goal of a single working case definition would mean at the very least that all researchers and clinicians produce work in line with that common definition. Until this is done clinicians and researchers will inevitably compare studies of patients who are diagnosed against different definitions and until this is achieved, it is inevitable that research efforts will be undermined.

In attempt to consolidate research efforts, a group at DePaul University (USA) developed a standard questionnaire named The DePaul Symptom Questionnaire (DSQ). The DSQ is a self-administered questionnaire that assesses core 54 ME/CFS symptoms. It is self-administered to avoid practitioner presence bias and assesses standardised symptomatology as defined by ME, ME/CFS and/ or Fukuda's Criteria (Jason, So, Brown, Sunnquist & Evans et al., 2015d). A standard assessment of symptomatology also reduces criterion variance which is the largest source of unreliability when there is no agreed set of biomarkers (Jason et al., 2015e). It has good to excellent test-retest reliability of 54 symptoms (Jason et al., 2015e). It is increasingly used as a diagnostic tool and is advocated by a number of ME/CFS charities and research groups (<http://www.mereseearch.org.uk/our-research/ongoing-studies/depaul-symptom-questionnaire>, 2017).

1.3. Aetiology

Although the aetiology of ME/CFS remains unresolved, the prevailing view is that it is likely to be a disorder of the autoimmune system. Whilst it is noteworthy that the World Health Organisation classifies ME/CFS as one of neurological origin, (Morris, Berk, Galecki, & Maes, 2014), it is also of note that the NICE guidelines briefly mentioned above appear to list numerous clinical features that are flu-like suggesting an infectious aetiology with immune/auto-immune tendency. Indeed, ME/CFS sufferers often recount stories of emotional stress or significant turmoil followed by an acute flu-like episode followed by relapsing fatigue (Bansal, Bradley, Bishop, Kiani-Alikhan, & Ford, 2012; Chia, Chia, Voeller, Lee, & Chang, 2010).

A number of recent studies support the hypothesis that ME/CFS is a disease of the immune and autoimmune system. For example, a recent immunological study took place in Norway and looked into influenza A (H1N1) vaccination during a flu pandemic. It was postulated that the 2009 H1N1 swine flu vaccination increased the prevalence of ME/CFS (Magnus et al., 2015). Norwegian patients were assessed via access to the unique personal identification number which allowed for mass surveillance. It was found that the flu virus H1N1 increased the risk of ME/CFS by more than 2 fold whereas the vaccination didn't (Magnus et al., 2015). Another notable study took place in Norway in 2015; the anti-cancer drug Rituximab was investigated after reports of ME/CFS symptom improvement upon administration in cancer patients who suffered synchronously with ME/CFS. This apparent decline in symptoms provided clues into the aetiology of ME/CFS. Rituximab's primary

role as an anti-cancer drug is to eliminate B-lymphocyte cells that produce antibodies thereby removing B-lymphocyte cells from circulation (B-lymphocyte cell depletion) (Fluge et al., 2011). B-lineage cells are responsible for the production of plasmablasts which secrete antibodies and other antibody type agents (Dörner, Jacobi, & Lipsky, 2009). The goal of B-lymphocyte depletion is to destroy B-lineage cells to reduce exaggerated autoimmunity whilst concurrently retaining some desirable aspects of autoimmunity (Clark & Ledbetter, 2005). In this double blind placebo controlled phase II study, 30 ME/CFS participants were injected with the antibody 500mg/m² Rituximab or saline and recalled at regular intervals until 36 months. Improvements were noted across the range of ME/CFS associated symptomatology and lasting improvements were noted in 67% (10 of 15) of the Rituximab group. The Norwegian group confirmed a rapid depletion in B-cells which allowed the team to confirm that ME/CFS was an auto-immune in origin (Fluge et al., 2011).

Another related body of research examined the potential role of cytokines in the cause and maintenance of ME/CFS. This work also supports the notion that ME/CFS is likely to be a disorder of the immune system, in keeping with the notion that inflammation plays a key role. Inflammation is one of the first immune system responses to infection. Inflammation is caused by eicosanoids and cytokines, which are released by injured or infected cells. Cytokines are responsible for the production of small proteins that have an immunomodulatory role in the body and chemokines are a variation of smaller cytokines that are found in the body which can recruit other immune cells to the site of infection (Bickel, 1993; Furie & Randolph, 1995). Pro-inflammatory cytokines (PIC) are associated with fever, inflammation and tissue destruction. Although recent studies have

shown cytokine levels are affected in ME/CFS, the precise nature of their role is unresolved. Maes et al., (2012) measured the plasma interleukin-1 (IL-1), Tumor necrosis factor alpha (TNF α), Polymorphonuclear elastase (PMN-elastase), serum neopterin and the lysozyme levels in 107 patients with ME/CFS and 20 normal controls. Serum Interleukin-1 (IL-1), TNF α , neopterin and lysozyme were significantly higher in patients with ME/CFS than in controls. A similar study (Landi, Broadhurst, Vernon, Tyrrell, & Houghton, 2016) examined 34 cytokines, chemokines and growth factors in 100 chronic ME/CFS sufferers and in 79 age and gender matched controls. 3 biomarkers, namely, Interleukin-16 (IL-16), Interleukin-17 (IL-17) and vascular endothelial growth factor (VEGF) were all significantly reduced in ME/CFS and furthermore a difference was found in levels between sufferers of less than 3 years and > 3 years. This is a potentially significant finding as reductions in these biomarkers were not present in chronic infectious and autoimmune liver disease with the presence of fatigue. The authors concluded that there is an association between ME/CFS and certain PIC's. Similarly, Peterson et al., (2015) took samples from the cerebrospinal fluid (CSF) of 18 CFS and 5 healthy controls. Of the 27 cytokines tested, namely, Interleukin- (IL-) IL-1ra, IL-2, IL-4, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17 and 1 β , basic Fibroblast Growth Factor (FGF), eotaxin, Granulocyte-Colony stimulating Factor (G-CSF), Granulocyte Macrophage -Colony stimulating Factor (GM-CSF), Interferon Gamma (IFN- γ), Inducible Protein-10 (IP-10), Monocyte chemoattractant protein-1 (monocyte chemotactic and activating factor) (MCP-1) (MCAF), Macrophage inflammatory protein 1-alpha (MIP-1 α), Macrophage inflammatory protein 1-Beta (MIP-1 β), platelet-derived growth factor BB (PDGF-BB), (Regulated on Activation, Normal T Cell Expressed and Secreted (RANTES), TNF- α and VEGF. Only IL-10 was significantly reduced. A difference has also been found between the

cerebrospinal fluid of 32 ME/CFS who at the advent of their condition experienced symptoms consistent with infection alongside 27 “atypical” ME/CFS patients who didn’t experience any flu-like prodromal attacks. In other words, 32 “classical” ME/CFS individuals were compared against and 27 “atypical” ME/CFS individuals. The “classical” ME/CFS group had showed changes consistent with disturbances in IL-1 signalling. The “atypical” group in comparison had lower levels of the inflammatory mediators Interleukin-17a and Chemokine (C-X-C motif) ligand 9 (CXCL9). Lower cytokine levels in “atypical” ME/CFS suggested that ME/CFS is associated with immunological dysregulation and that there may be a pathophysiological explanation between the subsets (Hornig et al., 2017).

The existence of subsets is often theorised due to the variance in symptomatology. One longitudinal study looked at how immune cells adapted over a 6 month period with the aim of discovering why there was such a variation experienced in sufferers of ME/CFS and if indeed subsets existed. The groups tested were 22 healthy controls, 22 ME/CFS with moderate symptoms 19 ME/CFS patients that experienced severe symptoms. Patients were divided in accordance to severity. All controls and patients had their and serum immunoglobins and cytokines analysed. Severe ME/CFS sufferers showed significantly reduced levels of cytokine and Interleukin-1 Beta (IL-1 β) yet Interleukin-7 (IL-7) and Interleukin-8 (IL-8) and Interferon type II (IFN- γ in humans) were found to be significantly higher. Interleukin- 6 (IL-6) was significantly reduced in moderate suffers. The results offered an insight into why some ME/CFS sufferers were mildly affected and some heavily burdened (Hardcastle et al., 2015). Indeed, a recent study (Montoya et al., 2017) has suggested that cytokine signatures vary with disease

severity in ME/CFS – they examined inflammatory mediators in the serum of 192 ME/CFS and 392 controls. They significantly found reduced Resistin levels and higher levels of Transforming Growth Factor- β 1 (TGF- β 1). Furthermore 17 cytokines: eosinophil chemotactic protein and C-C Motif Chemokine Ligand -11 (CCL11) / (Eotaxin-1), chemokine (C-X-C motif) ligand-1 (CXCL1 (GRO α)), chemokine (C-X-C motif) ligand-10 (CXCL10 (IP-10)), Interferon Gamma (IFN- γ), Interleukin- (IL-), IL-4, IL-5, IL-7, IL-12p70, IL-13, IL-17F, leptin, granulocyte colony stimulating factor (G-CSF), Granulocyte-macrophage colony-stimulating factor (GM-CSF), leukaemia inhibitory factor (LIF), Nerve growth factor (NGF), Stem cell factor (SCF) and transforming growth factor alpha (TGF- α) appeared to correlate with the severity of ME/CFS. Interestingly, 13 of the 17 cytokines are pro- inflammatory. Whilst this body of research is producing promising results, at present, the precise relationship between PICs and ME/CS is unclear. As such, further studies are needed.

Some of the most compelling behavioural evidence that ME/CFS is likely to reflect immune dysfunction and associated inflammation comes from studies that have compared symptom characteristics in ME/CFS against other established inflammatory autoimmune disorders. For example, ME/CFS shares a number of characteristics with Multiple Sclerosis, an established progressive neurological, inflammatory autoimmune condition with episodes of relapse (Ewing & Bernard, 1998). Shared symptoms include physical exertion causing varying levels of fatigue and postural hypotension. Visual disturbances are also reported in both conditions (Morris & Maes, 2013b).

Visual disturbances in ME/CFS represent an under-researched group of symptoms. People with ME/CFS consistently report a range of symptoms related to the quality of their vision, the most common of which include pain in the eyes, difficulty focusing on images, slow eye movements, difficulty tracking object movement and difficulty directing attention (Badham & Hutchinson, 2013; Hutchinson, Maltby, Badham, & Jason, 2013; Leslie, 1997; Loew, Marsh, & Watson, 2014; Mastropasqua et al., 2000; Potaznick & Kozol, 1992; Vedelago, 1997).

As outlined above, visual symptoms are also common in a range of neurological disorders such as multiple sclerosis (Robin et al., 2008). These include, for example, colour vision defects (Harrison, Becker, & Stell, 1987) visual disturbances and double vision (Costello, 2016). The similarities lend weight to the hypothesis that ME/CFS has an autoimmune and neurological component. Visual problems involve altered sensory and/or cognitive processing and reflect ME/CFS-related changes to relevant neural pathways in the brain. They also contribute to, and exacerbate, other ME/CFS-related symptoms such as chronic and debilitating headaches. Moreover, they are not present in other conditions, such as depression, with which people with ME/CFS are commonly misdiagnosed. As such, visual symptoms may represent a set of easily-identifiable ME/CFS-related symptoms that could prove useful in diagnosis.

1.4. Visual Problems in ME/CFS

One of the first studies to document visual symptoms in ME/CFS was that of Potaznick and Kozol's (1994). They conducted a self-administered questionnaire-based study to determine the nature of visual symptoms in ME/CFS. Respondents included 199 ME/CFS patients and 198 controls. Questions were grouped into: (1) Entopic symptoms (black spots, white spots, coloured spots, flashing lights, halos), (2) anterior segment symptoms (burning, itchy, gritty, teary, dry, scratchy feelings in the eye), (3) functional symptoms (distance & near vision symptoms, foggy vision, problems related to changes from distance to near and changes from near to distance, diplopia & shadowed vision), and (4) neuro-sensory symptoms (headaches related near vision work, headaches with distance vision, photophobia, poor depth perception and difficulty looking at moving objects). People with ME/CFS reported significantly more difficulties on almost all the aspects of vision included in the questionnaire. They also reported reduced driving frequency or complete cessation of driving as a result of their illness.

Other studies have also found evidence of visual problems in ME/CFS. In a study of 25 ME/CFS patients, Caffery et al., (1994) found that the most frequently reported visual symptoms were those related to accommodation (18 patients) and tear layer instability (19 patients). As such, they suggested that healthcare practitioners should investigate both accommodation, tear surface instability and ocular surface disease in this patient group. Single case studies have also raised the possibility of ME/CFS-related problems with colour sensitivity, accommodative dysfunction, and reduced visual fields (e.g. Leslie, 1997).

Vedelago, (1997) reported visual symptoms and the Optometric Outcome in 141 CFS patients referred for Optometric assessment as a result of visual complaints. Visual symptom reporting was high and there was evidence of optometric dysfunction across a range of aspects of vision and vision-related functions (see Tables 1.1 and 1.2 for synopses of key findings).

Symptom	Prevalence (% patients)
Poor concentration	78.7
Near blur	65.9
Visuo-Spatial balance/coordination	62.2
Poor memory	59.6
Headache	55.8
Photophobia	53.9
Distance blur	44.7
Near/Far/Near blur	39.7
Dizziness. Difficulty with moving objects	38.3
Sore Eyes	35.5
Stopped work because of vision	31.9
Neck pain	26.9
Spots/floaters/halos/ flashes	20.6
Stopped driving because of vision	12.6
Diplopia	9.9

Table 1.1. Prevalence of visual symptoms reported by 141 CFS patients (from Vedelago, 1997)

Visual Test	Abnormal/Normal	Finding
Ocular Motility Test	Abnormal	Slow saccades Marked jerkiness Conscious effort into changing visual fixation Discomfort Nausea
Binocular Function	Abnormal	Greater Exophoria (XOP with slow recovery Compensation of XOP with typical AHP
Remote Near Point of Convergence	Abnormal	Slow recovery Painful
Reach/ Grasp Tests	Abnormal	Reduced ability to reach and grasp after release One eye diverges Nausea/discomfort /dizziness reported
Near/Far/Near fixations	Abnormal	Poor convergence and one eye usually diverges, slow and uncomfortable.
Constricted peripheral fields	Abnormal	Constricted visual fields
Blink rate	Abnormal	Slow blink rate Incomplete blinking and straining appearance
Pupils	Abnormal	Small
Light sensitivity	Abnormal	Sensitivity to light
Tear Film		Low but normally associated with reduced mucus and lipid production
Anterior Segment Assessment	Abnormal	Chronic allergic conjunctivitis – low grade
Mid-line	Abnormal	Visual mid-line shift

Table 1.2. Outcome of visual examination (from Vedelago, 1997)

Mastropasqua et al., (2000) carried out a case control study involving 37 patients with CFS. They reported that the most commonly reported symptoms in CFS were blurring when refocussing from near to distance and distance to near, floaters, double vision, photophobia, dry eye symptoms e.g. gritty sore eyes, non-specific eye pain and ocular burning. In addition to the collation of symptoms, Orthoptic and Optometric measurements were also taken. These included corrected and uncorrected visual acuities, examination of the movement of the extra ocular muscles, auto-refraction results after instillation with 1% Cyclopentolate, fusional-vergence, near point of convergence and pupil diameters. Slit-lamp biomicroscopy of the anterior segment, Schirmer Test, and Goldmann tonometry were also undertaken and deficits were apparent on the following visual measures: Schirmer Tear Break Up Time (TBUT) and Hyperaemia. In conclusion, Mastropasqua et al., (2000) found a significant relationship between ME/CFS and foggy and shadowed vision, blurred vision at near, headaches and photophobia. They attributed photophobia and foggy vision to tear layer dysfunction.

