Abstract

Objective Electrophysiological Assessment of Neural Functioning in Multiple Sclerosis and Healthy Control Participants

Multiple sclerosis (MS) is a chronic, immune-mediated disease of the central nervous system with an unpredictable course. The heterogenous nature of the disease course and unpredictable nature of clinical exacerbations make MS notoriously difficult to diagnose. The current study used a battery of short-duration visual evoked potentials (VEPs) administered to individuals with multiple sclerosis (MS) (n = 20) and healthy control participants (CN) (n = 18). VEP responses examined specific neural mechanisms and potential group differences. Steady-state VEPs were elicited from contrast-sweep conditions (bright and dark isolated checks) monocularly to examine the integrity of the visual pathways. Signal-to-noise ratios, amplitude, and sine and cosine coefficients were analyzed under each condition for each group. Visual inspection of the data via clustered bar graphs indicate that the expected pattern was evident for the outcome measures, with the MS group's responses generally more deficient when compared to the control group. Univariate and multivariate statistics were used to examine patterns of responses and non-parametric tests were used to assess group differences. Linear mixed effects modeling (LMM) was used to identify significant differences for the outcome measures of signal-to-noise (SNR) and amplitude (AMP). In an exploratory analysis, LMM modeling was used to analyze contrast-response functions with the biophysical model for three parameters: initial contrast gain, shunting coefficient, and phase. Classification accuracy was assessed for all measures using receiver-operating-characteristic (ROC) curve analysis and logistic regression. LMM analysis of SNR revealed

significant differences for the fixed effects of depth of modulation (log_2DOM) (Δ-2LL = 296.343, *df* = 1, *p* < .01), Group x Log_2DOM interaction (Δ-2LL = 7.463, *df* = 1, *p* < .01), and test condition (bright versus dark checks) (Δ-2LL = 37.579, *df* = 1, *p* < .01). Similarly, LMM analysis of amplitude revealed significant differences for the fixed effects of log_2DOM (Δ-2LL = 323.031, *df* = 1, *p* < .01), Group x Log_2DOM interaction (Δ-2LL = 18.917, *df* = 1, *p* < .01), test condition (bright versus dark checks) (Δ-2LL = 31.022, *df* = 1, *p* < .01), and Test x Log_2DOM interaction effect (Δ-2LL = 8.939, *df* = 1, *p* < .01). LMM analysis of initial contrast gain revealed a significant difference for the fixed effect of test condition (bright versus dark checks) (Δ-2LL = 9.732, *df* = 1, *p* < .01). Similarly, LMM analysis of phase also revealed a significant difference for the fixed effect of test condition (Δ-2LL = 7.642, *df* = 1, *p* < .01). A final LMM analysis for the shunting coefficient revealed a significant difference for the main effect of group (Δ-2LL = 4.906, *df* = 1, *p* < .05). An assessment for heterogeneity of regression coefficients for the sine versus cosine coefficients of the fundamental frequency component of the response (at the stimulus frequency of 10 Hz) revealed a significant interaction effect for the bright check condition, Group x Cosine Coefficient (*F* (2, 72) = 23.14, *p* < .001). For the dark check condition, there was also a significant interaction effect of Group x Cosine Coefficient (*F* (2, 72) = 26.05, *p* < .001). ROC curve analysis and logistic regression revealed moderate predictive accuracy for classification of individuals with MS and healthy controls. The current study findings support the use of bright and dark isolated-check VEP conditions in the assessment of neuronal dysfunction in patients with MS. While this study is small, the significant differences identified between the MS and control populations justify continued research utilizing these

novel VEP techniques as a paraclinical tool in the diagnosis and assessment of neuronal dysfunction in individuals with multiple sclerosis.

Objective Electrophysiological Assessment of Neural Functioning in Multiple Sclerosis and Healthy Control Participants

by

Roseann Archetti

Submitted in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

in the Ferkauf Graduate School of Psychology

Yeshiva University

August 2020

The committee for this dissertation consists of:

Frederick W. Foley, Ph.D., Chairperson, Ferkauf Graduate School of Psychology, Yeshiva University

Vance Zemon, Ph.D., Ferkauf Graduate School of Psychology, Yeshiva University

James Gordon, Ph.D., Hunter College of the City University of New York

Acknowledgements

I gratefully acknowledge those who supported me throughout my graduate career. Dr. Foley, your passion for training is truly unmatched and your enthusiasm for research has proven to be contagious. Despite much reluctance, I can say you converted me to a believer, “Grad school is lots of fun”.

Vance, you truly are one of the most genuine and intelligent people I know. From the moment you first lugged the massive suitcase containing the EvokeDx machine to Holy Name to the hours spent at your kitchen table and on the phone analyzing data, I am so grateful for your persistence and eagerness to guide me through this process.

I wish to acknowledge committee member, Dr. Gordon, for his flexibility and willingness to be part of this project. I also wish to acknowledge my readers, Dr. Weinberger and Dr. Swencionis, whose guidance during the dissertation process and the courses throughout the program helped me to develop the research and clinical skills needed to complete this project.

Most importantly, I wish to acknowledge the individuals who served as participants in this study, particularly those who are diagnosed with MS, who participated solely for the mission of advancing our understanding of this disease.

Dedication

To my Mom, Dad, and brothers, Dan and Rob, who have been putting up with my inability to take the easy road from day one. Mom and Dad, I do not have the words to express how grateful I am for the life you have provided me and the support you give me each day. Mom, I know that I have conditioned a stress response in you every time your cell phone rings, and I pledge to try my hardest to decondition that response upon completion of my graduate career.

To my partner Steve, for providing unwavering support and a logical, level-headed approach to each crisis I introduce into our lives.

To my dearest friends and to my Chicken, for providing a never-ending supply of love, support, entertainment, and purpose to my life.

Table of Contents

List of Tables

List of Figures

Chapter I: Introduction

Multiple sclerosis (MS) is a chronic, immune-mediated disease of the central nervous system (CNS) with an unpredictable course. As of 2017, there were estimated to be nearly one million adults living with MS in the United States, a 362.6 per 100,000 rate (Wallin et al., 2019). MS disease progression can take several courses, but regardless of subtype the majority of cases trend toward increased lesion burden in the CNS as well as clinically worsening symptoms over time (Filippi et al., 2018; Lublin et al., 2014). Advancements in medications, specifically disease-modifying therapies (DMT), have drastically altered outcomes for many individuals with MS, serving to more optimally manage relapses and lower the accumulation of disability, in turn improving overall quality of life (Rae-Grant et al., 2018).

Symptom presentation is variable due to the unpredictable nature of lesion location, making MS notoriously difficult to diagnose. The diagnostic process for MS is further complicated by the heterogeneity of clinical presentations, and so clinicians use the assistance of rule-out techniques while also examining the presence of "typical" versus "atypical" symptoms of the disease (Giesser, 2011). Typical presenting symptoms are not unique to MS, therefore, further examination into the disease process is required before a more definitive diagnosis can be made. The McDonald Criteria for the diagnosis of multiple sclerosis, developed by the International Panel on Diagnosis of Multiple Sclerosis, has been widely used in research and clinical practice (Thompson et al, 2018). Historically, MS diagnostic criteria have required fulfillment of dissemination in time (DIT) and dissemination in space (DIS) of lesions in the CNS. The McDonald Criteria have undergone multiple revisions to incorporate technological advancements, including clinical, imaging, and laboratory evidence, with the goal of providing faster and more accurate proof of dissemination in time and space, so that individuals can begin

treatment regimens sooner. Accuracy of diagnosis is paramount, as misdiagnosis can result in adverse physical, psychosocial, and financial consequences (Solomon et al., 2016). The most recent revision of the McDonald Criteria, completed in 2017, emphasized an increased utilization of paraclinical assessments to supplement clinical findings in order to allow for earlier and more accurate diagnosis (Thompson et al, 2018).