Visual discomfort related to reading is commonly reported by those with ME/CFS (Leslie, 1997; Mastropasqua et al., 2000; Potaznick & Kozol, 1992; Vedelago, 1997). Reading-related problems have a profound impact on vision-related quality of life (Hazel et al., 2000). In ME/CFS, main reading-related problems include difficulty tracking lines of print and severe headaches, after just short periods of reading. Many of the symptoms reported during reading by ME/CFS patients are commensurate with those associated with visual stress/pattern glare. In a questionnaire study of 20 ME patients, Loew et al., (2013) found significantly more reports of symptoms commonly associated with visual stress in patients compared to

controls, such as slow reading, eyestrain and fatigue, print distortions, preference for reading on coloured paper, lack of depth perception and clumsiness, photophobia, dislike of reading under fluorescent lights. Furthermore, in a study of 20 ME/CFS patients and matched controls, it was found there was greater susceptibility to pattern-related visual stress in ME/CFS. Specifically, ME/CFS experienced significantly more pattern glare for mid (2.3 cycles per degree) and mid-high (between 9.4 and 2.3 cycles per degree) spatial frequencies (Wilson, Paterson & Hutchinson, 2015).

Based on patients' report that their eye movements are sluggish and that they have difficulties tracking moving objects, Badham and Hutchinson et al., (2013) objectively examined eye movements in those suffering from ME/CFS. Twenty patients were compared to matched controls for their ability to generate saccades and smooth pursuit eye movements. Patients and controls exhibited similar error rates and saccade latencies (response times). Patients could accurately fixate the target (prosaccades) but fixations were impaired when required to focus accurately in a specific position opposite the target (antisaccades). Patients also showed deficits in smooth pursuit eye movements. The authors proposed that the effects of ME/CFS can be overcome briefly for completion of saccades, but that continuous pursuit activity (accurately tracking a moving object) even for a short time period highlights dysfunctional eye movement behaviour in ME/CFS patients (Badham & Hutchinson, 2013).

Some self-report studies also show that visual problems associated with ME/CFS have a negative effect on everyday life. In a study of 59 ME/CFS patients, Hutchinson et al. (2013) looked at responses to the four vision-related items of The DePaul Symptom (DSQ)

questionnaire: eye pain, hypersensitivity to light, inability to focus vision and/or attention, and poor depth perception. Responses on each item revealed that vision-related problems were frequently experienced, the most frequent being sensitivity to bright lights (92%) followed by being unable to focus vision and/or attention (88%) and eye pain (86%). Loss of depth perception (61%) was least frequent. The more frequent the symptom, the greater the apparent severity/bother experienced by patients.

Most recently, in a study of 41 CFS patients and controls, Godts et al., (2016) have found evidence for CFS-related reductions in accommodative range, convergence and fusional amplitudes.

1.5. The Present Study

In summary, ME/CFS is a debilitating disorder. Its aetiology is poorly understood and likely to be multi-factorial. Its physiological impact can be extensive affecting the immune system, the central nervous system, and the cardiovascular system. Those suffering from ME/CFS experience a range of symptoms including disabling fatigue, flu-like symptoms, cognitive impairment, dizziness and headaches. Diagnosis of ME/CFS is controversial. With the exception of disabling fatigue and PEM, there are few definitive clinical features of the condition and its core symptoms, such as those outlined above overlap with those often prevalent in other conditions, particularly depression. As a result, ME/CFS is often a diagnosis of exclusion, being made as a last resort and possibly after a patient has experienced a series of inappropriate treatments of misdiagnosed disorders. It is imperative therefore that research

focuses on identifying significant clinical features of ME/CFS with a view to elucidating its underlying pathology and delineating it from other illnesses. Doing so will help researchers and healthcare professionals gain important insights into the condition, aid diagnosis and inform evidence-based therapeutic interventions.

Vision related problems in ME/CFS have a marked impact on quality of life. They also represent distinct, quantifiable, clinical features that could significantly improve diagnosis, provide insights into underlying pathology and represent a candidate for treatment, thereby improving the everyday lives of patients. However, attempts to verify patients' self-reports using objective, experimental and clinical methods have been relatively limited. The work presented in this thesis is therefore concerned with the outcomes of Ophthalmological and Optometric investigation in ME/CFS patients. The experiments outlined in Chapter 3 determined patients' performance on a range of tests of visual sensitivity. Chapter 4 reports the effects of ME/CFS on the morphology of the retina.

2.0 General Methodology

Details relating to participants and their recruitment are given. Specific experimental details are provided in the relevant experimental chapters.

2.1 Participants

The research took the form of a case control study of mixed experimental design with two between-subjects comparison groups, a patient group consisting of participants with ME/CFS (n=20) and a

matched (age & gender) control group (n=20) (Table 2.1).

All patients had an ME/CFS diagnosis, confirmed with the self-administered DePaul Symptom Questionnaire (Jason et al., 2010) (Appendix 1) that assesses ME/CFS related standardised symptomatology as defined by the CFS Case Definition (Fukuda et al., 1994), the Canadian ME/CFS Case Definition (Carruthers et al., 2003) and by the International Consensus Criteria (Carruthers, 2011). The DePaul Symptom Questionnaire was created for researchers and clinicians to reduced criterion variance, which is the largest source of unreliability when there is no agreed set of biomarkers (Jason et al., 2015d). It has good to excellent test-retest reliability of 54 symptoms which achieved Pearson's or kappa correlation coefficients of 0.7 or higher (Jason et al., 2015d).

Only participants who fulfilled these criteria were included. Furthermore, 1 patient with ME/CFS had a history of ocular disease (diagnosed Pituitary tumour) and excluded only from Colour Vision assessments and another patient suffered a road traffic accident during childhood and was excluded only from the Ocular Motility test. 1 control was diagnosed with a unilateral existing macula condition and consequently 1 eye was excluded from OCT and Amlser Grid findings. Ethical approval for the study was granted by the University of Leicester. All experimental methods adhered to the tenets of the Declaration of Helsinki. A Participant Information Sheet was supplied in advance of testing (Appendix 2. Informed consent was obtained before the study commenced (Appendix 3).

	Mean age (\pm SD)	Age range	Gender split (f:m)
Patients	49.80 (10.596)	33-68	17:3
Controls	49.300 (10.317)	34-70	17:3

Table 2.1. Mean (\pm 1 SD) age, age range and gender-split for patients and controls.

2.2. Recruitment

The majority of patients were recruited from a previous ME/CFS University of Leicester study, which commenced in 2014 and travelled from all over the UK (Table 2.2). The 2014 study recruited via ME/CFS local support groups such as ME Positive, Action for ME who assisted by making announcements through social media and via their own monthly newsletter (Wilson, 2016). All 19 of the original patients completed the DePaul Symptom Questionnaire as part of the 2014 study. However, it was a requirement that all 19 of the original ME/CFS participants repeated the DePaul Symptom Questionnaire prior to the present research study. The 20th ME/CFS sufferer contacted us after coming across the press releases from the University of Leicester.

Region	Patients	Controls
Leicestershire	6	17
Nottinghamshire	2	0
Northamptonshire	1	0
West Midlands	4	2
Cheltenham & Gloucestershire	1	0
Greater Manchester	1	0
Tyne & Wear	1	0
Greater London	2	0
Cambridgeshire	1	0
Lincolnshire	1	1

Table 2.2 Geographical locations of patients and controls

All controls were age and gender matched to patients. The controls were recruited via the 'Insider' which are sent to all members of staff at the University and a standard recruitment email was sent to all staff at the Leicester Royal Infirmary. Posters with a direct email address were also placed around the University of Leicester and Leicester Royal Infirmary buildings and were asked to respond if they were +/- 2 years of the patient group. ME/CFS patients were reimbursed with travel costs. Controls were paid £20 for participation regardless of the distance they travelled.

3. Tests of Basic Visual Function

3.1. Overview

The purpose of administering the tests outlined in this chapter was to determine basic sensory and sensory motor function in ME/CFS.

Monocular near and distance visual acuity was determined for each eye using the Institute of Optometry Near Test chart (Institute of Optometry, UK) for near vision and the Precision Vision Visual Acuity Testing chart (PVVAT, USA), for distance vision. Because the ME/CFS community often report distortions within their central field, an Amsler Grid was used to quantify these symptoms further. Difficulty discerning certain colours is also well documented and as such, red-green colour vision deficiencies were determined using the Ishihara Colour Vision Test and colour discrimination was determined using the Farnsworth-Munsell Hue-100 Test (FM-H100). Photosensitivity is also a commonly reported symptom within the ME/CFS Community so pupil function was also determined. The Ocular Motility test allowed further exploration of symptoms such as those associated with difficulty following objects.

Based on initial assessment, as outline above, the study was extended to include an objective measure of the Pupil Function because unusual pupil responses to a near target were found. A measure of Absolute Accommodation to investigate near focusing difficulties was also added. Absolute Accommodation measures

were achieved using an instrument comparable to an RAF rule and objective pupil measurements were taken using Pupillometry.

Finally, Contrast Sensitivity was determined because patients report difficulty night driving despite having perfectly good vision. It is well known that it is possible for drivers to have good visual acuities but also have poor sensitivity to stimulus contrast (Owsley & McGwin, 2010).

3.2. Visual Acuity

3.2.1. Introduction

ME/CFS patients commonly report symptoms like transient blurring at distance and near vision (Potaznick & Kozol, 1992; Leslie, 1997; Vedelago, 1997; Mastropasqua et al., 2000). Slow reading (Loew, Marsh & Watson, 2014), fatigue and eyestrain with prolonged reading and in particular discomfort reading under fluorescent lights has also been highlighted (Loew et al., 2014). Although near blur has been shown in a previous study of ME/CFS patients (Mastropasqua et al., 2000), other studies show no (overall) differences in basic distance visual acuity between patients and controls e.g. Badham & Hutchinson, (2013) and Wilson (2016) suggesting that other visual factors may be responsible for the symptoms outlined above.

Visual Acuities were measured monocularly primarily as a screening measure for patients and their age matched controls

and included in the visual test battery (hence each eye was tested individually) in that only participants with acuities within the normal range were admitted onto the study.

3.2.2. Methods

Visual Acuities at near were determined using the Institute of Optometry Near Test Card (I.O.O.N.T, Institute of Optometry, UK). The I.O.O.N.T is comprised of unrelated words of equal logarithmic progression size and of equal complexity but designed to appear random to participants (Evans & Wilkins, 2001). In contrast to traditional reading charts that require patients to read a series of prose, there are no contextual clues in the I.O.O.N.T and therefore the I.O.O.N.T is considered more accurate as there is no influence of intellectual ability or linguistic levels (Evans, & Wilkins, 2001). We considered the I.O.O.N.T to be the most suitable Near Chart for our participants as they were not screened for socio-economic status or intellectual ability. Patients were asked to bring in their most recent spectacles. Near Visual Acuities were taken with the most current reading spectacles or varifocals. If near work was habitually performed without distance spectacle correction, then this was also permitted. Participants read aloud the words on the card at a viewing distance of 40cm.



Figure 3.1 Institute of Optometry Near Test Card (i.O.O Sales Limited, UK).

Visual Acuity at distance was determined by taking LogMar acuities using the single line letters on a LogMar acuity (Early Treatment Diabetic Retinopathy Screening chart (ETDRS), (Precision Vision Visual Acuity Testing, PVMAT, USA), installed on high resolution Apple Macintosh under standard lighting conditions.



Figure 3.2. Example of an Early Treatment Diabetic Retinopathy Screening Distance Chart or ETDRS (Precision Vision, USA).

The ETDRS chart was chosen in favour of other distance charts to allow for comparability with the Near Acuity data in that where the principle behind the I.O.O.N.T relied upon logarithmic progression between paragraphs, so too was logarithmic progression the principle behind ETDRS acuities. The ETDRS contains letters of equal difficulty and there is a logarithmic difference between the lines (Lim, Frost, Powell, & Hewson, 2010). Participants were spectacle-corrected and read aloud the optotypes to their best ability at a distance of 4 metres. All patients brought in their distance spectacles.

3.2.3. Results

Mean near and distance Visual Acuities are shown in Table 3.1. A 2 (group: patients, controls) by 2 (eye: left, right) ANOVA confirmed that there were no significant differences in corrected Visual Acuity at near between patients and controls ($F(1,38)=1.113$; $p=.298$) or between the two eyes ($F(1,38)=.538$; $p=.468$), and no significant interaction between group and eye ($F(1,38)=3.177$; $p=.083$). A 2 (group: patients, controls) by 2 (eye: left, right) also confirmed that there were no significant differences in corrected Visual Acuity at distance between patients and controls ($F(1,38)=.281$; $p=.102$) or between the two eyes ($F(1,38)=.229$; $p=.635$), and there was no significant interaction between group and eye ($F(1,38)=.315$; $p=.578$).

	Patients		Controls	
	Right Eye	Left Eye	Right Eye	Left Eye
Near	0.715(0.032)	0.663(0.048)	0.715 (0.036)	0.775 (0.017)
Distance	0.0568(0.196)	-0.97(0.255)	-0.063(0.972)	-0.038 (0.183)

Table 3.1. Mean near and distance correct Visual Acuities in ME/CFS patients and controls. Standard Deviations are given in parentheses.

3.2.4. Discussion

Near and distance Visual Acuities for both groups were in the normal range and there were no significant differences between groups, in keeping with the findings of previous studies (Badham & Hutchinson, 2013; Wilson, 2016). This suggests other factors are a likely cause of the visual symptoms in this patient group.

3.3. Visual Distortions

3.3.1. Introduction

People with ME/CFS commonly report central visual distortions, examples of which include double vision (Vedelago, 1997) shadowed vision (Potaznick & Kozol, 1992) and reports that written text appears to move or vibrate (Loew, Marsh & Watson , 2014). For example, in a study of twenty ME/CFS patients, Loew et al., (2014) found that 40% of patients reported print distortions when viewing black text on a white background. Furthermore, a recent

study Wilson, et al., (2015) has also shown that ME/CFS patients experience significantly more distortions than controls when viewing horizontal black and white stripes on The Pattern Glare Test; for example found there were more pattern distortions reported at intermediate (2.3 c/deg) and mid-high spatial frequencies (9.4 c/deg) in a group of ME/CFS patients compared to controls, indicative of visual stress.

Central visual distortions (or metamorphopsia) and scotomas at near can be checked conveniently and accurately using an Amsler Grid and can be used to detect many maculae diseases (Frisen, 2009; Midená & Vujosevic, 2015). The Amsler Grid checks the integrity of the macula at the central 20 degrees of visual field (Crossland & Rubin, 2007; Nassar, Badawi, & Diab, 2015) and quick observation can give clues into potential pathology at the macular level (Midená & Vujosevic, 2016; Nassar et al., 2015).

The Amsler Grid is widely available, inexpensive and a non-invasive test and its ability to detect metamorphopsia has been found to approach a sensitivity between 88 - 93% (Chamorro, Cedrun & Portero, 2011; Nassar et al., 2015). While there are limitations with the Amsler Grid such (Schwartz & Loewenstein, 2015) as false negatives and poor compliance, the Amsler Grid is still an established qualitative self-measure and is often given to the susceptible patient by eyecare practitioners (Crossland & Rubin, 2007).

Metamorphopsia can be perceived as doubling and/or distortion of the lines on the Amsler grid (Nassar et al., 2015) and is well

documented in disorders such as Age-Related Macular Degeneration (ARMD) (Schawrtz & Loewenstein, 2015), Diabetic Maculopathy (Midena & Vujosevic, 2015), long-term use of anti-malarial medications containing Hydroxychloroquine (Midena & Vujosevic, 2015; Peponis, Kyttaris, Chalkiadakis Bonovas, & Sitaras, 2010), and Stargardt's Macular Dystrophy (Chamorro, Cedrún, & Portero, 2011). An example is given in Figure 3.3.

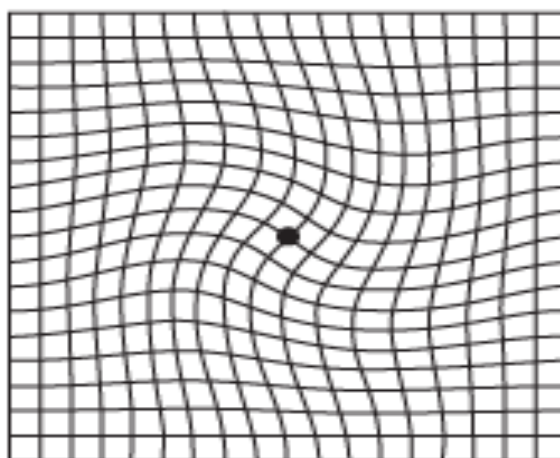


Figure 3.3. Example of an Amsler Grid with central metamorphopsia

(Bausch & Lomb @2017 Bausch & Lomb Incorporated)

3.3.2. Methods

There are altogether 7 designs of Amsler Grid (Crossland & Rubin, 2007), all of which contain a pattern (usually squares) printed within the confines of a 10cm x 10cm square. A white grid printed against a black background and a red grid on black background describe a few designs available (Crossland & Rubin, 2007) but given previous studies and reviews outlined above, the Amsler Chart employed in the present study was the design consisting of 5mm x 5mm black squares printed against a white background.