The ability for a skilled clinician to characterize clinical symptoms serves as an invaluable tool in the diagnostic process, but the MS disease process can be unforgiving and disease activity can be ongoing, even in the absence of observable manifestations of brain inflammation and demyelination. The presence of subclinical, silent lesions has been identified in autopsy studies, with research to support that these types of inflammatory lesions can occur up to ten times as often as clinical relapses (Filippi, Rocca, Martino, Horsfield, & Comi, 1998; Lebrun et al., 2008; Miller, Barkof, & Nauta, 1993; O'Riordan et., al, 1998). At this point, medical teams rely on radiological and paraclinical technology to further evaluate aspects of the CNS that are not observable from clinical examination alone. Radiological techniques can be used early in the diagnostic process to assist with the fulfillment of dissemination in time and space. Magnetic resonance imaging (MRI) is currently the most widely accepted paraclinical test to aid in the diagnosis of MS and can be used as a substitute for clinical findings in the determination of dissemination in time (DIT) or dissemination in space (DIS) in patients with an initial inflammatory demyelinating event of the CNS, also known as clinically isolated syndrome (CIS) (Thompson et al, 2018; Filippi et al., 2016). Other diagnostic tests that have been used to diagnose MS are optical coherence tomography (OCT), identification of cerebrospinal fluid specific oligoclonal bands, and neurophysiological testing, such as visual evoked potentials (VEP). Supplemental tools serve to detect disease activity that may not have presented clinically,

and in doing so, clinicians can build confidence in their diagnosis, subsequently initiating disease altering treatments earlier.

MRIs are capable of detecting gross abnormalities in brain structure at a single point in time, but a number of limitations to their clinical utility have emerged in the literature. Conventional MRI techniques lack a direct examination of function, have limited associations with clinical status, and, depending on the techniques used, may involve injection of gadolinium-based contrast agents (Bakshi et al., 2008). Electroencephalography (EEG) has the ability to measure electrical activity of the brain on a time scale of milliseconds, providing a map of brain activity with high temporal resolution. Examination of visual evoked potentials, or VEPs, is a technique that reflects electrical responses of the brain to visual stimulation extracted from the ongoing EEG. VEPs reveal the sum of excitatory and inhibitory postsynaptic potentials in cortical neurons and offer the opportunity to detect subtle changes in brain dynamics that are sensitive to disease processes and neural deficits. Historically, conventional VEP testing has required subjective analysis by an expert electrophysiologist examining the waveform and making judgements to identify peak times. This method requires a determination be made in a time-domain response, which is often embedded in considerable noise, thus limiting the clinician's ability to interpret the data appropriately. Hundreds of points make up a transient response, but conventional analysis focuses on just a few select deflections, losing much of the informational content. Research conducted in our laboratory over many years has resulted in electrophysiological tests that examine the VEP by quantifying entire responses objectively in the frequency domain, both retaining the data for the entire response while also removing subjective analysis. These techniques provide clinicians with an objective, non-invasive tool that can be used to detect neuronal dysfunction.

Much of the existing MS literature has been conducted with the conventional contrast-reversing checkerboard stimulus, which is limited in its ability to separate overlapping contributions from the various brain mechanisms. Research using VEPs to evaluate visual function in MS has revealed several consistent findings related to the disease process, which include increases in response latency to low contrast stimuli, as well as decreases in VEP amplitude attributed to axonal loss (Balcer, Miller, Reingold, & Cohen, 2015; Galetta & Balcer, 2013; Hamurcu, Orhan, Sarıcaoğlu, Mungan & Duru, 2017; Sakai et al., 2011; Thurtell et al., 2009). Sakai et al. (2011) suggest that low contrast stimuli may be most sensitive in detection of delayed responses within the visual system of individuals with MS. Increased latency in peak times with a normal appearing waveform (e.g., normal amplitude) is hypothesized to be a result of conduction block caused by demyelination (Blacer et al., 2015). Blacer et al., (2015) also suggested reduced VEP amplitude may result from conduction block due to either demyelination or damage to/loss of axons. While theories can be drawn based on amplitudes and peak times in the waveform, conventional VEPs lack the resolution needed to examine more specific mechanisms and pathways (Regan, 1989; Zemon et al., 1995). Deficits found in VEPs designed to target magnocellular and parvocellular pathways of individuals with MS suggest that the disease process may impact both of these neural subsystems (Thurtell et al., 2009). Novel isolated-check patterns, developed in our laboratory, are designed to tap the two prominent pathways (magnocellular and parvocellular) in the visual system along with subsystems that process bright (positive contrast) and dark (negative contrast) information (ON and OFF pathways, respectively) (Zemon & Gordon, 2006).

The current study seeks to compare individuals diagnosed with MS and healthy controls to investigate the functional integrity of select visual pathways using novel, isolated-check VEPs.

By better characterizing the differences between individuals with MS and healthy controls, we can begin to develop classification systems that utilize VEP measures. With increased classification accuracy, the VEP may be used as a reliable paraclinical tool to aid clinicians in diagnosing MS with increased speed and precision. We expect to identify disparate patterns of visual responses to isolated-check stimuli between the groups that reflect select deficits in distinct neural mechanisms. The long-term goals of this project include future longitudinal studies to evaluate the conversion of CIS to MS and enhance the diagnostic efficiency of MS to improve disease prognosis.

Background and Significance

Multiple sclerosis (MS) is an immune-mediated, multifocal disease of the central nervous system described in writings from as far back as the 14th century (Zalc, 2018). Jean Martin Charcot, typically credited with the first comprehensive account of MS in the 1800s, helped establish multiple sclerosis as a novel disease by virtue of rigorous clinical observation and pathologic investigation (Zalc, 2018). Today, MS is one of the most common disabling neurological disorders and leading causes of nontraumatic neurological disability in young adults (Noseworthy, Lucchinetti, Rodriguez, & Weinshenker, 2000). Mean age of onset for MS is around 33 years old, with 70% of cases emerging between ages 21 and 40 (Kaufman, Geyer, & Milstein, 2016). MS is at least two to three times more common in women than men and MS is particularly prevalent where white people of Nordic origin live, in temperate zones, and in high income countries, suggesting that population genetics, gene expression in a given physical environment, and socioeconomic structure may all serve as contributing factors (Koch-Henriksen, & Sorensen, 2010; Rotstein et al., 2018; Weinshenker et al., 1989). Research has suggested that the MS disease process differs across racial and ethnic groups, with demonstration

of cortical thickness and thalamic volume differences observed between African American and Caucasian individuals (Al-Kawaz et al., 2017; Johnson et al., 2010; Lichtman-Mikol et al., 2019).

The number of individuals with MS has increased by 9.5% from 2008 to 2013, with an estimated 2.3 million people thought to have MS worldwide (Browne et al., 2014). The continued increase in MS prevalence rates is attributed, in part, to improving survival rates. But increased incidence is thought to be a factor as well, aided by improvements in the diagnosis and reporting of MS (Browne et al., 2014).

Disease course and clinical patterns. Over the years, our understanding of the underlying mechanisms of MS has evolved, and we now believe the illness generally begins with areas of inflammation developing within the oligodendrocyte-generated myelin sheaths of CNS axons (Kaufman et al., 2016). T-cell-mediated inflammatory processes attack myelin, resulting in the demyelination of axons and the eventual development of sclerotic plaques throughout the brain and spinal cord resulting in blocked, delayed, or intermittent signal conduction (Kaufman et al., 2016; McDonald & Sears, 1969). MS comes with a wide range of phenotypic expressions, lending itself to several proposed theories regarding underlying mechanisms. Inflammatory mechanisms are thought to influence the relapsing aspects of the disease, which the majority of current medical treatments target for intervention (Costello, 2013). However, there is evidence to suggest that neurodegeneration within the sclerotic plaques may account for the more progressive and disabling aspects of the disease (Frohman, Racke, & Raine, 2006). The underlying mechanisms for these two phases of the disease are thought to be different, warranting further examination into the mechanism of action.