Twenty ME/CFS and their matched controls took part in the study but one control was not considered due to a pre-existing macular condition. Participants viewed the Amsler Grid at a distance of 30cm, at which each square on the grid subtended 1 degree of visual angle and the overall grid subtended 20 degrees (Crossland & Rubin, 2007). Participants were instructed to fixate on a dot in the centre of the grid with their spectacle correction. If spectacles were forgotten, participants were asked to adjust the working distance to achieve clarity. Amsler Grid observations were conducted monocularly (binocular viewing would mask potential pathology in an affected eye) and participants were asked to report any distortion or doubling of lines and blurry areas or areas missing. Any observations were drawn directly onto the Amsler Grid.

3.3.3. Results

40 % of patients described double/shadowed lines whereas no controls reported any Metamorphopsia. In the case of 3 patients, when asked to draw the distortion directly onto the Amsler Grid, the pattern of distortion appeared to become dynamic, i.e. the final area exceeded the areas of doubling/shadowing initially reported thus quantification by recording the number of squares distorted/missed would not portray this finding not least because some drawings covered the entire grid. This pattern of dynamic distortion is not a typical finding and, whilst normally any drawings of Metamorphopsia are completed within a few seconds, 3 patients were allowed to complete their drawings which took minutes rather than seconds. No scotoma was reported.

3.3.4. Discussion

That almost half the patients reported distortions on the Amsler Grid yet exhibited Visual Acuities at near were normal which raises the possibility of retinal dysfunction in this group. This is explored in the following chapter (Chapter 4) in which optical coherence tomography was employed to determine the effects, if any, of ME/CFS on the morphology of the retina.

3.4. Ocular Motility

3.4.1. Introduction

ME/CFS patients consistently report that they have difficulty moving their eyes when tracking objects in their visual field (Potaznick & Kozol, 1992; Vedelago, 1997). Problems related to eye movements have also been shown experimentally where ME/CFS patients performed worse than controls on tasks that require anti-saccadic fixations (Badham & Hutchinson, 2013). Smooth pursuit eye movements also become worse over time (Badham & Hutchinson, 2013).

The precise control of movements of the extraocular muscles (EOMs) is necessary for efficient eye movements/binocular vision which is controlled voluntarily as well as influenced by the Vestibular-Ocular, Pursuit, Saccadic and Vergence reflexes (Bedell & Stevenson, 2013; Brueckner, Ashby, Prichard, & Porter, 1999). A brief description of the functioning of the EOM's is as follows: the

right and left lateral rectus muscles (RLR & LLR) are responsible for abduction and adduction respectively. The Superior and Inferior rectus muscles (SR & IR) are responsible for elevation and depression and the right and left Superior (RSO & LSO) and Inferior Oblique (LIO & RIO) muscles are responsible for the diagonal movements Optokinetic, (Purves, Augustine, Fitzpatrick, 2001) (Figure 3.4).

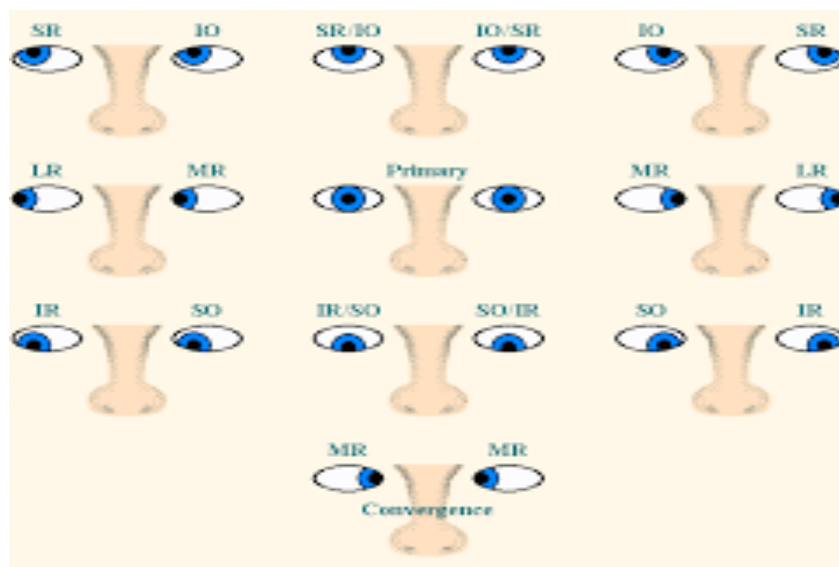


Figure 3.4. Schematic of the extra ocular muscles involved in the 8 fields of gaze test. (Anatomy, Physiology and Pathology of the Human Eye, Montgomery, T, 1998- 2017).

The 6 finely tuned EOM's surrounding the eyes, as well as their corresponding innervating nerves (III, IV and VI) can be affected by trauma, fatigue and disease causing changes in their shape and size and result in EOM paresis (Lacey, Chang, & Rootman, 1999; Poonyathalang, Khanna, & Leigh, 2007). Paresis of the EOM's can be observed by examining conjugate eye movements of the 6 EOM's in the 8 cardinal positions of gaze and are characterised by a

restricted movement in a particular direction of gaze (Danchaivijitr & Kennard, 2004) and the paretic EOM(s) can be often be deduced by observation when followed up by the Cover/ Uncover test (Evans, 1997; Danchaivijitr & Kennard, 2004).

The Ocular Motility test was included in the battery of tests because in addition to allowing basic observation of pursuit movements and the potential uncovering of paretic muscles, the functioning of the Levator Palpebrae Superioris, which controls elevation and retraction of the upper lids could also be observed at start of the test. Myasthenia Gravis could be of particular interest in the context of ME/CFS as it is caused by fatigue and can manifest as fatigued EOM(s) and bilateral ptosis due to the reduced function of the Levator Palpebrae Superiori (Smith & Lee, 2017), Superior Oblique paresis (Rush & Shafrin, 1982), Inferior Oblique paresis (Almog, Ben-David, & Nemet, 2016) and partial 3rd and 6th nerve (Gounden, Lee, Mellick, Rutkowski, & Middleton, 2016).

3.4.2. Methods

Participants sat uncorrected in a chair (to achieve an uninterrupted field of view which could be disrupted by spectacle frames) whilst examination took place at the level of fixation at a distance of 1m. Participants were asked to fixate on a small plastic target of 5 mm diameter for 10 minutes whilst maintaining a static head position and asked to report any symptoms of tiredness, discomfort, pain and diplopia. Restriction and smoothness of the movement of the EOM's were observed. Three ME/CFS patients were excluded. One was excluded due a previous road traffic accident that caused physical

trauma to the eye muscle(s), the 2nd because of a Botox injection for a squint correction and the 3rd patient was excluded because there were difficulties following task instructions. As such, the sample size for this part of the study was 17 patients and 17 matched controls.

3.4.3. Results

88% of patients reported some description of discomfort whereas only 5% of controls complained of the same. Of the ME/CFS who reported superior gaze discomfort, only 20% of them had observable jerky movements.

Figure 3.5 shows the specificity of patients' discomfort. The most commonly reported area of discomfort lay above the horizontal meridian i.e. above the line of fixation. This superior gaze discomfort was reported in 71% of ME/CFS patients. Upon further inspection of upper gaze discomfort reports, there were more symptoms of discomfort in the upper right hand gaze 59% (Dextro-Elevation) compared to the upper left hand gaze 52.9% (Laevo-Elevation). In comparison, only 1 control experienced discomfort superiorly. Levator Palpebrae Superiori appeared to be functioning normally in both groups. There were slightly more reports of discomfort in the lower right hand gaze quadrant (Laevo-Depression) at 17.6% compared to the lower left hand gaze quadrant which was reported ay (Dextro-Depression) at 11.8%. The controls reported no Laevo-Depression or Dextro-Depression gaze symptoms. Lateral (Levo-Version and Dextro-Version) gaze symptoms were reported in 11.7% of ME/CFS patients compared to 0% of lateral gaze discomfort in the controls.

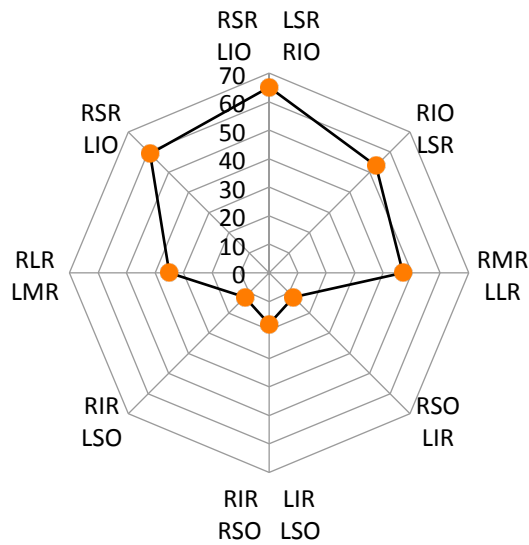


Figure 3.5. Schematic illustrating percentage of patients who reported discomfort in each gaze direction of the 8 fields of gaze test.

Of the ME/CFS patients who reported symptoms of discomfort, all reported symptoms as early as the 1st or 2nd repetition but the level of discomfort stayed the same rather than progress with subsequent repetitions. Only one control participant reported progressive difficulty over the duration of 10 minutes.

3.4.4. Discussion

Parallels can be drawn between ME/CFS and Myasthenia Gravis (MG) which is a chronic autoimmune neuromuscular disease (Vernino, Cheshire, & Lennon, 2001) associated with fatigue (Avidan, Le Panse, Berrih-Aknin, & Miller, 2014; Drachman, 2003; Meriggioli & Sanders, 2009). MG is thought to be caused when

pathogenic anti-bodies attach themselves to acetylcholine receptors on the post synaptic receptors at the neuromuscular junction which leads to fewer acetylcholine receptors post- synaptically which interferes with signals that contract muscles (Avidan et al., 2014; Drachman, 2003; Meriggioli & Sanders, 2009)

Skeletal muscles are affected in 80% of cases of MG (Avidan et al., 2014). Muscle weakness of the limbs is intermittent and followed by asymptomatic periods (Barton & Fouladvand, 2000). First reported symptoms however often implicate the eye (Avidan et al., 2014) (and so named Ocular Myasthenia Gravis (OMG). EOM involvement has been reported in 90% of individuals with MG (Gunji et al., 1998).

Notable symptoms in OMG range from blurry vision, weakness and painless fatigue of EOM, with normal visual acuity, diplopia, normal pupils with ocular ptosis (Barton & Fouladvand, 2000; Smith & Lee, 2017) or without ocular ptosis (Smith & Lee, 2017).

Much like ME/CFS, MG sufferers report a worsening in the condition with greater effort (Avidan et al., 2014). Although there was no worsening over time seen during the Ocular Motility Test, the greater inherent work involved in superior gazes to counter gravitational effects could be considered a greater effort spatially rather than over time. Indeed this was shown to be the case in 88% of super gaze symptoms. 20% of ME/CFS in total observed jerky movements which might be indicative of poor nerve innervation, partial trauma to a muscle(s), tiredness or underlying pathology (Evans, 2007). However, as there was no actual paresis observed, it might be more appropriate to attribute the jerky movements to fatigue.

In the context of ocular motility, ME/CFS and MG might share some aspect of clinical presentation (with the exception of ptosis in OMG) including the fact that they are both incurable (Barton & Fouladvand, 2000; Smith & Lee, 2017), however, where MG is diagnosed by electrophysiological and pharmacological procedures, the diagnosis of ME/CFS remains inconclusive. For now, tests such as the Acetylcholine test, (the effect of cholinesterase inhibitors on certain autoantibodies) might only help diagnose MG/OMG but the prospect of its existence alone may give hope to the ME/CFS community of a future diagnostic test(s) given that MG/OMG shares many clinical features (Avidan et al., 2014; Barton & Fouladvand, 2000).

3.5. Pupil Function

3.5.1. Introduction

Photosensitivity is a common complaint of ME/CFS (Loew et al., 2014; Mastropasqua et al., 2000; Potaznik & Kozol, 1992; Vedelago, 1997; Underhill, 2015). Hutchinson et al., (2013) found that 92% of ME/CFS complained of photosensitivity in a DePaul Symptom Questionnaire study.

Photosensitivity could be a sign of tear dysfunction (Pflugfelder, 2011), a symptom of conjunctivitis (Noble & Lloyd, 2011), corneal disruption or Dry Eye syndrome (Digre & Brennan, 2012; Javadi & Feizi, 2011; Kanellopoulos & Asimellis, 2016). Inflammation of the Uveal tissues are also known causes of photosensitivity e.g. Iritis and Uveitis (Digre & Brennan, 2012). These examples, which are not exhaustive, generally focus on active and/or acute inflammatory

diseases of the corneal and uveal tissues but other known causes of photosensitivity could be grouped under autonomic (primarily) parasympathetic nervous dysfunction (Bremner & Smith, 2006; Wilhelm, 2011). In fact, the connection between inflammation and the Argyll- Robertson Pupil disorder and bacterial inflammation is already established in the Argyll Robertson Pupil (Thompson & Kardon, 2006). Given that bacterial infections can compromise the parasympathetic pathway, it is quite possible that viral inflammation could also affect the parasympathetic pupil reactions and the pupils may consequently be particularly relevant in ME/CFS given the widely accepted viral roots.

There are a number of documented pupil conditions caused by viruses or bacterium. The Argyll-Robertson Pupil is a well-known pupil condition and characterised by bilateral small irregular pupils which react to accommodation but don't constrict to light. This phenomenon is well documented in Neurosyphilis, a disease believed to be of bacterial origin (Pate, 2016; Sakai, Shikishima, Mizobuchi, Yoshida, & Kitahara, 2003; Spector, 1990). The Holmes-Adie Pupil is another well established pupil anomaly which is found unilaterally in 80% of cases (Crowell, Feldman & Tripath, 2014; Martinelli & Minardi, 2001; Mayer, 2014). It is caused by postganglionic parasympathetic damage (Spector, 1990) and 70 % of patients tend to be female (Arciniegas-Perasso, Díaz-Céspedes, Manfreda-Domínguez, & Toro-Giraldo, 2016; Bremner & Smith, 2006). Sympathetic disorders such as Horner's syndrome for example, result in facial anhidrosis, miosed pupil with a ptosis (Spector, 1990; Pate, 2016). Photophobia does not appear to be documented.

There was a 2-fold reason for checking Pupil Function in the present work: firstly to establish any autonomic pupil disorders as described

above and secondarily because pupil responses may help in the diagnosis of binocular difficulties which may have manifested during the Ocular Motility Test (Evans, 1997).

3.5.2. Methods

A brief description of the anatomy is essential for the understanding of the Pupil Function tests. Normal pupillary function exists by virtue of the Afferent and Efferent pathways (Broadway, 2012; McDougal & Gamlin, 2015). The Afferent Pathway carries the photoreceptor signals from the Retinal Ganglion Cells via the Optic Nerve to the Optic Chiasm where there is a decussation of nasal fibres so that some of the signal continues along the right tract and the other nasal fibres continue along the left tract. The temporal fibres and contralateral nasal fibres synapse in the corresponding Pre-Tectal Nucleus. The Pre-Tectal Nuclei are located within the mid- brain at the level of the Superior Colliculus. These temporal ipsilateral fibres and the contralateral nasal signals then travel to the Edinger Westphal Nuclei (EWN) of the Oculomotor Nerve (Wilhelm & Kardon, 1997; Spector, 1990; Pate, 2016).