For individuals with MS, the inflammatory mediated demyelination of the central nervous system is coupled with significant axonal damage and neuronal loss (Kilstorner et al., 2013; Costello, 2013). This frequently results in symptoms that may include neurological, physical, psychological, and cognitive impairments (Giesser, 2011). There is great variability of clinically present symptoms among patients with MS, depending on where inflammation and demyelination have settled in the brain. In the process of diagnosing MS, clinicians are guided by typically presenting symptoms as well as symptoms that may represent a "red flag," as those help lead a clinician away from diagnosing an individual with MS. Despite varying presentations, the most commonly observed typical symptoms of MS include optic neuritis, internuclear ophthalmoplegia, L'hermitte's sign, sensory level paraparesis or quadriparesis, pyramidal tract signs, and neurogenic bladder (Giesser, 2011). While there is theoretically no area of the CNS free from attack, grey matter symptoms, such as dementia, seizure, or aphasia, are less common at the time of diagnosis. Initial presentations with normal neurological examination, abnormalities in only a single location, persistent or slowly progressive symptoms, peripheral symptoms, normal brain magnetic resonance imaging (MRI), normal spinal fluid, and/or the lack of typical symptoms as described above, are key indicators that the individual may not have MS. Atypical symptoms function as "red flags" or alarm bells, alerting the clinician that there may be an alternate explanation for the presenting symptoms. For differential diagnosis, the lack of typical symptoms present at onset can serve as the most important diagnostic feature for individuals who do not have MS (Boster et al., 2008; Giesser, 2011).

Typically, symptoms of MS will have an abrupt onset, increasing for several days before reaching a plateau, followed by recovery over several weeks to months. Over the course of the disease, clinical manifestations may be expressed as neuro-ophthalmologic, cerebellar/brainstem,

urinary/bowel, and sexual dysfunction. Additionally, individuals may experience cognitive impairment, pain, fatigue, and sleep difficulties (Giesser, 2011).

Visual disturbances are among the most common in MS, as lesions can affect any point along the afferent or efferent visual pathways (Beh, Frohman, & Frohman, 2016; Costello, 2013). An estimated 21% of individuals diagnosed with MS present with optic neuritis as their first clinical symptom and at least one-third of those with MS have visual symptoms which persist over the course of the disease (Costello, 2013; Jasse et al., 2013). Commonly observed visual symptoms of MS also include blurred vision, visual loss, eye pain, and oscillopsia (Costello, 2016).

Unfortunately for those with MS, the brain is not only exposed to inflammatory processes when there is an outward clinical sign of exacerbation. Rather, research indicates ongoing disruption within neural circuits in the absence of clinically significant symptoms. Autopsy studies have indicated the presence of subclinical, silent lesions present in individuals with no obvious clinical correlates (O'Riordan et., al, 1998; Lebrun et al., 2008). There is evidence that these inflammatory lesions occur nearly ten times as often as clinical relapses occur (Miller et al., 1993; Filippi et al., 1998). Thus, it is believed that a certain level of conduction may be restored among demyelinated pathways. The presence of silent lesions supports the notion that recovery of function may be possible due to suspension of inflammation and/or plasticity and reorganization within remaining nerve cells (Foster, Whalen, & Waxman, 1980). Although the restructuring of additional sodium channels is believed to restore functioning of demyelinated axons, conduction along these pathways remains inferior than that of healthy axons (Smith & McDonald, 1999). The disease process remains active in the central nervous system of individuals with MS regardless of clinical manifestations, so relying solely on clinical

observations for diagnosis handicaps the clinician. Incorporating paraclinical assessment in diagnosis may assist the clinician in more accurately measuring these disruptions to neuronal functioning. However, our ability remains limited in classifying subclinical diseases processes, and diagnosticians continue to rely on assessment of clinical observations to establish the disease course and specific MS phenotype.

Multiple sclerosis phenotypes. Though clinical characteristics of MS are diverse, the disease can be classified based on the observable clinical course. For 85% of MS patients, the disease course begins with a first clinical event consistent with demyelinating etiology, known as CIS (Miller, Chard, & Ciccarelli, 2012). CIS, by definition, is isolated in time and most commonly isolated in space, though multifocal presentations of CIS can occur. CIS commonly presents in young adults, between the ages of 20 and 40, with an acute onset of symptoms reaching a peak within two to three weeks. Symptoms that are most typical at disease onset are optic neuritis, spasticity, weakness, sensory disruptions, nystagmus, neurogenic bladder symptoms, Lhermitte's sign, and Babinski's sign (Miller et al, 1993; Filippi et al., 1998). The most common CIS features typically seen in those who eventually convert to MS affect the optic nerve, brain stem, and spinal cord (Miller et al., 2008). A CIS episode lasts at least 24 hours and is not better accounted for by fever, infection, or encephalopathy (Miller et al., 2012). For individuals with CIS, approximately 84% will develop a second demyelinating event, consistent with a diagnosis of MS, within 20 years (Hou, Jia, & Hou, 2018; O'Riordan et al., 1998).

At onset, approximately 85-90% of MS cases demonstrate a relapsing and remitting disease pattern, known as relapsing-remitting MS (RRMS) (Miller and Leary, 2007). RRMS is characterized by clinical attacks followed by periods of partial or complete recovery. RRMS can be described as active or not active, depending on if the individual is experiencing a relapse or

has new MRI activity (Lublin et al., 2014). For some individuals with RRMS, particularly when MS is left untreated, disease activity transforms to a progressive worsening pattern, with few to no relapses, known as secondary progressive MS (SPMS). SPMS commonly follows an initial relapsing-remitting course with eventual progression of worsening neurologic functioning and accumulation of disability over time. Traditionally, an estimated 90% of individuals with RRMS transition to SPMS after 25 years, though in the era of disease modifying treatments, these conversion rates are believed to be dropping (Confavreux, Aimard, & Devic, 1980; Ebers, 2001; Weinshenker et al., 1989). Approximately 15% of the MS population fall into the category of primary-progressive MS (PPMS), which is characterized by slow, progressive disability from the onset of the disease (Hurwitz, 2009). Like RRMS, both SPMS and PPMS can be further classified as active or not active at different points in time. SPMS and PPMS phenotypes of MS can also be further classified as with progression or without progression, depending on whether there is objective evidence of sustained worsening over time (Lublin et al., 2014). The MS subtype classification system has evolved in conjunction with our knowledge and understanding of MS pathology. A consensus paper, originally developed in 1996 by the US National Multiple Sclerosis Society Advisory Committee on Clinical Trials in Multiple Sclerosis, was later revised in 2014 to reflect increased understanding of MS and further clarify the definition of patient groups (Lublin & Reingold, 1996; Lublin et al., 2014). The revision eliminated progressive relapsing MS (PRMS) from the clinical course descriptions and is instead now classified as PPMS with activity (Lublin et al., 2014).

Therapy for multiple sclerosis. Prognosis for MS has significantly changed since the introduction of disease modifying treatments, or DMTs (Bates, 2011; Coyle, 2008; Filippi et al., 2004; Kappos et al., 2016; Rot, Ledinek, & Jazbec, 2008). Enhanced control of disease

progression was found by implementing treatments that targeted key mechanisms involved in immune modulation within the brain and spinal cord (Polman et al., 2006; Rudick et al., 2006; Tennakoon et al., 2006). Before the introduction of DMTs, it was estimated that 80-90% of those diagnosed with RRMS would progress to SPMS within 25 years (Confavreux et al., 1980; Weinshenker et al., 1989). RRMS relapses were shown to be more common in individuals with younger age of onset and those who were closer to the start of the disease course (Tremlett, Zhao, Joseph, Devonshire, & UBCMS Clinic Neurologists, 2008). Accumulating effects of MS relapses result in clinical disability over time, and nearly half of MS patients required assistance walking by 15 years after disease onset (Dixon & Robertson, 2018; Weinshenker et al., 1989). Conversion from RRMS to SPMS is considered a crucial determinant of long-term prognosis and targeting treatment toward preventing this conversion remains critical (Brown et al., 2019; Scalfari, Neuhaus, Daumer, Muraro, & Ebers, 2014). Initiating disease-modifying therapy before the second clinical attack has been shown to reduce the risk of MS disability accumulation (Tintore et al., 2015). As such, significant emphasis has been placed on early and accurate diagnosis, so that individuals with confirmed cases of MS can start DMTs, with the goal of delaying disability progression.

For many individuals with MS, DMTs may only be partially effective, or do little to address symptoms like gait disturbance, sexual dysfunction, or psychiatric problems (Giesser, 2011). Alternate and supplemental treatments focus on symptom management without altering the disease course. At the onset of an acute relapse, a typical first-line treatment option is corticosteroid therapy (Thrower, 2000). Additional symptomatic MS therapy used over the course of the disease targets reduction or elimination of symptoms that impact an individual's quality of life or daily functioning. Henze, Rieckmann, and Toyka (2006), completed a review of

symptomatic treatments for individuals with MS, and found that symptoms of fatigue are best targeted by lowering the body temperature, physical training, rehabilitation, and drug therapy. For MS related pain syndromes, drug therapy, psychological intervention, and physical therapy can be beneficial (Henze, Rieckmann, & Toyka, 2006). Henze et al., (2006) identified that bladder and bowel dysfunction, affecting up to 80% of individuals with MS during their disease course, is often treated with drug therapy, bladder and toilet training, counseling on aids/devices, and physical therapy. Sexual dysfunction treatments included psychological intervention, surgical treatments, physical devices/aids, and drug therapy (Henze et al., 2006). Henze et al., (2006) noted physical therapy, occupational therapy, drug therapy, and surgical intervention to be beneficial for managing ataxia and tremors. For cognitive dysfunction, cognitive rehabilitation and drug therapy are often used (Henze et al., 2006). Psychotherapy and drug therapy for psychiatric symptoms, particularly depression, were identified as beneficial (Henze et al., 2006). Dysarthria, dysphonia, and dysphagia are typically treated with speech therapy, prosthetic/technical aids, drug therapy, and surgical treatment (Henze et al., 2006). Finally, spasticity, which can lead to symptoms such as gait disturbance and muscle weakness, is usually treated with physical therapy and drug therapy (Henze et al., 2006).

Diagnosing multiple sclerosis. Diagnostic criteria for MS have historically included the need to demonstrate dissemination of central nervous system lesions in time and space, while ruling out alternate diagnoses. The now widely used McDonald Criteria, developed by an international panel of experts in association with the National Multiple Sclerosis Society of America, were initially established in 2001, with updated versions published in 2005, 2010 and, most recently, 2017 (McDonald et al., 2001; Polman et al., 2005; Polman et al., 2011, Thompson et al., 2018). The McDonald Criteria incorporate the older Poser criteria of demonstrating

dissemination of lesions in space (DIS) and time (DIT) for a diagnosis of MS (Poser et al, 1983). Diagnosis remains a rule-out processes and if the clinical presentation fulfills the 2017 McDonald Criteria, without a better explanation for the symptoms, the diagnosis is MS. Throughout each revision, the McDonald Criteria have incorporated the use of supplemental techniques, such as magnetic resonance imaging findings and CFS analysis, to prove dissemination in time and/or space when clinical information is deficient. Each revision has targeted earlier diagnosis and is designed to better predict conversion from CIS to definite MS (Thompson et al., 2018).

Use of MRI in the 2017 McDonald criteria. Standardized brain and spinal cord MRI protocols have been developed for the MS diagnostic process by a European collaborative research program, Magnetic Resonance Imaging in Multiple Sclerosis (MAGNIMS), and the Consortium of Multiple Sclerosis Centers (Filippi et al., 2016; Rovira et al., 2015; Traboulsee et al., 2016). MRI is recommended for all individuals for whom a diagnosis of MS is being considered and is often used to demonstrate impact of the disease on the CNS when clinical data is lacking (Thompson et al., 2018). Revised 2017 McDonald Criteria outline the patterns of both symptomatic and asymptomatic MRI evidence deemed sufficient for determination of dissemination in time and space, in absence of clinical correlates, for individuals with clinically isolated syndrome. However, an exception was made to these MRI criteria for optic neuritis patients with lesions in the optic nerve, due to lack of sufficient evidence in the literature for that particular lesion site (Thompson et al., 2018).

To demonstrate DIS, one or more T2-hyperintense lesions on MRI is required (greater than or equal to 3 mm in long axis) in two or more of the following locations: periventricular, cortical/juxtacortical, infratentorial, and/or spinal cord. DIT can be demonstrated by MRI in two

ways. First, DIT can be represented by a new gadolinium-enhancing or T2-hyperintense lesion when compared to a previous MRI. A second way to demonstrate DIT on MRI is with the presence of both a gadolinium-enhancing lesion and a non-enhancing T2-hyperintense lesion in a single imaging scan (Thompson et al., 2018).

MRI findings do not always align with observable clinical findings. Brain features that are suggestive of demyelination, but which lack clinical manifestations have been termed radiologically isolated syndrome, or RIS (Granberg, Martola, Aspelin, Kristoffersen-Wiberg, & Fredrikson, 2013). In certain instances, RIS may be the first visible manifestation of MS, with approximately one-third clinically converting to CIS and/or MS (Granberg, Martola, Kristoffersen-Wiberg, Aspelin, & Fredrikson, 2013). Management of RIS remains a debate among practicing clinicians, particularly because RIS is a relatively new entity lacking sufficient research pertaining to prevalence and long-term prognosis of the syndrome (Granberg et al., 2013).

Overall, MRI is a vital tool frequently used in diagnosis; however, it can introduce diagnostic errors due to poor specificity. In fact, hyperintense lesions found in CNS white matter are also commonly seen in migraine populations, microvascular disease, and even among healthy adults (Kruit, van Buchem, Launer, Terwindt, & Ferrari, 2010; Morris et al., 2009; Vernooij et al., 2007). The correlations between MRI lesion load and patient disability remain weak, as the clinically silent lesions do not always translate to disease disability or prognosis, which limits interpretability and insight into MS-related brain changes (Miller, Grossman, Reingold, & McFarland, 1998). Conventional MRI techniques are not specific to underlying pathology, however advanced techniques, such as diffusion tensor imaging (DTI), have been able to detect subtleties not observable with conventional MRI. A rapidly developing area of research utilizes

DTI to examine normal-appearing white matter, or NAWM. Within this area of research, NAWM refers to the diseased tissue surrounding white matter hyperintensities that appear normal on conventional MRI (Filippi, Absinta, & Rocca, 2013). DTI has been shown to be sensitive to microstructural abnormalities occurring in NAWM tracts and research has demonstrated an association with these NAWM areas and disability progression over time. Advanced MRI techniques, such as DTI, demonstrate high sensitivity in detecting diffuse brain abnormalities and show promise as a potential biomarker for MS disease progression, however, additional research is needed to standardize methodological approaches for this technique (Filippi et al., 2013; Kolasa et al., 2019).