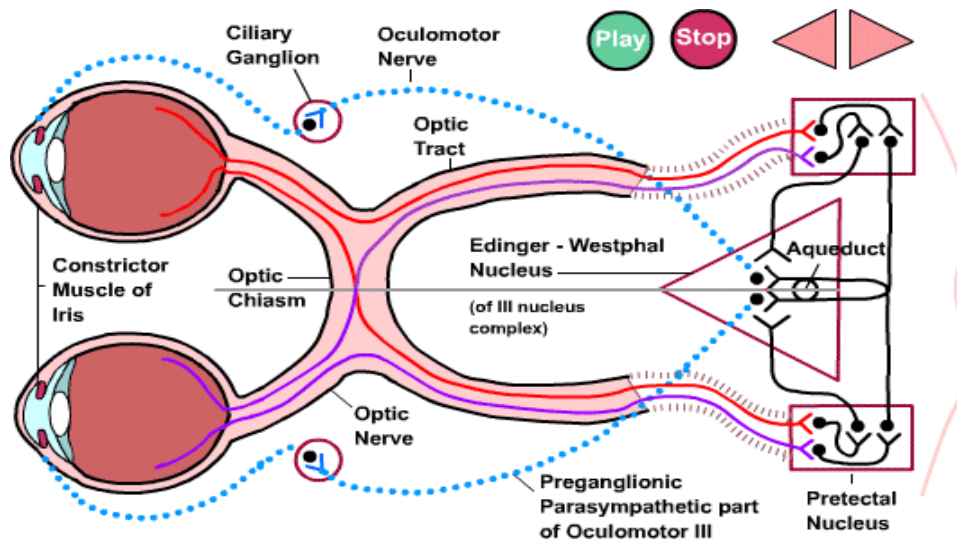


Figure 3.6. A Schematic of the parasympathetic pathways: Pupillary Light Reflex. (Anatomy, Physiology and Pathology of the Human Eye, Montgomery, T, 1998- 2017)

The Efferent limb describes the relaying of the signal to both pupils and these leave the EWN to supply the Ciliary Ganglion of which 97% of the post ganglionic fibres innervate the Ciliary Ganglion (Pate, 2016) and 3 % of the Post Ganglionic fibres innervate the iris sphincter muscle along the same path as the 3rd nerve (Wilhelm & Kardon, 1997). Any dysfunction of these pathways offers clues into potential deficits, the location of which can often be pinpointed along the visual pathway (Figure 3.6).

Autonomic dysfunction photosensitivity can be determined by employing established optometric methods that investigate pupil responses (Table 3.2); Pupillary Light Reflex (PLR) which confirms the presence of direct and consensual responses, Swinging Flashlight test (SFL) which confirms the presence of Relative afferent Pupillary Defect (RAPD) and Pupil Responses at Near (PRN) which is observed by a constriction of the pupils to a near target (Broadway, 2012; Pate, 2016; Spector, 1990). PLR, SFL, PRN will be collectively referred to as 'Pupil Function', which was assessed using a Keeler Ophthalmoscope.

Test/Measurement	Expected outcome
PLR - Direct Response	If light is shone into the right eye, we expect the right eye to constrict in a healthy patient (Pate, 2016; Wilhelm & Kardon, 1997).
PLR - Consensual Response	Participants were asked to fixate at 6m for distance. If light is shone into the right eye, we expect the left eye to also constrict confirming the presence of a normal consensual response. If there is no consensual response, damage is expected along the Efferent Pathway (Pate, 2016; Wilhelm & Kardon, 1997).
Swinging Flashlight Test	If light is shone into the right eye, the pupil should constrict normally. If this light is switched quickly to the left eye, a constriction should also be seen and a maintained constriction in the right eye should also be observed in a healthy patient (Broadway, 2012; Pate, 2016; Spector, 1990; Wilhelm & Kardon, 1997). In the case of a Relative Afferent Pupillary Defects (RAPD). The eye that the light has just been transferred to will appear to dilate.
Pupil Response at Near (PRN)	An equal constriction is expected in both eyes at a near target in a normal healthy patient (Pate, 2016; Wilhelm & Kardon, 1997).

Table 3.2. Tests/Measurements of Pupil Function and their expected outcome.

Figure 3.7 illustrates a left RAPD. The eye that the light has just been transferred to will appear to dilate. This is because the dilation of the RE overpowers the constriction of the LE i.e. consensual response is greater than the direct response in the eye with RAPD. In a normal eye the constriction will be maintained (Broadway, 2012; Pate, 2016; Spector, 1990; Wilhelm & Kardon, 1997).

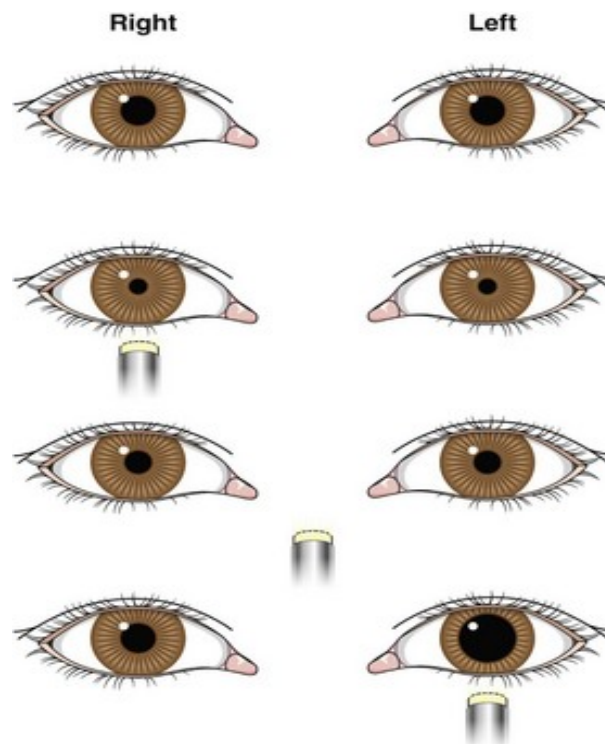


Figure 3.7. A Schematic of a Left Relative Afferent Pupillary Defect from Health & Medicine, Ophthalmology Eponyms! Dr A. Alsherbiny January, 2016)

3.5.3. Results

Normal direct and consensual responses were found in both patients and controls. No RAPD was evident in either group. There appeared however to be unusual accommodative response at near in our ME/CFS patients in that the right pupil appeared larger and reacted more slowly to a near target.

To objectively confirm the unusual reactions to a near target in which the right pupil in the ME/CFS group appeared slightly larger in 35 % of patients, patients were called back to an additional testing session. Of the original twenty patients, 13 returned to be tested with the SWIFT Pupillometer. Testing was undertaken with the automated SWIFT in near black-out conditions. The only light sources were from the monitor and the red and green LED lamps but this was within the requirements of 2 cd/m² and therefore sufficient for participants to view the fluorescent fixation target. The distance between the eyepieces and chinrest rail was fully extended in accordance with the manufacturer's instructions and the participants were effectively tested at 128 cm i.e. the distance from the orbital notch to the fixation target.

RAPD was determined using an automated version of the Swinging Flash Light test, made up of a sequence of 2 seconds of illumination followed by a 0.5 second period of darkness which switched between eyes. The difference in light intensity required to equalise the extent of pupil response corresponds to the level RAPD (Cayless & Bende, 2016).

Figure 3.8 shows mean pupil diameters for each eye in response to illumination taken by the SWIFT Pupillometer. A 2 (group: ME/CFS, controls) by 2 (eye: left, right) mixed repeated measures ANOVA revealed a main effect of group ($F(1,24) = 6.216, p < .05$) in that the ME/CFS group exhibited larger pupil diameters than controls. Although there was a tendency towards a particularly large right pupil in the ME/CFS group, this effect was not significant. This was confirmed by the ANOVA which showed no significant difference in pupil diameter between the left and right eyes ($F(1,24) = .176, p = .678$) and no interaction between group and eye ($F(1,24) = 2.971; p = .98$).

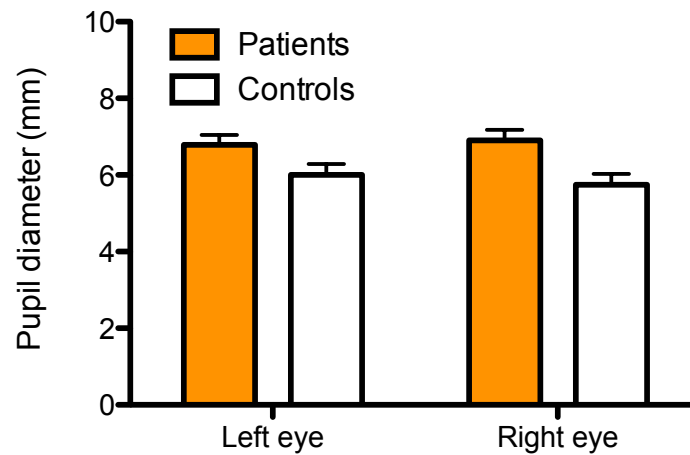


Figure 3.8. Mean Pupil Diameters (mm) for the left and right eyes of patients and controls. Error bars represent ± 1 S.E.M.

Figure 3.9 Shows RAPD values for patients and controls. A paired samples t-test confirmed that there was no difference between patients and controls ($t=0.4782, df =12; p=0.641$).

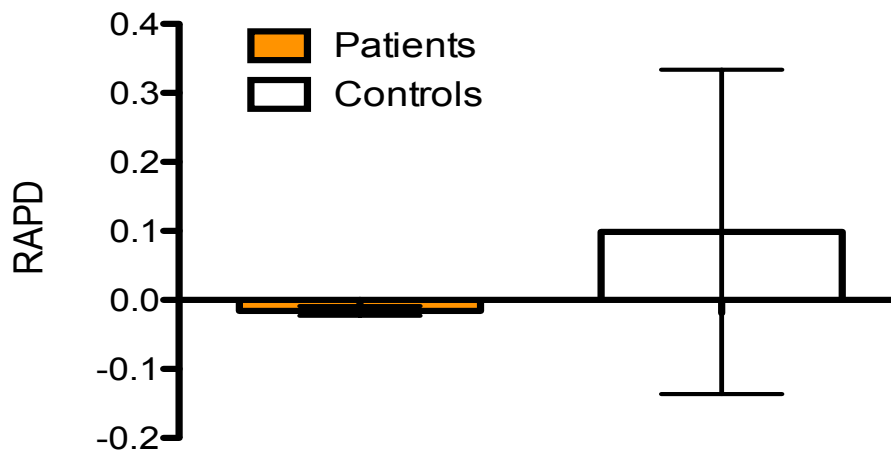


Figure 3.9. RAPD values for patients and controls. Error bars represent ± 1 S.E.M.

3.5.4. Discussion

The Pupil Function data showed potentially interesting findings both in the practitioner observations as well as the in Video-Pupillometry data. Practitioner observations are as follows; miosis of both eyes were observed when focusing at a near target, however, in 35% of ME/CFS cases, the right eye was underwent a slower rate of miosis even though both pupils achieved miosis to the same extent all cases. As there were no unusual reactions found at far i.e. (direct, consensual and swinging flashlight reactions at 6m were normal), we suggest that there may be accommodative element to this finding and that our findings are at least, in part, identifiable with the Holmes- Adie Pupil.

The exact aetiology of the Holmes- Adie Pupil is unknown (Pate, 2016; Mayer, 2014) although there is a strong consensus that damage leads to a reduction in neurones or denervation of Ciliary Ganglion cells

(Crowell et al., 2014; Mayer, 2014; Pate, 2016; Spector, 1990). A reduction in these cells has been proven histologically (Crowell et al., 2014). Holmes-Adie Tonic Pupils are thought to be caused by viral or bacterial infection e.g. Syphilis, Variella Zoster Virus (VZV) (Arciniegas-Perasso et al., 2016; Pate, 2016). Diabetes can also cause bilateral tonic pupils (Pate, 2016). Further features of Holmes-Adie include a slow constriction to light due to denervation of the iris sphincter (Crowell et al., 2014) accompanied by loss of accommodation (Pate, 2016; Spector, 1990) and sometimes a paralysis of accommodation (Mayer, 2014) where pupils react slowly to a near target and report blurred vision as a chief complaint. Segmental paralysis of the iris sphincter is also a feature (Pate, 2016; Thompson, 1977).

Patients with Holmes- Adie may be totally asymptomatic, however 80% of 122 patients in a retrospective study had symptoms of dark adaptation, photophobia and anisocoria of which 35 % said they complained of brow ache, blurred vision, with near work (Spector, 1990). Reports of symptoms of reduced accommodative are also documented (Spector, 1990). Ciliary Ganglion damage in the Holmes- Adie Pupil is responsible for an attenuated response to light, slow reactions to accommodation and miosis is achieved at a larger amplitude (Bremner & Smith, 2006) We, on the other hand, recorded no attenuation to light, a similar amplitude of miosis accompanied with a slow reaction to accommodation.

Over time, numerous reports of aberrant re-innervation of the Ciliary body (and of the pupil to a lesser extent) results in the classic “tonic” pupil (Spector, 1990). The tonic pupil in the affected eye is documented to appear smaller than the normal eye and is best

distinguishable when the affected pupil re-dilates slower over time when focus shifts from near to far (Spector, 1990). The pupil diameter data revealed that pupil diameter in patients are generally larger than controls and the right pupil diameters in particular was larger in patients than controls, although this was not found to be significant. Differences in pupil diameter may be due to aberrant re-innervation. However, even if we assume that all ME/CFS group had a diagnosis of Holmes-Adie Pupil, given that this process occurs over time, it would be difficult to say with any degree of certainty what stage these ME/CFS group were. The literature states that the “tonic” pupil is as expected to be visible around 2 years (Baran, Balbaba, Demir, & Özdemir, 2014) but the only certainty we can provide is that all our patients were diagnosed over 2 years (notwithstanding delays achieving diagnosis). Further study with larger sample sizes is necessary before more definitive interpretation would be possible.

3.6. Accommodation

3.6.1. Introduction

Whilst measuring the Near Acuity, patients would often push the Near Chart away to achieve clarity. The pushing away of reading material is a well-established sign of loss of accommodation which normally indicates the onset of Presbyopia (Patel & West 2007; Bonilla-Warford, 2012). Presbyopia is the reduced ability of the eye to focus due to hardening of the crystalline lens due to age (Glasser & Campbell, 1998; Heys, Cram, & Truscott, 2004). Presbyopes often report symptoms of blurred vision at near, eye strain, discomfort of the eyes, trouble re-focusing from distance to near or from near to

distance, headache, fatigue and the requirement for brighter light for near tasks (Mancil et al., 2010).

Epidemiological studies show the mean onset of presbyopia in England is around 45.5 years in both sexes (Jain, Ram, & Gupta, 1982) and in the United States of America, the average age of presbyopia onset is 41 years (Miranda, 1979). An apparent reduction in accommodation was unexpectedly observed during the Near Acuity screening process in patients under the age of 41 of the ME/CFS group. Furthermore, whilst reduced accommodation was expected and found within the typical presbyopic age range, the pushing away of material appeared to be apparent in patients who had even recently updated their spectacles. Healthy patients in practice usually notice a reduction in the near vision every 2 years (du Toit, 2006). Notably, in both the pre-presbyopic and presbyopic ME/CFS sufferers, the pushing away of reading material to achieve near focus appeared to be fluctuating i.e. reading material would be pushed away and near focus achieved only to be shortly re-adjusted for near focus and re-adjusted for near focus again. This transient change of focus was not evident in controls. There also appeared to be more effort required to settle on the point of near clarity compared to the controls.

These observations, coupled with previous reports of reduced accommodation in ME/CFS (Caffery et al., 1994; Godts, Moorkens, Mathysen, 2016; Mastropasqua et al., 2000) led us to measure accommodation in all members, presbyopic and pre-presbyopic of the ME/CFS and control groups. Given the indication from the pupil

responses that there may be differences between the eyes, absolute accommodation is reported.¹

3.6.2. Methods

Unfortunately, only a small number of patients (n=9) returned for accommodation testing from the original cohort. Amplitude of accommodation was measured by adopting the Push-Up/Push-Down Test as it was non-automated, simple and therefore quick to complete. There is evidence to show that the Push-Up test can result in slightly higher amplitudes than the Push-Down test (Antona, Barra, Barrio, Gonzalez, & Sanchez, 2009) however, the Push-Up and Push-Down methods to be quick, easy and comparable to the Modified Push-Up test and Minus Lens method e.g. (Momeni-Moghaddam, Kundart, & Askarizadeh, 2014). To take potential biases into account Absolute Accommodation was determined using both the Push-Up and Push-Down methods, after which a mean was taken. Measurements were taken monocularly. Participants reported points of “1st blur” and “1st clear – the midpoint taken if the points were non-coincidental.

3.6.3. Results

Figure 3.10 shows mean Absolute Accommodation for the ME/ CFS group and controls. There was a significant reduction in patients’

¹ Amplitude of accommodation is the total accommodative power of the eye i.e. the accommodation of each eye (Encyclopedia and Dictionary of Medicine, Nursing, and Allied Health, Seventh Edition (2003).

accommodation compared to their age matched controls. A paired samples t-test confirmed that, compared to controls, accommodation was significantly reduced in the ME/CFS group ($t=3.236$; $df=6$; $p<.05$).

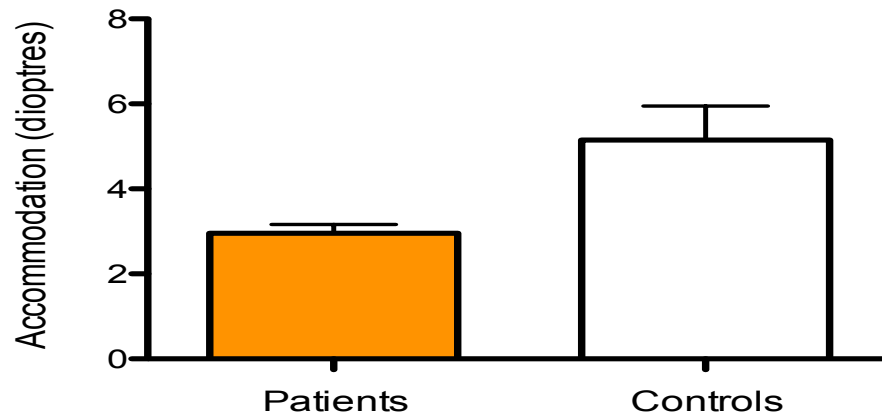


Figure 3.10. Mean Absolute Accommodation for the ME/CFS group and controls. Error bars represent ± 1 S.E.M

There was a general reduction in Absolute Accommodation across the patient group with the only exception occurring in the case of the 61 year old patient who had 0.15D more than her age matched control. This accommodation was most notably reduced for the younger individuals with ME/CFS but less obvious in age >45 year olds. The Absolute Accommodation was Figure 3.11 shows that the reduction in accommodation was greater the younger the patients were and this was particularly evident in the pre-presbyopes. This graph, as expected, followed a typical curvilinear reduction of accommodation (Momeni-Moghaddam, 2014). Thus, in those >45 years and above the reduction is less apparent due to the natural decline in accommodation levels.

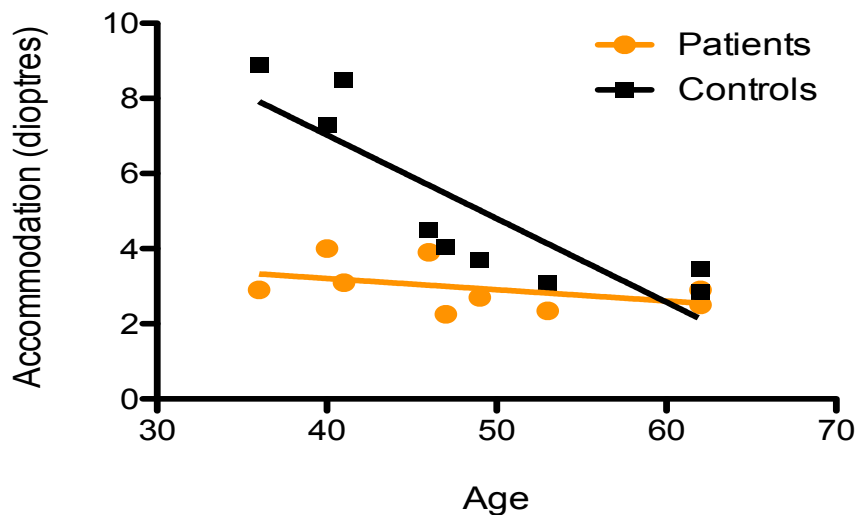


Figure 3.11. Absolute Accommodation in ME/CFS patients and matched controls as a function of age.

3.6.4. Discussion

Our findings of low reduced accommodation are supported by the previous studies (Godts et al., 2016) Accommodation anomalies have also been noted in earlier reviews for example The Macintyre study described by Leslie, (1997) details how a 10 year old girl presented with extreme variability in accommodation with varying Retinoscope results varied from 0.00 to -6.00 yet the young girl was clinically Orthophoric.

Our study not only supports earlier findings, but offers more comprehensive analyses particularly within the pre-presbyopic patient group in that the accommodation seems proportionally reduced compared to Controls. Our findings also detail how both the pre-presbyopes and presbyopes experienced a transient blurring

when achieving near focus, requiring greater effort (compared to controls) even when spectacles were updated within the last 2 years.

Certain medications, in particular anticholinergic medications could potentially contribute to reduced accommodation. Anticholinergics work by selectively blocking the binding of acetylcholine to the acetylcholine receptor sites thereby inhibiting parasympathetic Ciliary muscle contraction. Anticholinergic side effects such as confusion and poor memory are therefore prescribed with great care (Lieberman, 2004). The patients and controls in this Study however were not prescribed anticholinergic medications at the time of testing.

The advice to Optometrists echoes those of Leslie's (1997); practitioners should assess all ocular symptoms and general health in their entirety and additionally actively look for early signs of reduced accommodation in pre-presbyopics. Optometrists should also look out for a transient blurring and frequently changing near addition taking into account effects of early loss of accommodation due to disease, medication and trauma (Dentone & Ashfari, 2015).

3.7. Colour vision

3.7.1. Introduction

There have been few previous reports of colour vision disturbances ME/CFS patients. These include coloured spots were reported

(Potaznik & Kozol, 1992) and a suspicion of red desaturation after the Red Dot Test revealed the possibility of a mild red desaturation (Leslie, 1997).

The connection between acquired Colour vision deficiencies and diseases is well known. Some examples include alcoholism (Verriest, Francq, & Pierart, 1980) which can result in colour loss in the Blue-Yellow hues (Mergler, Blain, Lemaire, & Lalande, 1988). Tritan like defects are reported in Glaucoma, (Pacheco-Cutillas, Edgar, & Sahraie, 1999; Papaconstantinou et al., 2009) and Red-Green (R-G) defects in pituitary tumours (Herse, 2014). Diabetics also have demonstrable colour vision defects, which occur with greater incidence with greater severity of diabetic retinopathy (Green et al., 1985). For example, Fong, Barton & Bresnick (1999) found 50 % of 2701 diabetics had Tritan like defects. Multiple Sclerosis sufferers are also known to acquire colour vision deficiencies. Harrison et al., (1987) found 45 % of patients were recorded to have R-G defects as confirmed by the ICVT and 42.5% were found to have poor colour discrimination with Farnsworth Munsell Hue-100 arrangement test, regardless of optic neuritis. It is accepted that colour vision deficiencies can serve as a marker in Retinal Ganglion Cell (RGC) damage in Multiple Sclerosis (MS) patients (Lampert et al., 2015) where both Red-Green and Yellow-Blue deficiencies can occur (Shaygannejad et al., 2012) affecting both parvocellular and koniocellular pathways (Moura et al., 2008)

In the present study, colour vision was determined using the two standardised tests, the Ishihara Colour Vision Test (ICVT) and the Farnsworth Munsell Hue-100 (FM-H100) arrangement test.

3.7.2. Methods

3.7.2.1. The Ishihara Colour Vision Test

The Ishihara Colour Vision Test (ICVT) is a colour perception test for R-G deficiency and normally used in practice to detect the X-chromosome linked colour vision deficiency which manifests as Protanopia, Deuteranopia (a total lack of red and green cones respectively) and Protanomoly and Deuteranomoly which describe mutations of the red and green retinal photoreceptors (Purves et al., 2001). Acquired blue-yellow deficits that precede red-green anomalies are often indicative of retinal disease, whereas red-green deficits, which are often accompanied by some hue-yellow loss, are more likely to reflect damage to the retinostriate pathway (including the Optic Nerve). This is a rule of thumb assumed by Kollner (Hasrod & Rubin, 2016).

The Ishihara test contains 38 plate edition pseudo-isochromatic plates (Birch, 1997) which are made up of the Demonstration plate, Transformation plate, Vanishing, Hidden and finally the Diagnostic plates which differentiate between Protanopes from Deuteranopes (Birch, 1997).

The ICVT was administered according to the Manufacturer's Instructions i.e. each plate was held at arms' length, constituting a viewing distance of approximately 75 cm (Kanehara Trading, Tokyo). Testing was performed monocularly given the purpose was to detect pathology rather than screen for congenital R-G blindness

and only a 5 second viewing time per plate was allowed. 4 or more incorrectly read plates constituted a fail.

3.7.2.2. The Farnsworth Munsell Hue-100 Test

It was important to investigate symptoms across as many hues as possible and as outlined above, the ICVT is limited to determining R-G deficiencies. The Farnsworth Munsell Hue-100 Test (FM-H100) is an arrangement test that determines deficits in subtle colour discrimination across yellow, green, orange and purple hues (Cranwell, Pearce, Loveridge, & Hurlbert, 2015; Kinnear & Sahraie, 2002). The FM-H100 is useful for monitoring the progress of acquired deficiency because of its sensitivity in detecting subtle changes (Kinnear & Sahraie, 2002; Pacheco-Cutillas et al., 1999).



Fig 3.12. An example to illustrate the Farnsworth Munsell Hue-100 Test (Colour max.org)

The FM-H100 consists of 4 colour palettes each of which contain 22 coloured tiles. The coloured tiles are grouped into yellow/green, blue/purple, orange/magenta and purple/ magenta hues (Figure 3.13). All 22 colour tiles were distributed in random order but within the confines of each colour hue (Kinnear & Sahraie, 2002).

Whilst 16 patients and their matched controls took part in this part of the Study, one was excluded because of a diagnosed pituitary tumour and a second patient was excluded due to a diagnosed macular condition. Therefore 14 patients and their controls were included.

Participants were asked to arrange the coloured tiles in colour sequence using the fixed tiles at either end as the reference point. There was no time limit and near correction was worn. The pre-assigned numbers on the back of the tiles were entered in the FM-H100 software which produced a Total Error Score (TES) which is calculated by adding Error Scores (ES) or the sum of the numerical difference between each coloured cap (Kinnear & Sahraie, 2002; Pacheco-Cutillas et al., 1999). As the TES gives us a value across all 4 colour palettes and not just one colour group, diagnosis for a specific colour vision deficiency e.g. R-G or Tritan deficiencies is not possible. The FM-H100 does however provide a polar diagram illustrating the hues where the errors occurred.

3.7.3. Results

R-G colour vision as determined by the ICVT as normal in all patients and controls (except in the case of the patient with the Pituitary

tumour). However, the FM-H100 revealed that the ME/CFS group exhibited poorer colour hue discrimination in the yellow/ green and blue/green hues compared to the control group ($t=3.269$; $df=14$; $p<.01$). Mean data are shown in Figure 3.13.

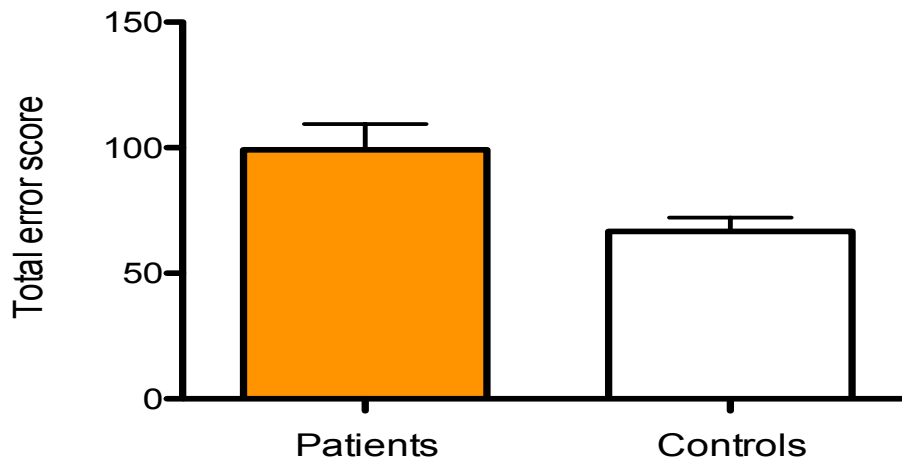


Figure 3.13. Total error scores for patients and controls. Error bars represent ± 1 S.E.M.

3.7.4. Discussion

With the exception of the patient with the pituitary tumour, the ICVT did not detect any R-G defect in either group. The ES provided a gross measure of colour discrimination, representing the numerical difference between two adjacent caps. The ES's were then added together to give the Total Error Score (TES), (Pacheco-Cutillas et al., 1999). TES revealed that both patients and control groups made most errors in the yellow/green and blue/ green hues but patient errors occurred more significantly in those hues. Given that the FM-H100 provides no specific diagnostic TES value indicative of either a

Tritan or R- Green deficit, the FM-H100 may best serve ME/CFS patients as a tool whereby the progression of disease is monitored rather than revealing specific underlying R-G and Tritan visual system changes *per se*.

3.8. Contrast Sensitivity

3.8.1. Introduction

It is well documented that the human visual system decomposes a visual scene into a set of sinusoidal components of specific spatial frequencies (levels of image detail), orientations, phase positions and contrasts (Blakemore & Campbell, 1969; Campbell & Robson, 1968; Chung & Legge, 2016). This is evident in the human Contrast Sensitivity Function (CSF), which provides a measure of the range of spatial detail that is visible (resolvable) to the visual system and the relative sensitivity to stimulus contrast within this range. Contrast Sensitivity deficits can be present even when there is no detectable impairment in visual acuity. They provide a sensitive clinical measure of visual function and can reveal abnormal visual processing at the level of the retina and in the cortical and subcortical visual pathways.

The ability of an observer to detect spatial contrast can be determined by measuring a contrast threshold. This refers to the minimum difference between the light and dark transition at a border or an edge of a pattern or object that allows an observer to reliably detect its presence. Assessment of human sensitivity to image contrast typically involves determining contrast thresholds across a

range of spatial frequencies. Contrast thresholds can be easily quantified by recording whether participants can detect carefully-controlled, computer-generated sinusoidal gratings presented at a range of different contrast levels from a very low contrast that participants cannot detect to a high contrast that is clearly detectable, and from which a contrast threshold (75-79% correct) is calculated. The human CSF takes the form of bandpass (inverted u-shaped) spatial tuning function, with maximal sensitivity occurring with the middle range of spatial frequencies (~2-6 cycles per degree of visual angle - cpd). Changes in CS are well documented in ageing (reviewed in Owsley, 2011) and are evident in a range of retinal diseases (Kiser, Mladenovich, Eshraghi, Bourdeau, & Dagnelie, 2005). They are also present in a number of 'non-visual' diseases, such as Parkinson's disease (Archibald, Clarke, Mosimann, & Burn, 2011) and Schizophrenia (Slaghuis, 1998).

In the present study, Contrast Sensitivity was determined for stationary luminance-defined sinusoidal gratings spanning a 5-octave range of spatial frequencies (0.5 to 16 cpd) in ME/CFS patients and controls.

3.8.2. Methods

Stimuli were sinusoidal gratings subtended 6 degrees (horizontally & vertically) and were generated using a Macintosh G4 and presented on a Sony Trinitron CRT monitor with an update rate of 75 Hz using the C programming language. The monitor was gamma-corrected using a spot photometer (LS-100, Konica Minolta) and look-up-tables (LUT). For precise control of luminance contrast the number

of intensity levels available was increased from 8 to 14 bits using a Bits++ attenuator (Cambridge Research Systems). The mean luminance of the display was $\sim 44 \text{ cd/m}^2$ and the monitor was the only light source.