Use of cerebrospinal fluid (CSF) analysis in the 2017 McDonald criteria. To assist with ruling out infectious or neoplastic etiologies of presenting symptoms, laboratory testing can include examination of CSF. A patient's CSF is examined for oligoclonal bands, which are bands of immunoglobulins. Evidence of intrathecal antibody synthesis, demonstrated by two or more CSF-specific oligoclonal bands, supports, but is not specific to, a diagnosis of MS (Andersson et al., 1994). For adults with clinically isolated syndrome, CSF oligoclonal bands were strongly and independently associated with conversion to clinically definite MS (Kuhle et al., 2015). Lack of oligoclonal bands has a high negative predictive value, suggesting that an alternate diagnosis to MS should be considered (Deisenhammer, Zetterberg, Fitzner, & Zettl, 2019). CSF analysis can be used to support the diagnosis of an alternative disease process when findings are atypical for MS, including and elevated protein concentration of > 100 mg/dL, pleocytosis with > 50 cells per mm^3, or the presence of neutrophils, eosinophils, or atypical cells (Thompson et al., 2018). According to the 2017 McDonald Criteria, the presence of CSF oligoclonal bands permits the

diagnosis of MS specifically when an individual presents with a typical clinically isolated syndrome and clinical, or MRI, demonstration of DIS (Thompson et al., 2018).

Heterogeneity in multiple sclerosis diagnosis. Heterogeneity of disease onset requires special consideration of diagnostic criteria to ensure accuracy of diagnosis. Individuals may present at different points in the disease course, providing varying amounts of clinical or paraclinical evidence for a diagnostician to analyze. For instance, if an individual presents with two or more objective clinical attacks, with clinical evidence of two or more lesions in the brain, no additional information is required to fulfill the 2017 McDonald Criteria. In this case, the disease has demonstrated DIT (two or more clinical attacks) as well as DIS (two or more lesions). While this type of presentation may be more straightforward in RRMS cases, a disease course with progressive onset can make it more challenging to meet these criteria. In the absence of clear-cut clinical attacks which demonstrate dissemination in both time and space, additional data are required for diagnosis, and MRI and CSF examination should be utilized. If imaging or CSF analysis are negative, the diagnostician should reconsider the diagnosis of MS (Thompson et al., 2018). In the case of progressive disease onsets, such as those seen in PPMS, the 2017 McDonald Criteria require over a year of disability progression with two of the following: one or more characteristic MS T2-hyperintene lesion, two or more T2-hyperintense spinal lesions, and/or the presence of CSF-specific oligoclonal bands (Thompson et al., 2018).

Differential diagnosis and multiple sclerosis misdiagnosis. Misdiagnosis of MS is not uncommon and due to a long list of differentials, the diagnostician must be mindful of alternate diagnoses which mimic MS, such as neurologic disorders, vascular disease, inflammatory processes, infections, and metabolic disorders (Solomon, Naismith, & Cross, 2019). A critical component of MS diagnostic criteria is ruling out alternate medical explanations for the

neurologic symptoms experienced. For instance, the experience of numbness or tingling in the extremities, a common symptom seen in MS, may be the result of a vitamin B12 deficiency, which can be ruled out with a simple blood test. However, other differential diagnoses may be more challenging to discriminate. The literature suggests between 30%-67% of new referrals to MS subspecialty clinics were ultimately determined to not have MS (Solomon et al., 2016; Yamout et al., 2017). A multicenter study that evaluated individuals who were determined to have been misdiagnosed with MS revealed that alternate diagnoses included migraine or migraine in combination with other diagnoses (22%), fibromyalgia (15%), nonspecific or non-localizing neurologic symptoms with abnormal MRI (12%), conversion or psychogenic disorders (11%), and neuromyelitis optica spectrum disorder (6%) (Solomon et al., 2016).

Primary reasons for misdiagnosis of MS include misinterpretation of clinical symptomology and inappropriate application of the McDonald Criteria (Solomon et al., 2019; Solomon, Klein, & Bourdette, 2012). The revised 2017 McDonald Criteria emphasize the importance of incorporating the judgement of a physician who has MS-related expertise, aided by paraclinical tests, in order to optimize diagnostic accuracy due to the prevalence of misdiagnosis in this population (Thompson et al., 2018). Evaluation by a clinician with specific experience with MS would be ideal, however, access to care, particularly to specialized medicine, remains a barrier for many individuals. Discrimination between typical and atypical symptoms remains a key factor in MS diagnosis, with an estimated two-thirds of misdiagnosed patients exhibiting an atypical presentation (Solomon et al., 2016). For those who are potentially misdiagnosed with MS, unnecessary exposure to the side-effects of DMTs and the average yearly cost of about $60,000 can be harmful to both a patient's physical and financial well-being (Hartung, Bourdette, Ahmed, & Whitham, 2015). DMTs are also contraindicated for some

differential diagnoses, such as neuromyelitis optica spectrum disorder, making it potentially harmful for those individuals to receive MS related therapies (Thompson et al., 2018). Those who receive an inaccurate diagnosis also go without treatment for their true diagnosis, leading them further away from potential symptom resolution. Thus, a trade-off remains between efficient diagnosis of true MS and avoidance of erroneous diagnosis in individuals who do not have MS.

In the era of disease-modifying therapeutic medications, with their potential to significantly alter the disease course for those with confirmed cases of MS, increased emphasis is continually made toward developing strategies that will allow for earlier and more accurate diagnosis. Limitations in application of the McDonald Criteria remain, as they were developed to predict risk of conversion to MS, not to differentiate MS from other demyelinating diseases. While researchers continue to pursue a more specific biomarker for MS, incorporation of imaging and laboratory techniques are utilized to aid clinicians in diagnosis. The inclusion of paraclinical assessments, such as imaging, has improved the diagnostic process, however, there remains room for enhancement to further develop diagnostic specificity and sensitivity (Brownlee, Hardy, Fazekas, & Miller, 2017). An array of diagnostic tools has shown promising utility for assisting MS diagnosis through examination of the anterior visual system, including the MRI, optical coherence tomography (OCT), and visual evoked potentials (VEP). The International Panel on Diagnosis of Multiple Sclerosis identified studies that validate the use of MRI, OCT, and VEP in fulfillment of DIT or DIS as a high priority (Balcer et al., 2015; Thompson et al., 2018).

Assessment of the visual system in multiple sclerosis. The adage that “the eyes are the window to the soul,” or in this case to the brain, holds true with the MS population. Most people

with MS experience some form of visual disturbance along the course of their disease, therefore, there is high likelihood of the visual system being affected. In fact, post-mortem studies have suggested that about 90% of individuals with MS have lesions along their visual pathway, irrespective of a clinical history of optic neuritis (Ikuta & Zimmerman, 1976; Toussaint, Périer, Verstappen, & Bervoets, 1983). The visual system has consistently provided an early clinical sign of multiple sclerosis and the 2016 MAGNIMS criteria proposed the optic nerve as a fifth anatomical location for fulfillment of MRI criteria to prove dissemination in space (Thompson et al., 2018; Filippi et al., 2016). Cells located in the back of the eye relay information directly into the brain and certain tools, such as MRI, OCT, and VEP, can measure the health of these nerves in the back of the eye and throughout the visual system.

Assessment of the visual system with MRI. Expert consensus has confirmed MRI evidence of optic nerve inflammation constitutes an additional item to meet dissemination in time according to the 2017 McDonald Criteria (Filippi et al., 2016). Specifically, clinicians may observe increased T2 signal, gadolinium enhancement, and optic nerve swelling on MRI (Thompson et al., 2018; Filippi et al., 2016). When examining individuals with CIS, the presence of clinically silent brain lesions on MRI considerably increased the likelihood of conversion to clinically definite MS (Tintore et al., 2015). Advanced MRI techniques, such as DTI, provide more sensitive detection of inflammatory demyelination and axonal degeneration, however, the findings remain inconsistent, and further research is needed (Andersen et al., 2017). MRI findings provide informative diagnostic information by detecting gross abnormalities in brain structure and have proven as a vital clinical tool for MS diagnosis, yet, as discussed previously, there remain limitations in specificity and this tool offers limited application toward understanding neuronal functioning.

Assessment of visual system with OCT. Optical coherence tomography, or OTC, enables non-invasive imaging of the anterior eye via light to provide cross-sectional images of tissue structure on the micron scale in real time (Fujimoto, Pitris, Boppart, & Brezinski, 2000). OCT examination of the macular retinal nerve fiber layer (RNFL) and ganglion cell layer (GCL) have been suggested as possible biomarkers of axonal damage in MS (Alonso, Gonzalez-Moron, & Garcea, 2018). MS patients with greater brain atrophy have considerably larger retinal involvement, as measured by RNFL loss (Gordon-Lipkin et al., 2007). Additionally, within MS patients, emerging evidence suggests that subclinical RNFL thinning and retinal ganglion cell axonal loss occur separately from acute optic neuritis, serving as a measure of silent lesions (Fisher et al., 2006; Khanifar et al., 2010; Pulicken et al., 2007; Naismith et al., 2009). Analysis of RNFL thinning can also be used to assist the differentiation of MS from diseases such as neuromyelitis optica and Susac's syndrome (Bennett et al., 2015; Ringelstein et al., 2015). OCT can be used to detect early nerve degenerations, which is suggested to have predictive value for future disability progression (Britze & Frederiksen, 2018). Given this evidence, it is suggested that examination of the anterior visual pathway, which is a frequent target of MS pathology, can be used to detect and monitor neurodegeneration (Galetta, Calabresi, Frohman, & Balcer, 2011). Though there are promising results from existing data, this new area of study still lacks substantial scientific evidence for clinical diagnostic use in MS. Additionally, like magnetic resonance imaging, OCT provides structural analysis, with limited application toward functional processes.

Assessment of the visual system with low contrast measures. Specificity remains a limitation of imaging techniques, as MRIs have been known to reveal structural abnormalities that do not have a perceived clinical correlate. Individuals with MS who show clinical

manifestations of visual disturbance, typically in the form of optic neuritis, often experience prolonged impairment to contrast vision, even after other aspects of vision, including visual acuity, color vision, and field loss, have been restored (Beck, Ruchman, Savino, & Schatz, 1984; Zimmern, Campbell, & Wilkinson, 1979). Contrast vision has been shown to correlate with vison-specific quality of life measures, demonstrating an ability to provide clinically meaningful information (Balcer et al., 2015; Thurtell et al., 2009; Zimmern et al., 1979). Contrast vision and the ability to distinguish adjacent areas of varying luminance has been clinically quantified in MS populations by low contrast letter acuity techniques. The literature supports the use of low contrast letter acuity testing with Sloan letter charts as a sensitive measure of afferent visual dysfunction in MS (Balcer et al., 2015; Baier et al., 2005).

The visual system: exploration of neural mechanisms through visual evoked potentials. The visual system is comprised of parallel neural pathways that originate in the retina and carry excitatory and inhibitory signals to the brain (Ratliff & Zemon, 1984; Zemon & Ratliff, 1982, 1984). Photoreceptors in the retina convert visual information into electrical signals and send them into an intermediate layer, which in turn relays signals to various types of retinal ganglion cells. These retinal ganglion cells account for approximately 90% of the fibers in the optic nerve and transmit potentials into the cortex (Kaplan, 2013). Visual input is carried into the cortex by three main cellular pathways: M (magnocellular), P (parvocellular), and K (koniocellular). It is generally accepted that the visual system functions primarily through two main cortical streams, the ventral (P dominated, with K input) and dorsal (M dominated) (Kaplan, 2013). The M cells form a large-cell pathway that projects to the two ventral (magnocellular) layers of the lateral geniculate nucleus (LGN). The P cells form a small-cell pathway that projects to the four dorsal (parvocellular) layers of the LGN (Carlson, 2013;

Kaplan, 2004). Literature in the field reports that each pathway has distinct properties with regard to contrast; in a general sense, the magnocellular pathway displays sensitivity to low contrast stimuli whereas the parvocellular pathway displays sensitivity to high-contrast stimuli (Kaplan, 1991; Kaplan & Shapley, 1986; Zemon & Gordon, 2006). The current study examined the pairs of parallel pathways that transmit contrast-dependent signals, with emphasis on whether a deficit in response to certain stimuli is reflective of neuronal dysfunction.

Both the magnocellular and parvocellular pathways have subtypes of cells, labeled on-center (ON) and off-center (OFF), that process separately positive and negative contrast information, respectively (Kuffler, 1953; Hubel & Wiesel,1961; Schiller, 2010). The physically larger ON cells contribute to the perception of brightness (positive-contrast), while smaller OFF cells contribute to the perception of darkness (negative-contrast), and this information is disseminated separately to the brain by ON and OFF pathways (Fiorentini, Baumgartner, Magnussen, Schiller, & Thomas, 1990; Schiller, 1982). Evidence supports greater contrast gain and finer spatial tuning in OFF pathways compared to ON pathways and evoked potentials elicited by positive or negative contrasts could advance diagnostic practices for disorders where these pathways are affected (Zemon, Gordon, & Welch, 1988).

Electrogenesis of the visual evoked potential. The VEP reflects the electrical response of the brain to visual stimulation extracted from the electroencephalogram (EEG). The signal that is generated after viewing a stimulus can be recorded from the surface of the head, either with a large array of electrodes (multi-channel) or with a single channel of electrodes placed appropriately. Although multi-channel, high-density EEG recording is often used for the purposes of research and certain clinical applications, much information can actually be obtained from single-channel recording which has the key advantage of speed in a clinical setting. The

rate of visual stimulation can be altered to elicit either a transient or steady-state response. Transient VEPs (tVEP) are produced by abrupt changes in stimuli with sufficient time between stimulus change for the response to settle. Steady-state VEPs (ssVEP) are produced by stimuli that change more frequently so that responses overlap and generate an oscillatory waveform. There is substantial support in the literature that the primary sources of the evoked potentials are the apical dendrites of pyramidal neurons that reside in the primary visual cortex (V1) (Creutzfeldt & Kuhnt, 1973; Ducati, Fava, & Motti, 1988; Nakamura, Kakigi, Okusa, Hoshiyama, & Watanabe, 2000; Schroeder, Tenke, Givre, Arezzo, & Vaughan, 1991; Shigeto, Tobimatsu, Yamamoto, Kobayashi, & Kato, 1998). The resultant extracellular currents are volume conducted to the surface of the head to produce the scalp potentials. Both excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs) contribute to the VEP recording and research indicates that net extracellular currents produce the EPSPs and IPSPs that occur in the neocortex and give rise to the scalp-recorded electrical responses (Creutzfeldt & Kuhnt, 1973; Eccles, 1951; Purpura, 1959; Zemon, Kaplan, & Ratliff, 1980). These studies have elucidated the electrogenesis of the VEP, in response to either electrical stimulation to the optic pathways or flash/pattern visual stimuli, beginning with an initial positive peak (P60) which represents afferent activity arriving in the cortex from the LGN. This initial depolarization occurs in cortex layer 4c of the primary visual cortex. The subsequent negative deflection (N75) is thought to represent the summed EPSPs associated with the spread of depolarization to more superficial layers of the apical dendrites (layers 2 and 3). The later positive wave (P100) reflects summed IPSPs and is associated with superficial hyperpolarization. Further support for the excitatory/inhibitory basis of the VEP was explored in a study which blocked GABAergic inhibition in the visual cortex of cats, revealing absence of a prominent positive deflection and

enhancement of the early negative deflection following application of a GABAa antagonist (Zemon, Kaplan, & Ratliff, 1980). These key findings are critical to the applications of VEPs, as excitatory and inhibitory processes are not limited to the visual cortex, rather, they are common neural processes to other systems within the brain. As such, abnormalities identified throughout these mechanisms reveal deficits in brain function in general, rather than only the visual system.