The luminance contrast of the pattern could be varied according to the following equation:

$$\text{Luminance contrast} = (L_{\max} - L_{\min}) / (L_{\max} + L_{\min}),$$

[3.1]

where L_{\max} and L_{\min} are the maximum and the minimum luminances of the grating, in the range 0-1.

Threshold measurements were taken using a single-interval, forced-choice procedure. On each trial, participants were presented with a fixation cross, followed by the presentation of the grating, upon which they were required to judge its orientation (vertical or horizontal). Participants were allowed a short practice run and the testing was performed in the dark. The luminance contrast of the test stimulus was varied from trial to trial according to a modified 1-up 3-down staircase designed to converge on the contrast corresponding to 79.4 correct (Levitt, 1971; Wetherill & Levitt, 1965). At the beginning of each run of trials the contrast of the test pattern was initially set to a suprathreshold level (typically $\sim 6 \text{ dB}$ above threshold) and the initial staircase step size was chosen to be half this value. On subsequent reversals the step size was halved and testing was terminated after a total of 16 reversals. Threshold estimates were taken as the mean of the last 4 reversals in each staircase. Each observer completed 2 staircases per condition and

the order of testing was randomised. Contrast thresholds were converted into log Contrast Sensitivity (logCS).

3.8.3. Results

Patients appeared to exhibit poorer logCS than controls towards lower spatial frequencies (Figure 3.14). The following statistically significant differences emerged. A 6 (spatial frequency: 0.5, 1, 2, 4, 8, 16 cpd) x 2 (group: patients, controls) ANOVA confirmed main effects of spatial frequency ($F(4.015, 136.521)=22.091$; $p<.001$) and group ($F(1,34)=4.442$; $p>.05$). Post-hoc paired samples t-tests showed that logCS at 0.5 and 1 cpd was significantly worse in patients compared to controls (0.5 cpd: $t(18) = 2.528$, $p<.05$); 1 cpd: $t(18) = 2.440$, $p<.05$).

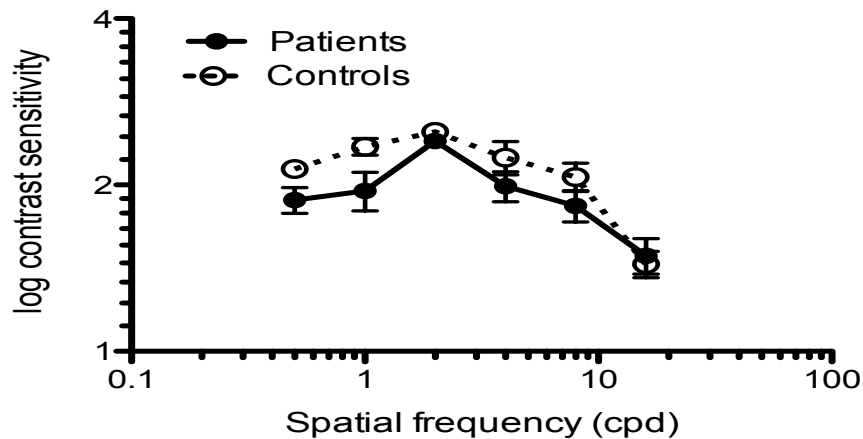


Figure 3.14. Contrast Sensitivity (log) for patients (closed symbols) and controls (open symbols) across a 5-octave range of spatial frequencies (0.5 to 16 cpd). Error bars represent ± 1 S.E.M.

3.8.4. Discussion

Contrast Sensitivity was significantly depressed at 0.5cpd and 1cpd in the ME/CFS group and may reflect processing of information in the ganglion cells of the retina. Ganglion cells are divided into Parvocellular or P-cells and Magnocellular cells or M-cells which have spatio-temporal characteristics (Plainis & Murray, 2005). M-cells carry neural information along the upper Dorsal stream to the Lateral Geniculate Nucleus (LGN) (Wang et al., 2016). The upper Dorsal Stream is responsible for hand and eye movements which allows for orientation i.e. the upper Dorsal Stream is concerned with “how” and “where” we move (Plainis & Murray, 2005) and responsible for locating objects in a field of view.

P-cells account for 70 % of ganglion cells, and are responsible for high spatial resolution and carry signals along the ventral stream to the LGN (Plainis & Murray, 2005; Willows, Corcos & Kruk, 1993). M-cells account for 10 % of ganglion cells and are sensitive to low contrast and saturate when the contrast is too high (Plainis & Murray, 2005; Willows et al., 1993). Given that we found a reduction of Contrast Sensitivity toward the lower spatial frequencies, we suggest that there is a potential reduction in M-cells in the ME/CFS group compared to controls.

There are many examples of disease that reduce Contrast Sensitivity much like our ME/CFS curve (Pelli & Bex, 2013). Age Related Macular Degeneration (ARMD) patients were found to have low Contrast Sensitivity at high spatial frequencies (Sokol et al., 1985). Glaucoma is associated with low Contrast Sensitivity at low spatial frequencies (McKendrick, Sampson, Walland, & Badcock, 2007) and we can draw parallels with our patient group. The idea that Contrast Sensitivity studies can act as a potential diagnostic test

has already being investigated for other conditions such as diabetes (Ong, Ripley, Newsom, & Casswell, 2003). Diabetics have been shown to have variable Contrast Sensitivity depending on the severity of disease (Sokol et al., 1985; Verrotti et al., 1998).

Given that our ME/CFS patients appear to experience low Contrast Sensitivity, at least at relatively low spatial frequencies, damage to the retinae of ME/CFS may be implicated. This notion is investigated further in Chapter 4.

3.9. Summary

Patients' visual responses differed significantly from those of controls on the following measures: Accommodation, Pupil Diameter, Colour Discrimination, and Contrast Sensitivity. Gaining a more comprehensive understanding of visual deficits in ME/CFS is fundamental to identifying measurable perceptual markers that can be used to aid accurate diagnosis and identifying treatable symptoms to improve patients' quality of life. If these data can be replicated in larger numbers of ME/CFS, a set of Guidelines could potentially direct those in the medical and optometric fields to refer suspect ME/CFS for further in-depth optometric assessment.

Chapter 4. The Effects of ME/CFS on the Morphology of the Retina

4.1 Overview

The purpose of Chapter 4 was to determine the effects (if any) of ME/CFS on the morphology of the retina, namely the retinal processing layers, the photoreceptor layers and the Optic Nerve head.

4.2. Background

As outlined in Chapter 1, there is growing literature demonstrating that vision-related problems represent a measurable class of symptoms that are commonly reported by patients with ME/CFS. Self-report studies have highlighted the existence of ME/CFS-related visual problems which include blurred vision, diplopia, floaters, photophobia, dry, gritty and tired eyes, ocular burning and non-specific eye pain, poor oculomotor control, poor depth perception, spots, lights and halos in the visual field, and vision-related headaches (Potaznick & Kozol, 1992; Leslie, 1997; Vedelego, 1997; Hutchinson et al., 2014). Other studies have revealed abnormalities of the pre-ocular surface (Caffery et al., 1994) and vascular pathology in the eye (Frolov & Petrunia, 2003). There is also evidence for a significantly higher distribution of exophoria, lower functional vergence (near and far), poor convergence, lower tear secretion and low Tear Break Up Times (TBUT) in ME/CFS patients, compared to healthy controls (Mastropasqua et al., 2000). Others

have found reduced accommodation (Caffery et al., 1994; Godts et al., 2016), impaired anti-saccadic and smooth pursuit eye movements (Badham & Hutchinson, 2013), deficits in visual attention (determined using visual cueing, visual search & selective visual attention tasks) (Hutchinson & Badham, 2013) and increased susceptibility to pattern-related visual stress (Loew et al., 2014; Wilson et al., 2015). In addition, the results of experiments outlined in Chapter 3 of this thesis revealed that ME/CFS is associated with visual distortions on the Amsler Grid, larger pupil diameters, poor colour discrimination and abnormal Contrast Sensitivity (poor Contrast Sensitivity towards lower spatial frequencies – 0.5, 1 c/deg).

The visual markers of ME/CFS revealed in this thesis and in other studies may be indicative of abnormal visual processing at the level of the retina and in the cortical and subcortical visual pathways. The purpose of the study outlined in this chapter was therefore to examine this possibility further by determining in the effects of ME/CFS on the morphology of the retina using optical coherence tomography (OCT).

Spectral-domain, high-resolution, optical coherence tomography (SD-OCT) uses back-scattered light to create images of the retina and the Optic Nerve Head (ONH) It provides cross-sectional, real-time, images which can be taken in micrometre (μm) resolution. (Fujimoto, Pitris, Boppart, & Brezinski, 2000). It has been used successfully to reveal disease-related changes to the retinal layers and ONH in a number of Neuropsychiatric and Neurodegenerative disorders including Schizophrenia, Alzheimer's Disease, Parkinson's Disease and Multiple Sclerosis (MS). In Schizophrenia, significant retinal nerve fibre layer (RNFL) thinning, decreased macular volume and

thinning of the macular ring have been documented. Furthermore, these effects appear to be related to illness duration (Ascaso et al., Lee, Tajunisah, Sharmilla, Peyman & Subrayan, 2013). RNFL thinning is also present in Alzheimer's Disease (Boeke, Rosen, Mastrianni, Xie, & Bernard, 2016; Cunha et al., 2016). RNFL thinning has also been documented in Parkinson's Disease where the duration and severity of the disease also appears to be related to the extent of RNFL thinning observed (Boeke, 2016; Lee, Ahn, Kim & Jeon, 2014). Of particular relevance to ME/CFS, given its association with autoimmune dysfunction, RNFL thinning has also been documented in MS (Gordon-Lipkin et al., 2007)

Due to the nature of some of the visual symptoms reported by ME/CFS patients (e.g. photosensitivity, chemical sensitivity), SD-OCT represents an effective means of investigating retinal abnormalities in this group, particularly, given that it is quick, non-invasive nature and pupil dilation is not required (Huang et al., 1991). As such, the experiments presented in this chapter use SD-OCT to compare the retinal layers and the Optic Nerve heads of a group of ME/CFS patients and controls.

4.3. Methods

4.3.1. Participants

Twenty ME/CFS patients and twenty matched controls took part (see Chapter 2 for further details).

4.3.2. Image Acquisition

Volumetric imaging (7 x 7 x 2mm) of the fovea and ONH region was performed using high-resolution spectral-domain OCT (Copernicus; Optopol Technology S.A., Zawiercie, Poland; wavelength = 850nm; theoretical axial resolution of 3.0 μ m, scan rate = 52,000 A-scans / second). Two images of the fovea and three images of the Optic Nerve of each eye were acquired and the best images selected for each. 5 images were excluded due to poor image quality such as motion artefacts or shadows. Each volume consisted of 75 horizontal B-scans with 743 A-scans per B-scan. To avoid possible bias during image analysis a code was allocated to each OCT scan and the researcher was masked to the identity of each scan.

4.3.3. Procedure

All patient and control groups were anonymised using only their initials. Two 3-d scans of the Optic Nerve and two 3d scans of the fovea were taken of each eye. 2 animation scans of the fovea were also taken of each eye. The best image for each participant was chosen for analysis. The chin rest adjust so that the pupil was clearly visible in the view finder. Focus was adjusted using the inbuilt calculator for image clarity.

Retinal region	Segmentation details
Retinal nerve fibre layer (RNFL)	From Internal Limiting Lamina to Retina Ganglion Cell Layer
Processing (inner) retinal layers	From the Internal Limiting Membrane (ILM) to the outer plexiform layer (OPL) / outer nuclear layer (ONL) border calculated for the parafovea (layers not present at the fovea)
Photoreceptor (outer) retinal layers	From the OPL / ONL border to the cone outer segment tips (COST) calculated for the fovea and parafovea.
Retinal pigment epithelium complex	From the cone outer segment tips to the Bruch's membrane averaged for the fovea and parafovea.

Table 4.1. Retinal Segmentation details (see also Figure 4.1)

Manual segmentation of retinal layers was performed on a single B scan selected at the centre of the fovea indicated by the deepest point of the foveal pit and where the cone outer segments were most elongated. A custom-written ImageJ macro was used to define retinal layer borders by locating points, which were fitted with a spline fit (<http://imagej.nih.gov/ij/>; provided in the public domain by the

National Institutes of Health, Bethesda, MD, USA, (2016). The retina was flattened along the Bruch's membrane by translating each A-scan vertically (Figure 4.1). To reduce the possibility of Type 1 errors due to multiple comparisons, the main statistical analysis was limited to the following measurements (photoreceptor (outer) retinal layers, processing (inner) retinal layers, retinal pigment epithelium, retinal nerve fibre layer) (see Figure 4.1 and Table 4.1 for details of retinal segmentation) which were calculated at the fovea and parafovea (average of 1000 μ m temporal and nasal to the foveal centre) and averaged across the two eyes. Table 4.2. provides a glossary of abbreviations for each part of the retina.

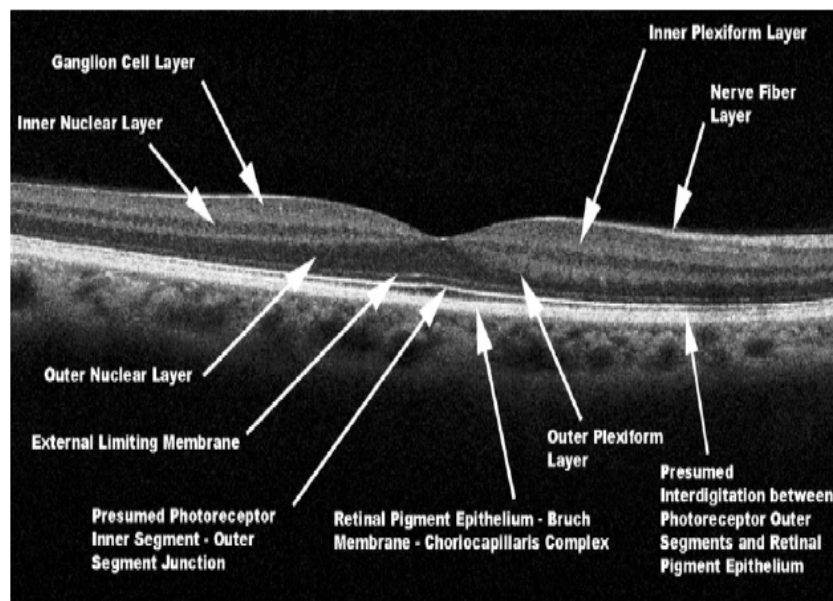


Figure 4.1. Example of retinal segmentation with Optical Coherence Tomography. See Table 4.2 for abbreviations. Source: Evaluation of Age-related Macular Degeneration With Optical Coherence Tomography, Research Gate, (Keane et al., 2012).

Section of retina	Abbreviation
Ganglion Cell Complex	GCC
Inner Nuclear Layer	INL
Inner Segment Layer	ISL (photoreceptor layer)
Optic Nerve Head	ONH
Outer Plexiform Layer	OPL
Outer Nuclear Layer	ONL (photoreceptor layer)
Outer Segment	OS(photoreceptor layer)
Peripapillary Retinal Nerve Fibre Layer	ppRNFL
Retinal Nerve Fibre Layer	RNFL
Retinal Pigment Epithelium	RPE

Table 4.2. Abbreviations for Retinal Layers.

Standard ONH measurements including cup, disc and rim diameters, areas and volumes and the thickness of the RNFL were measured using an automated algorithm. To minimize any inaccurate measurements by the software the disc margins, position of the internal limiting membrane and RNFL were adjusted manually. Peripapillary RNFL (ppRNFL) thickness was measured within the

temporal, superior, nasal and inferior quadrants of an annulus with internal diameter of 2.4 mm and width of 0.4 mm (default settings).

Retinal thickness and RNFL thickness in the macular area were measured using a semi-automated method. The manufacturer's software was used for flattening the B-scans along the retinal pigment epithelium (RPE). The position of the internal limiting membrane, the outer limit of the RNFL and the RPE were delineated using automated algorithms in the software and corrected manually to minimize segmentation inaccuracies. The retinal and RNFL thickness were measured in 3 standard circular zones as defined by Early Treatment Diabetic Retinopathy Study (Virgili et al., 2011) (central annulus (1mm), inner annulus (1 to 3 mm) and outer annulus (3 to 6mm) and the inner and outer annuli were separated into four quadrants (superior, inferior, temporal and nasal). For statistical analysis, thickness measurements in the centre of the foveal pit, paracentral area (averaged thickness of each layers from 250 μm nasally to 250 μm temporarily from the centre), nasal/temporal areas (averaged thickness of each layers from 500 to 2000 μm from the centre, nasally and temporarily respectively) were used. Exploratory analysis was also carried out on the following retinal layers: ganglion cell complex (GCC - combination of ganglion cell layer and inner plexiform, which is difficult to segment due to poor contrast between the two layers), inner nuclear (INL), outer plexiform (OPL), outer nuclear (ONL), inner segment (IS) and outer segment layer (OSL).

4.4. Results

4.4.1. Retinal layers

Mean retinal thickness (μm) for processing and photoreceptor retinal layers, and retinal pigment epithelium in patients and controls are shown in Figure 4.3. There were no significant differences between patients and controls in the thickness of the retinal processing layers ($t=1.073$; $df=38$; $p=.290$) or the retinal pigment epithelium ($t=.160$; $df=38$; $p=.874$). However, photoreceptor layers were thinner in patients compared to controls ($t=3.223$; $df=38$; $p=.003$).

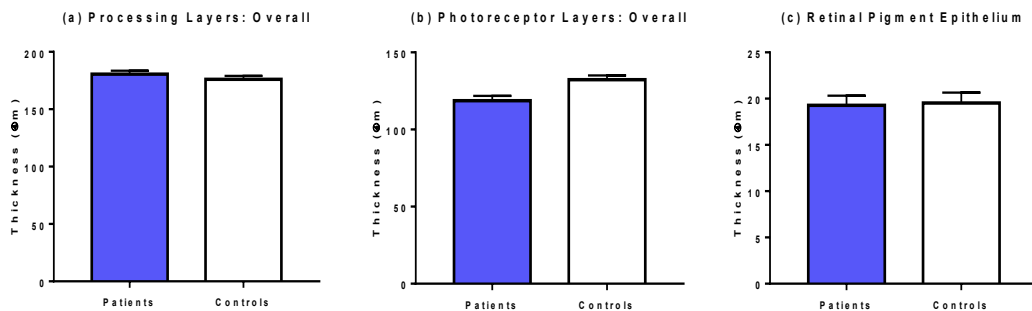


Figure 4.2. Mean thickness (μm) of (a) processing layers, (b) photoreceptor layers and (c) retinal pigment epithelium (RPE) for patients and controls. Error bars are 1 S.E.M.

To determine the specificity of this effect, the photoreceptor layers were divided into the following structures: outer nuclear layer, inner segment and outer segment layer (Figure 4.3). While there was no effect of ME/CFS on the inner segment ($t=4.88$; $df=38$; $p=.628$) there was significant thinning in the outer nuclear layer ($t=2.978$; $df=38$; $p=.005$) and the outer segment layer ($t=3.393$; $df=38$; $p=.002$).

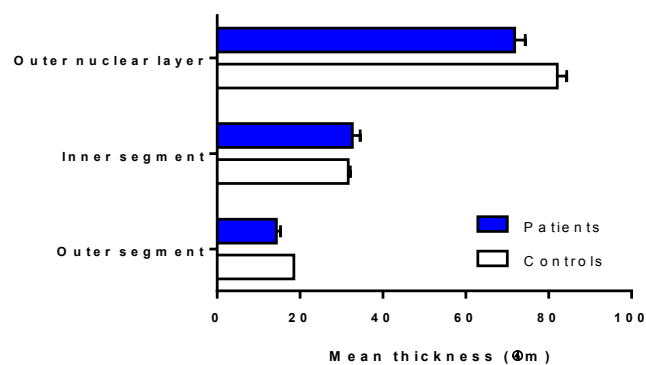


Figure 4.3 Mean thickness (μm) of outer nuclear layer, inner segment and outer segment of the photoreceptor layers of the retina for patients and controls. Error bars are 1 S.E.M.

4.4.2. Optic Nerve Head

Figure 4.4 shows Optic Nerve head measures for patients and controls. There were no significant differences between patients and controls (Table 4.3).

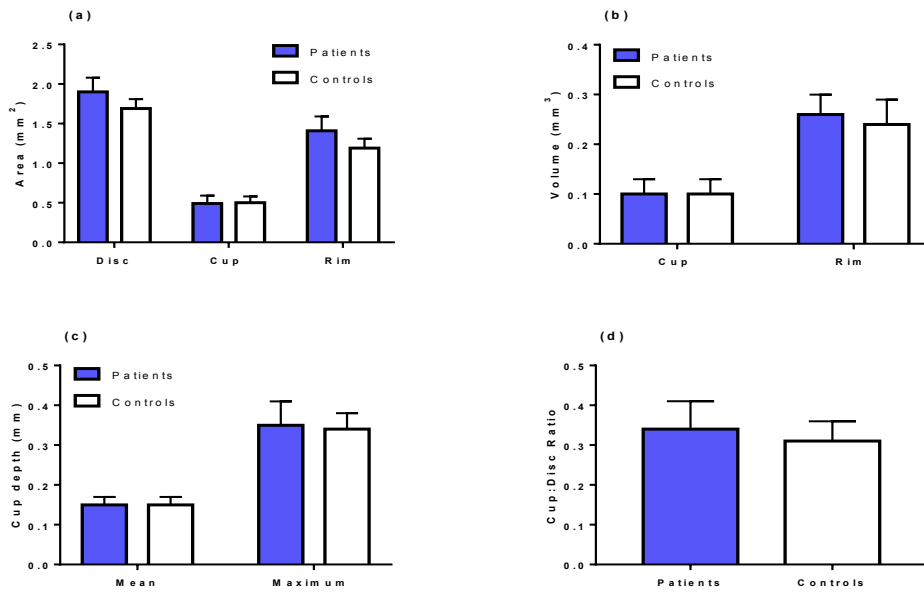


Figure 4.4 Optic Nerve head measures for patients and controls. (a) area (mm^2) of disc, cup and rim (b) volume of cup and rim (mm^3) (c) minimum and maximum cup depth (mm) (d) showing cup:disc ratios. Error bars are ± 1 S.E.M.

ONH Measure	T	df	Sig. (2-tailed)
Disc Area	1.009	30	0.321
Cup Area	-0.024	30	0.981
Rim Area	1.008	30	0.321
Cup:Disc Ratio	0.303	30	0.764
Cup Volume	0.047	30	0.963
Rim Volume	0.222	30	0.826
Mean Cup Depth	0.131	30	0.897
Max Cup Depth	0.178	30	0.860

Table 4.3. *t*-Test results (patients vs. controls) for each measure of the Optic Nerve head.

4.5. Discussion

The findings presented in this chapter revealed that ME/CFS was associated with thinning in the photoreceptor layers of the retina, namely the Outer Nuclear Layer (ONL) and outer segment (OS). Morphological changes to the retina may account for some of the differences in visual sensitivity outlined in Chapter 3. They may also account for hypersensitivity commonly reported by people with ME/CFS. The significant reduction in thickness in both the ONL and OS may also indicate a potential biomarker.

These findings may also account for some of the deficits shown in Chapter 3. For example, there is a good deal of evidence that Contrast Sensitivity is dependent upon the sensitivity of the retina, which sends excitatory signals to the LGN (Kaplan & Shapley, 1986). Indeed a number of studies have shown a correlation between poor Contrast Sensitivity and retinal thinning in ocular diseases such as glaucoma (Amanullah et al., 2017) and in neurodegenerative diseases such as MS (Bock et al., 2011).

Thinning in the photoreceptor layers of the retina may also account, at least in part, for ME/CFS-related deficits in colour discrimination. The ONL and OS are made up of the rods and cones. Rods are responsible for scotopic vision and cones are responsible for photopic colour vision anomalies. See Figure 4.5.

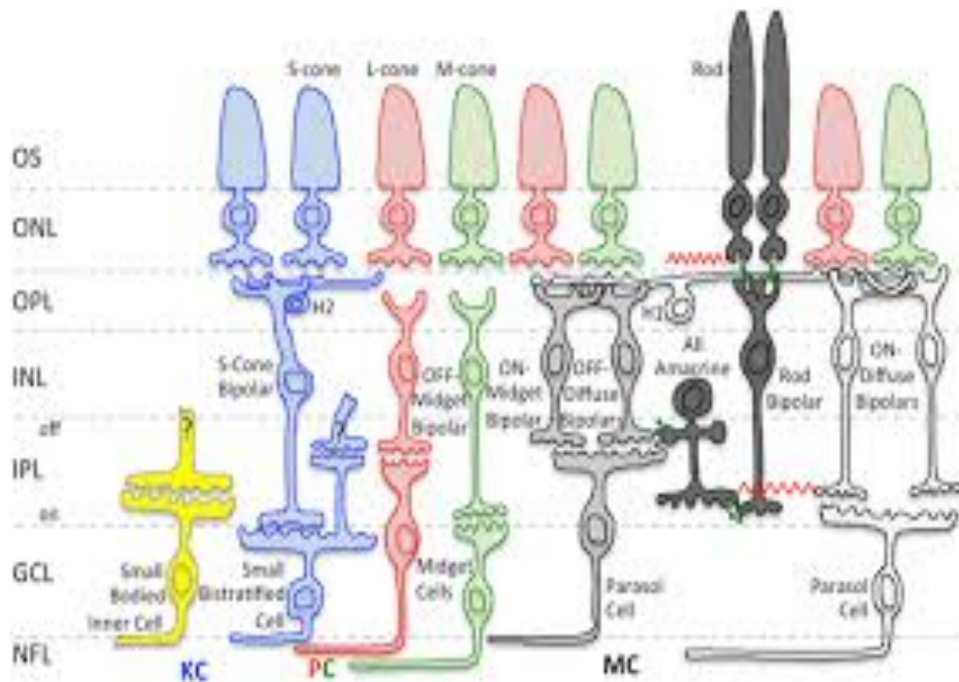


Figure 4.5. Rods and Cones Illustrated in Retinal Layers. *Frontiers:Vision under Mesopic Scotopic Illumination*, www.frontiersin.org, (Zele & Cao, 2005).

A thinning/shortening of those layers particularly, the cones are likely to inhibit the ability to discriminate colour perfectly. This present study highlights greater deficiencies in the yellow/green and blue/green hues but across the red hues. ME/CFS may only compromise blue and yellow cones but without additional testing with equipment with much higher resolution, this is currently unknown.

One potential caveat concerning the data presented here is that they represent the interpretation of the OCT scans by one observer. 5 images per eye were taken from group and the clearest image was chosen to manually segment. Typically a second blinded observer would be recruited to analyse the images, after which between-observer comparisons would be performed. On a related note, if this retinal segmentation were to be repeated, differences results may

arise due to fine discrepancies between images i.e. what might appear the clearest image to one researcher may not be the same to another. Consequently a different choice of image may mean a different retinal thicknesses are found. Indeed, (Lujan et al., 2015) found that outer receptor artefacts can be created by entry position at the pupil using SD – OCT readings. Specifically, they found that entry position artefacts can cause substantially different ONL and Henle fibre layer (HFL) thicknesses. They imaged 57 normal eyes and assimilated segmentation at top of the HFL, the external limiting membrane and at the junction of the HFL and the ONL and found the thicknesses of the HFL and ONL varied across different eccentricities. They concluded that differentiation between the HFL and ONL would be more accurate using directional OCT, method that can manipulate the beam depending on the pupil entry position. An example is given in Figure 4.6.

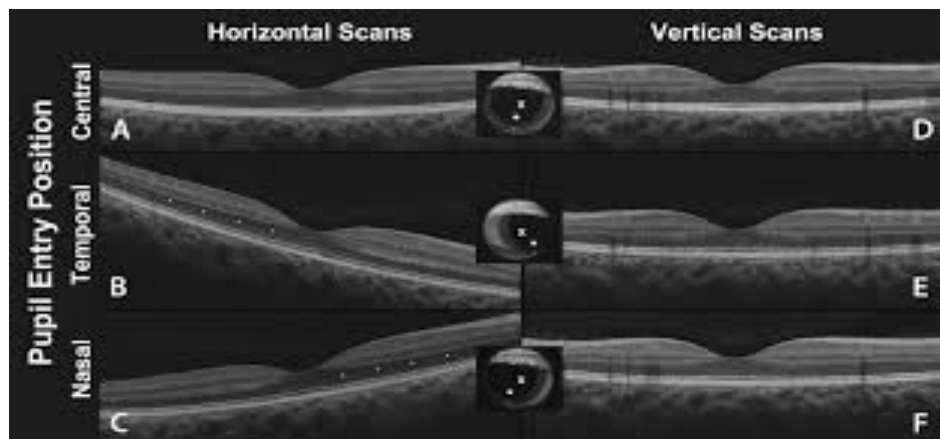


Fig 4.6. Revealing Henle's Fiber Layer Using Spectral Domain Optical Coherence Tomography (Brandon et al., 2011)

5. General Discussion and Future Directions

The experiments outlined in this thesis have shown that people with ME/CFS exhibit a range of visual markers associated with their illness. Principal differences included reduced absolute accommodation, larger pupil diameters, reduced colour discrimination, poor spatial Contrast Sensitivity and thinning in the photoreceptor layers of the retina.

Reduced Absolute Accommodation was observable in both the presbyopic and pre-presbyopic age range of the ME/CFS cohort in comparison to controls. These findings corroborated a previous study by Godts et al., (2016) who also reported reduced accommodation levels. Additionally, the present study found a pattern in proportionality between the patient cohort and control i.e. the younger ME/CFS individuals, the larger the discrepancy in accommodation levels. Transient blurring was also more likely to occur amongst patients during examination. On the basis of these findings, it might be reasonable to speculate people with ME/CFS may be more susceptible to an accelerated process of lens hardening. Theoretically, this could be confirmed best by biometric axial length measures of isolated donor lenses of the ME/CFS community but given that ME/CFS is not related unconditionally to premature death, biometric measures would prove fruitless. The correlation between a hard lens (lens sclerosis) and the inner nuclear colour of the lens is however well established (Bron, Vrensen, Koretz, Maraini & Harding, 2000). Nuclear sclerosis appears as a yellowing of the lens nucleus and is identifiable with a Slit-Lamp Biomicroscope. The greater the thickness of the nucleus,

the deeper the yellowing. Its severity can be categorised against a photographic grading scale and although open to inter and intra-practitioner variance, this method of classification is common practice. The correlation between nuclear sclerosis and visual symptoms is also well known e.g. sensitivity to light, glare, cloudy/blurred dim vision, fading of yellow colours, increased difficulty with night vision, halos around lights, brighter lights for near focus. Notably these symptoms match many of those highlighted in questionnaire studies (e.g. Potaznick & Kozol, 1992; Vedelago, 1997; Mastropasqua et al., 2000; Hutchinson et al., 2014).

Nuclear sclerosis could also account, at least in part, for the Contrast Sensitivity deficit at lower spatial frequencies. The prospect of earlier lens changes may well occur in isolation in the ME/CFS community. However, it is more likely that the symptoms are multifactorial and are accompanied by fatigued EOM's. Indeed, the Ocular Motility results presented in this thesis, although limited by both patient and practitioner subjectivity, indicate that there is an element of fatigability of the EOM's particularly in the superior gazes of which 71% of the ME/CFS cohort reported discomfort. The possibility of fatigued EOM's is strengthened by the findings of Godts et al., (2016) who also noted reduced convergence which is dependent upon primarily the yoked action of the medial recti. As such, this tentative hypothesis may warrant further study, where follow-up studies on larger numbers should include slit lamp examination specifically in the search for and classification of nuclear sclerosis and measures of absolute accommodation in the younger ME/CFS sufferers to explore if the pattern of proportionality exists in the under 35's.