Conventional VEP analysis: promise and pitfalls. The conventional VEP test conducted in medical centers throughout the world is the transient VEP to a contrast-reversing checkerboard of high contrast. Typically, peak-to-trough amplitudes and timing of prominent deflections are measured (Odom et al., 2016). Within the MS population, characteristic VEP findings include increases in response latency (e.g., P100) to low contrast stimuli, as well as decreases in VEP amplitude, which has been attributed to axonal loss (Galetta & Balcer, 2013; Kilstorner et al., 2013; Thurtell et al., 2009). When compared to OCT, assessment of the anterior visual system via visual evoked potentials is arguably the more preferred method for detecting clinical and subclinical optic disturbance (Naismith et al., 2009). When directly compared in an optic neuritis cohort, VEP P100 latency measures maintained relatively higher sensitivity for confirming clinical and subclinical optic neuritis compared to OCT; however, neither test in this particular study was specific for optic neuritis or MS (Naismith et al., 2009). Among individuals with MS, pattern reversal VEPs have been shown to assess optic neuritis damage (Halliday, McDonald, & Mushin, 1973). Increased response latency has been shown to detect demyelinating lesions in the optic nerve pathways 90% of the time (Thurtell et al., 2009).

Traditional analysis techniques are highly subjective and require a trained professional with electrophysiological expertise to evaluate the time-domain waveform. Careful selection of peak times is not always clear cut, as even among healthy controls, individual waveforms may

vary drastically, making clear identification of desired timepoints difficult and subject to interpretation. Determination of peak time points in a time-domain response can often be embedded in considerable noise when dealing with clinical populations and selection of those points may be ambiguous. Hundreds of data points make up a transient response, however, conventional techniques focus on just a few deflections. While transient VEP tests can provide a great deal of information, they do not do well to separate the overlapping contributions from various brain mechanisms on their own. Such limitations create space for unreliable results and inaccurate conclusions.

Frequency domain analysis of the VEP waveform. Frequency domain analysis has proven a reliable means with which to quantify the entire VEP waveform objectively (Gutowitz, Zemon, Victor, & Knight, 1986; Zemon et al., 1995; Zemon & Gordon, 2006; Zemon et al., 1988; Zemon, Gutowski, & Horton, 1983; Zemon, Hartmann, Gordon, & Prunte-Glowazki, 1997; Zemon, Kaplan, & Ratliff, 1980, 1986; Zemon, Pinkhasov, & Gordon, 1993; Zemon & Ratliff, 1982, 1984; Zemon et al., 2008; Zemon, Victor, & Ratliff, 1986; Zemon et al., 2012). This process is performed by a discrete Fourier transform applied to the time-domain VEP waveform, which in turn characterizes the entire response as a set of frequency components (amplitude and phase measures). To ensure a true response is being analyzed, frequency components that are known to contain noise are filtered out of the recording. For the transient VEP response to a contrast-reversing checkerboard stimulus, this can be done by eliminating odd-order harmonic frequency components, further allowing the response to be analyzed objectively and with an improved signal-to-noise ratio (SNR). Further multivariate statistical analyses are conducted on the frequency components to assess the significance of responses, strength and timing of responses, and difference between responses. These frequency-domain

analysis techniques have also proven reliable in a variety of disease populations, including but not limited to schizophrenia, autism, glaucoma, headaches, epilepsy, and retinitis pigmentosa (Alexander, Rajagopalan, Seiple, Zemon, & Fishman, 2005; Butler et al., 2001; Coppola et al., 2013; Conte & Victor, 2009; Greenstein, Seliger, Zemon, & Ritch, 1998; Siper et al., 2016; Weinger, Zemon, Soorya, & Gordon 2014). With data collected as part of the parent study for this research, frequency domain analysis was analyzed in an MS population for transient VEP responses (Siegel, 2019). In Siegel's published dissertation, she demonstrated the utility of frequency domain analysis of transient VEP to contrast-reversing checkerboard stimuli in the assessment of visual dysfunction in patients with MS (Siegel, 2019) Phase data generated in the frequency domain have been used to estimate delays in the VEP waveform (Duwaer & Spekreijse, 1978; Regan, 1966; Zemon & Gordon, 2018). Zemon and Gordon (2018) demonstrated that the square root of power in a frequency band (Band 2, even harmonics from 12-28 Hz) yielded accurate, objective estimates of N75-P100 amplitude with nearly 90% of the variance explained by the linear relation. Frequency-domain analysis has consistently proven to be an objective and reliable technique that removes the guesswork typically associated with conventional analysis of the VEP waveform without eliminating informational content of the response.

Stimulation of ON and OFF pathways with novel VEP techniques. Structural changes that occur in the nervous system, such as those resulting from trauma or a disease process, can alter neural functioning. Neural functioning can also be altered by changing the sensory stimulus. For example, presenting stimuli in high contrast opens ion channels, allowing electrical signals to cross and subsequently change the membrane potential and increase conductance across the membrane. As such, we can manipulate these functions based on the stimuli presented and

analyze the subsequent alteration in neural signals through the measurement of electrical responses. The frequency of stimuli can be increased to a level at which the neural activity does not return to baseline, creating a steady-state response. When an isolated-check pattern is temporally modulated, a dominant response at the stimulus frequency is generated. Isolated-check patterns have been used to drive particular visual pathways by displaying positive-contrast or negative-contrast checks, which have been thought to target ON and OFF pathways, respectively (Zemon & Gordon, 2006; Zemon et al., 1988).

Zemon and Gordon (2006) applied these patterns in humans to examine the properties of magnocellular and parvocellular systems. They investigated the use of low contrast stimuli to focus on contributions of the magnocellular pathway, as this pathway is thought to exhibit high-contrast sensitivity, responding to low contrast conditions (Kaplan & Shapley, 1986; Lee, Pokorny, Smith, Martin, & Valberg, 1990; Zemon & Gordon, 2006). These methods have been used to study visual pathways in individuals with schizophrenia, revealing deficits in the isolated-check responses under low contrast conditions (Butler et al., 2001). The isolated-check VEP (icVEP) technique has been used in several studies to assess glaucomatous neural deficits and was actually found to yield high diagnostic accuracy (Greenstein et al., 1998; Xu et al., 2017; Zemon et al., 2008). Weinger, Zemon, Soorya, and Gordon (2014) used icVEP techniques to examine early stage visual processing in ASD. The icVEP technique was also used in a study of retinitis pigmentosa, a genetic retinal degenerative disorder, to show selective deficits in magnocellular function (Alexander et al., 2005). Though relatively new, icVEPs have demonstrated clinical utility in a number of populations, yet there are no identified published studies that examine these techniques in an MS population. Interestingly, selective deficits have been found in magnocellular and parvocellular streams in MS, suggesting that the disease

process may differentially affect these neural subsystems (Evangelou, Konz, Esiri, Smith, Palace, & Matthews, 2001; Thurtell et al., 2009). Porciatti and Sartucci (1996) examined the differences between the magnocellular and parvocellular pathway via chromatic contrast stimuli in individuals with MS and optic neuritis. Results of the study suggested that when the parallel streams specific for color contrasts (parvocellular) and luminance contrasts (magnocellular) were examined with pattern electroretinograms and VEPs, resulted indicated higher vulnerability in the parvocellular system, when compared to the magnocellular system, noting, however, that these findings do not rule out potential contributions from magnocellular pathway deficits (Porciatti & Sartucci, 1996). Application of icVEP techniques may provide additional insight into these select pathways and may reveal deficits in MS populations that are not common in healthy controls.