In respect of our Pupil Function tests using the Ophthalmoscope, we found that 35% of ME/CFS patients possessed an unusual accommodative pupil response conducted at 40cm where the right pupil appeared larger and reacted more slowly to a near target. We then determined objective findings using SWIFT Pupillometer at 128cm and found the ME /CFS group to exhibit larger pupil diameters than controls. Furthermore, there was again a tendency for a larger right diameter but this was not found to be significant. These findings may be indicative of anisocoria in both the Pupil Response at Near (PRN) and SWIFT findings at 40cm and 128cm respectively. For example, the Holmes-Adie Pupil bears similarities with our PRN findings in that there is an accommodative element and it occurs unilaterally in 80% of cases (Crowell, Feldman & Tripath, 2014; Martinelli & Minardi, 2001; Mayer, 2014). Given that the stages of progression involved in the Holmes-Adie Pupil include a damaged Ciliary Ganglion and potential aberrant re-innervation of the Ciliary Body, the greater effort to accommodate to a target at 40cm may exaggerate any anisocoria leading to a 35% occurrence at near. The rate of anisocoria in the general population however is 19 % (Lam, Thompson, & Corbett, 1987). The 45% anisocoria may be a genuine find but together with an apparent predilection for the RE, there is no way of confirming that this is repeatable unless replicated in larger numbers. The other issue we have with assessing anisocoria is that the existing literature describes the Holmes- Adie Pupil as organic and changing in nature which makes classification using Pupil Function tests extremely difficult. The best way to confirm the possibility of the Holmes-Adie is by confirming the presence of segmental paralysis of the sphincter using the slit lamp (Kardon et.al 1998; Bremner & Smith 2007; Pate, 2016). Excessive constriction can also be observed after instillation of 0.1% Pilocarpine (parasympathomimetic) as 80 % of Holmes- Adie have show cholinergic super sensitivity. Examining for the absence of

and Deep tendon reflexes in the knee would also be required (Kardon et al 1998, Mayer 2014, Pate 2016).

With respect to colour vision, the FM-H100 revealed that both the patients and control groups made most errors within the yellow/green and blue/green areas but this was significant in the ME/CFS cohort. This indicated possible involvement of the outer retinal layers and indeed the initial OCT analyses confirmed the Outer Nuclear Layer and the Outer Segment layer appeared significantly thinner in patients compared to controls. These findings also support the notion that people with ME/CFS may be susceptible to premature nuclear sclerosis, particularly in the context of reports of fading among the yellow hues (Salvi et al., 2006).

5.1. Visual Symptoms and ME/CFS Diagnosis

The findings outlined above highlight the importance of Optometric assessment in ME/CFS. Of particular interest in this regard is accommodation where previous studies (Godts et al., 2016) and the present study have revealed abnormalities. In keeping with the recommendations of Godts et al., (2016), accommodation and the near point of convergence should routinely be included in any suspect or diagnosed ME/CFS sufferer. In addition, the findings outlined in this thesis also suggest that special consideration be given to the pre-presbyopes given that reduced accommodation is normally unexpected in this age group; an Optometrist may find they need to prescribe a near addition of around +0.75D in pre-presbyopic individuals with ME/CFS. It may also be the case that an Optometrist

may encounter difficulties when prescribing a Near Addition because the patient could be experiencing transient blur. Numerous “re-checks” may occur after the initial prescribing of the Near Addition because of transient blur. Similarly if a patient needs frequent increases in Near Addition only after a few months after a dispense of spectacles, suspicions should be raised and a referral to a specialist considered.

The Ocular Motility test should be routinely performed when presented with a patient experiencing symptoms of prolonged and unexplained fatigue. Particular attention may need to be paid on symptoms reported in the upper hemisphere and the Optometrist may consider a 10 minute Ocular Motility Test. A referral might be considered if discomfort is reported within the first cycle along with symptoms that do not get worse with repetition and are accompanied with symptoms with no visible signs of jerky movements. Similarly, we suggest that metamorphopsia seen on the Amsler Grid with an absence of pathology and/or dynamic metamorphopsia that seems to increase in size and takes longer than a few seconds to complete and which might eventually cover the entire expanse of the Amsler Grid should be not be dismissed and communicated to the specialist in the referral. The ability of the FM-H100 to detect subtle colour differences is known by the Optometric profession and can be completed at a fraction of the time and cost that would be incurred by the Hospital Eye Service and GP referrals. In this instance however we recommended the FM-H100 may best serve ME/CFS patients as a tool whereby the progression of disease is monitored rather than revealing specific underlying visual system changes *per se*.

Although the OCT data presented here is suggestive of ME/CFS-related pathology in the photoreceptor layers of the retina, this work is preliminary and should be followed up by a larger study and also, with directional OCT. Directional OCT allows the elimination of horizontal pupil entry artefacts (Lujan, 2011). This was not possible in the current study but should be controlled for in future studies to provide more accurate data.

In terms of the clinical utility of determining Contrast Sensitivity in ME/CFS, the psychophysical methods we have used in the present study are likely to be too time-consuming and standard eye charts such as the Pelli-Robson Test are unlikely to be sufficiently sensitive. Recent developments in Bayesian adaptive procedures which provide quick measurement of the Contrast Sensitivity function (Lesmes, Jeon, Lu, & Doshier, 2006; Lesmes, Lu, Baek, & Albright, 2010) are promising in this context. These procedures have been shown to achieve good agreement with Contrast Sensitivity functions determined using conventional psychophysical methods (such as those employed in the present study) in normal and clinical vision (Pelli & Bex, 2013), where such procedures have been successfully implemented for measuring of Contrast Sensitivity in amblyopia (Hua et al., 2010). They have also been suggested as a means of measuring the Contrast Sensitivity functions of low vision patients (Chung & Legge, 2016).

5.2. Visual Symptoms as a Window into ME/CFS as an Autoimmune Disease

Like people with ME/CFS, Multiple Sclerosis (MS) patients exhibit a wide range of visual symptoms. These, and other, similarities lend weight to the notion that ME/CFS is a disease of the autoimmune system. MS is an inflammatory, neurodegenerative autoimmune disease (Martínez-Lapiscina et al., 2014). MS and ME/CFS share phenomenology and neuroimmune characteristics (Morris & Maes, 2013b). The onset of ME/CFS has been associated with infections and autoimmune disorders and, like MS patients, people with ME/CFS exhibit a range of immune abnormalities indicating dysregulation of the immune system (Morris, Anderson, Galecki, Berk, & Maes, 2013). Optic neuritis is common in MS (Chen & Gordon, 2005) along with reports of loss of vision, colour vision disturbances, pain in the eyes, blurred vision, and visual fatigue (Martínez-Lapiscina et al., 2014). The Contrast Sensitivity deficits shown in this thesis also bear similarity to the profile of Contrast Sensitivity deficits recently reported for multiple sclerosis (MS) (Vieira-Gutemberg, Mendes-Santos, Cavalcanti-Galdino, Santos, & SIMAS, 2014). There was poorer Contrast Sensitivity at 0.5cpd, 1.25cpd and 2.5cpd in those diagnosed with MS and in the present ME/CFS group, Contrast Sensitivity was significantly depressed at 0.5cpd and 1cpd. In keeping with the OCT data reported here for ME/CFS, MS has also recently been associated with retinal thinning and consequently with brain atrophy (Gordon-Lipkin et al., 2007). MS is typically diagnosed by observations of sclerosis on the brain revealed by MRI scans. Similarly, findings could therefore be potentially extrapolated to suggest the scanning of ME/CFS brain using either traditional Magnetic Resonance Imaging (MRI) or

functional Magnetic Resonance Imaging (fMRI) which measures brain activity.

5.3. Future Directions

Because there is no established cause of the condition, no conclusive tests to determine its presence and no definitive outward signs that set it apart from other disorders, clinicians must rely on patients' self-perceptions and reports. As such, the findings reported, along with other visual problems experienced by patients with ME/CFS, may have important implications for diagnosis of ME/CFS and may provide some insights into its aetiology.

The data presented here are based on relatively small pilot studies in twenty ME/CFS patients and matched controls. This is also often the case in other previous studies that have examined visual signs and symptoms of ME/CFS. Future studies, therefore, should investigate key visual deficits (revealed here and also in previous small-scale studies) in larger groups of patients and controls. The inclusion of additional comparison groups such as a group of individuals suffering from fatigue unrelated to ME/CFS and a group with MS, are also likely to provide useful information.

6.0 Appendices

APPENDIX 1- DEPAUL SYMPTOM QUESTIONNAIRE

Vision Items – DePaul Symptom Questionnaire

(from Jason et al., 2010., American Journal of Biochemistry and Biotechnology 6:120-35)

For each of the symptoms listed below, please rate its **FREQUENCY** and **SEVERITY** (how much it has bothered you) within the past 6 months

SYMPTOM FREQUENCY

		None of the time	A little of the time	About half the time	Most of the time	All of the time
1	Eye pain	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
2	Sensitivity to bright lights	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
3	Unable to focus vision and/or attention	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
4	Loss of depth perception	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

SYMPTOM SEVERITY/BOTHER

		Symptom not present	Mild	Moderate	Severe	Very severe
1	Eye pain	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
2	Sensitivity to bright lights	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
3	Unable to focus vision and/or attention	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
4	Loss of depth perception	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

APPENDIX 1- DEPAUL SYMPTOM QUESTIONNAIRE

APPENDIX 1- DEPAUL SYMPTOM QUESTIONNAIRE

For each of the statements listed below, please indicate how much each symptom has **BOTHERED YOU** in the past month.

	Not at all	A little	Quite a lot	A lot	N/A (symptom not present)
1 Strong light makes me feel uncomfortable	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
2 Flicker makes me feel uneasy	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
3 I feel pain behind my eyes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
4 I feel pain in my eyes when I stare at something	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
5 I get double-vision when I stare at something	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
6 Looking bright lights gives me a headache	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7 My eyelids feel heavy	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
8 My eyes feel achy	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
9 I dislike looking at striped patterns	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
10 I find it difficult to open my eyes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
11 I see unusual colours when I look at things	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
12 I see unusual colours when looking at a white background	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

APPENDIX 1- DEPAUL SYMPTOM QUESTIONNAIRE

Photosensitivity Scale

(adapted from Shigihara et al., 2010, Behavioural Medicine 36:109-112)

For each of the statements listed below, please indicate the **FREQUENCY** with which you have experienced each symptom in the past month.

	Never	Rarely	Sometimes	Frequently	Always
1 Strong light makes me feel uncomfortable	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
2 Flicker makes me feel uneasy	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
3 I feel pain behind my eyes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
4 I feel pain in my eyes when I stare at something	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
5 I get double-vision when I stare at something	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
6 Looking bright lights gives me a headache	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7 My eyelids feel heavy	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
8 My eyes feel achy	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
9 I dislike looking at striped patterns	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
10 I find it difficult to open my eyes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
11 I see unusual colours when I look at things	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
12 I see unusual colours when looking at a white background	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

APPENDIX 1- DEPAUL SYMPTOM QUESTIONNAIRE

APPENDIX 1- DEPAUL SYMPTOM QUESTIONNAIRE

For each of the statements listed below, please indicate how much each symptom has **BOTHERED YOU** in the past month.

		Not at all	A little	Quite a lot	A lot	N/A (symptom not present)
1	Strong light makes me feel uncomfortable	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
2	Flicker makes me feel uneasy	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
3	I feel pain behind my eyes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
4	I feel pain in my eyes when I stare at something	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
5	I get double-vision when I stare at something	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
6	Looking bright lights gives me a headache	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7	My eyelids feel heavy	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
8	My eyes feel achy	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
9	I dislike looking at striped patterns	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
10	I find it difficult to open my eyes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
11	I see unusual colours when I look at things	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
12	I see unusual colours when looking at a white background	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

APPENDIX 1- DEPAUL SYMPTOM QUESTIONNAIRE

APPENDIX 2- PARTICIPANT INFORMATION SHEET

PARTICIPANT INFORMATION SHEET

Questionnaires

To accompany our experimental measures, we have included a number of questionnaires. One will be sent to you to be completed before you attend. This is the DePaul Symptom Questionnaire which we include in our participant screening section of any publications stemming from the work. The others contain questions about visual symptoms in ME/CFS, vision-related quality of life and fatigue. We will administer them when you arrive to take part in the study. The other questionnaires should take only a few minutes each to complete. We will use responses to correlate subjective visual problems against our experimental measures (outlined below). We have done this before and found very good agreement between self-reports and objective tests of vision.

Basic visual function

We will perform a few basic visual tests. These are very similar to those your optician might perform. In these tests, you will be asked to read letters from a chart so that we can ascertain your visual acuity (the smallest detail your eye can see).

Pupil Examination

The pupil examination is a simple but effective test. A light is shone into the eye and the reaction of the pupil is observed. Any disruption in the visual pathway may manifest as an abnormal pupil reaction.

APPENDIX 2- PARTICIPANT INFORMATION SHEET

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Spatial Contrast Sensitivity

Contrast sensitivity is a very useful measure of visual function. Even when visual acuity seems normal, the ability of the visual system to detect things that are very low in contrast might still be affected. This can tell us about what is happening beyond the optics of the eye, in the retina at the back of the eye and the visual pathways from the retina to the visual processing areas of the brain. In a contrast sensitivity task, participants look at striped patterns (gratings) on a computer screen and respond to a grating pattern each time it is seen by indicating its orientation (Vertical stripes or horizontal stripes). Some patterns will contain more stripes than others. This allows us to determine how sensitive people are to contrast at different levels of detail.

Dry Eye Test

Dry eye tends to be a reported symptom of ME/CSF. We will examine this using Schirmer's Test of Tear Flow. The Schirmer test involves placing a small strip of filter paper on the inner eyelid for 5 mins in both eyes. The length of moistened filter paper is then recorded.

Colour Vision

There are 2 colour vision tests that we will use. The 1st is the Ishihara test that is often used in clinical practice to test for colour vision deficits. The 2nd test is called the Farnsworth Munsell Hue 100 test. It is more sensitive test of colour discrimination requiring the participant to arrange coloured tiles in order of colour progression.

APPENDIX 2- PARTICIPANT INFORMATION SHEET

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Retinal Imaging

We will look at the retina in detail using a technique called Optical Coherence Tomography (OCT). The OCT machine gives us very detailed and useful images of the inside layers of the retina. It is not invasive in any way. All that is required is that participants look into the device.

APPENDIX 2- PARTICIPANT INFORMATION SHEET

APPENDIX 3- PARTICIPANT CONSENT FORM

Participant Consent Form

BACKGROUND INFORMATION

Title: Ophthalmic correlates of CFS/ME

Researchers: Nadia Ahmed, Claire Hutchinson, Frank Proudlock

Purpose of data collection: Research

Details of Participation: The present study is concerned with the outcomes of ophthalmological investigation in CFS/ME. In this study, we will determine performance on a range of tests of visual sensitivity, such as visual acuity, and sensitivity to contrast, colour and depth. We will also take high resolution images of the retinae (the back of the eye). We will establish the presence of dry eye syndrome (a problem related to tear production), which causes eye pain and itchiness in the eyes, problems commonly reported by people with CFS/ME. All measurements from CFS/ME participants will be compared to a group of matched control participants.

CONSENT STATEMENT

1. I understand that my participation is voluntary and that I may withdraw from the research at any time up until give specific date or other time point, without giving any reason.
2. I am aware of what my participation will involve.
3. My data are to be held confidentially and only name of researcher and/or supervisor will have access to them.
4. My data will be kept in a locked filing cabinet for a period of at least five years after the appearance of any associated publications. Any aggregate data (e.g. spreadsheets) will be kept in electronic form for up to one year, after which time they will be deleted.
5. The overall findings may be submitted for publication in a scientific journal, or presented at scientific conferences.
6. This study will take approximately 12 months to complete.
7. I will be able to obtain general information about the results of this research by giving the researcher my email address or postal address now. The duration of this study will be approximately 1 year (June 2015 to June 2016). As such, a summary of the findings will be provided in June 2016. A synopsis will also be published in Breakthrough Magazine, the official quarterly magazine of ME Research UK and submitted for publication in scientific journals.

I am giving my consent for data to be used for the outlined purposes of the present study

All questions that I have about the research have been satisfactorily answered.

I agree to participate.

Participant's signature: _____

Participant's name (please print): _____

APPENDIX 3- PARTICIPANT CONSENT FORM

7.0 References

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