The biophysical model. Zemon and Gordon (2006) provided an exploration of a nonlinear model to characterize contrast gain control in contrast-response functions obtained with VEP responses of adults. This biophysical model provided an explanation for how positive- and negative-contrast information drive selectively ON and OFF pathways in V1 through the process of rectification (Zemon & Gordon, 2006). Through this model, information on various parameters can be obtained, including contrast gain (i.e., the initial slope that reflects afferent excitatory input) and contrast gain control (i.e., nonlinear effects on the response that reflect shunting inhibition and alters time constants in the system). The change in slope of the amplitude and the change of phase of the response versus contrast functions makes up the mechanism of contrast gain control, which was originally observed in retinal ganglion cells of monkeys and cats (Shapley & Victor, 1978, 1979; Kaplan & Shapley, 1986). The gain control process enables the visual system, and other sensory systems throughout the body, to adapt to their environment

and optimize responses. In a study examining neurons in the visual system of cats, Borg-Graham, Monier, and Frégnac (1998) demonstrated that visual input can increase the conductance of cortical neurons by increasing $GABA_A$-mediated shunting inhibition, decreasing cellular temporal integration. The time constant of a recipient cell decreases as the stimulus contrast increases (Borg-Graham et al., 1998).

The biophysical model developed by Zemon and Gordon (2006) was explored in this study to examine estimates of contrast gain based on an individual's ssVEP contrast response function. An exploratory examination of the nonlinearity of the visual system, associated with increased speed of neural responses as contrast increases, were evaluated among individuals with MS and controls. To date, this study is the first application of the biophysical model for parameters obtained from isolated-check stimuli responses in an MS population. Contrast gain control is a mechanism found specifically in the magnocellular pathway, which is a pathway believed to be affected in MS populations, as such, presentation of these magnocellular-biased stimuli may elucidate further differences between MS and control groups (Thurtell et al., 2009).

Rationale for the Study

Understanding the prognosis of MS, particularly during the initial onset of the disease, remains a major research goal in the search for effective treatment practices. Several studies provide significant evidence supporting the use of DMTs early in the disease course of MS to not only delay the conversion to clinically definite MS, but also slow progression of brain tissue loss (Bates, 2011; Coyle, 2008; Filippi et al., 2004; Kappos et al., 2016; Rot et al. 2008). Current research has also provided evidence that suggests DMTs may be even more effective for individuals with clinically isolated syndrome (Miller et al., 2005; Kappos et al., 2006). Accuracy and confidence of early diagnosis are critical for avoiding mistreatment, as early DMT use can

have harmful effects if not administered properly. We aim to broaden our understanding of neural functioning at the earlier points of the clinical manifestation of the disease, as this remains a crucial point in the diagnostic timeline with regard to starting disease modifying treatments.

Diagnosis of MS can feel like a moving target, particularly when considering the heterogenous nature of clinical symptoms and imaging manifestations not only between patients, but also in the transformation of symptoms within one individual as the disease progresses. While navigating the labyrinth of MS diagnosis presents a unique set of challenges for clinicians, it is also important to consider the experience of the patient. Diagnostic testing can be painful or uncomfortable and last for months or longer, carrying with it a cloud of uncertainty surrounding what the future holds and how the results may impact life as one knows it. Improving diagnostic clarity not only carries benefits for providers and the medical field (e.g., reducing costs and time demands), but it can also serve to minimize the emotional, psychological, and financial burden placed on the patient should a more definitive diagnosis be made with fewer medical visits and less invasive technologies.

The International Panel on Diagnosis of Multiple Sclerosis has identified studies that validate the use of paraclinical measures, including VEP, for the fulfillment of DIT and DIS as a high priority (Thompson et al., 2018). VEP research in MS populations has revealed consistent findings related to the disease process, including increases in response latency to low contrast stimuli and decreases in VEP amplitude (Galetta & Balcer, 2013; Kilstorner et al., 2013; Thurtell et al., 2009). Selective deficits found in magnocellular and parvocellular streams in MS provide evidence to support disease impact on these neural subsystems (Thurtell et al., 2009). Examination of these distinct pathways via icVEP stimuli has proven valuable in other disease populations (e.g., glaucoma, autism spectrum disorder, schizophrenia, and retinitis pigmentosa),

with promising applications for the MS population. VEP measures have been proven useful in demonstrating the presence of CNS abnormalities, and may have the potential to fulfill diagnostic requirements of dissemination in time and space by providing a reliable and objective indication of neuronal abnormality due to disease process. Compared with gold standard paraclinical methods in MS, such as MRI and CSF analysis, VEP serves as a cost-effective, non-invasive alternative for clinicians and patients. As such, establishing a reliable and objective measure of neural dysfunction in individuals with MS can be used to facilitate the diagnostic decision-making process for physicians and track disease progression over time, leading to more efficient and accurate care for patients.

Innovation

VEPs have been used in the MS field for quite some time, however, the conventional time-domain measures rely on subjective judgments and ignore much of the informational content in the response, and therefore are limited in value. Novel icVEP techniques used in this study are based on frequency-domain measures that have been proven objective and capture the entire complement of neural information in a response. While conventional VEPs reflect activity of the visual system as a whole, research has informed the understanding that the visual system is comprised of various pathways. By modifying the stimuli presented to the visual system, we can begin to target more select visual pathways. IcVEP stimuli are designed to tap the two parallel pathways in the visual system that process bright (positive contrast) and dark (negative contrast) information. No published research was found that studied these techniques and stimuli in an MS population. This unique examination of the select visual pathways in the brain would help to expand our understanding of the underlying neural mechanisms at play in MS.

In summary, the current study hopes to expand the existing knowledge of structural and functioning relationships within the visual system of individuals diagnosed with MS. Expert consensus in the MS field has identified a need for additional research validating the use of paraclinical assessments, such as VEPs, to support a definitive diagnosis in MS. The literature to date has demonstrated the clinical utility of VEPs for MS populations, and novel icVEP techniques have yielded significant results in other disease populations, demonstrating promise for the use of these new methods in MS.

Hypotheses

Aim 1: The proposed cross-sectional project seeks to examine the parallel pathways of the visual system in adults with MS relative to healthy controls with novel, steady-state icVEP stimuli to assess for population differences in the parallel pathways that process bright (positive contrast) and dark (negative contrast) information. It sought to compare signal-to-noise ratios (SNR) of the steady-state isolated-check VEP between groups.

Hypothesis 1: We hypothesized that the MS population will display significantly lower signal-to-noise ratios compared to healthy controls. The VEP literature specific to MS populations has consistently demonstrated diseases processes targeting the visual system, particularly in response to low contrast stimuli, and we hope to further expand our understanding of how select ON and OFF cell activity may be preferentially impacted in MS populations (Galetta & Balcer, 2013; Kilstorner et al., 2013; Thurtell et al., 2009).

Aim 2: To compare amplitude measures of the steady-state isolated-check VEP between groups.

Hypothesis 2: It is hypothesized that the MS population will display significantly weaker amplitudes compared to healthy controls. The rationale for this hypothesis is based upon the

literature which suggests decreases in amplitude within MS populations (Blacer et al., 2015; Galetta & Balcer, 2013; Hamurcu et al., 2017; Kilstorner et al., 2013; Sakai et al., 2011; Thurtell et al., 2009).

Aim 3: To compare sine and cosine coefficients of the fundamental frequency component, which are used to compute the amplitude and phase measures, of the steady-state isolated-check VEP between groups.

Hypothesis 3: Is it hypothesized that adults with MS will display deficient VEP responses at the critical condition being examined. Conventional VEP analysis within the MS population have found characteristic responses which include increases in response latency (e.g., P100) to low contrast stimuli, as well as decreases in VEP amplitude (Galetta & Balcer, 2013; Kilstorner et al., 2013; Thurtell et al., Leigh, 2009).

Aim 4: Compare predictive classification accuracy of signal-to-noise and amplitude measures of the bright and dark isolated check VEPs for group membership.

Hypothesis 4: It is hypothesized that novel, bright and dark isolated check measures will yield significant classification accuracy of MS and control group membership. Given that responses at earlier depths of modulation are often clouded in noise, this analysis plans to target the response as it rises out of the noise for both groups, thus providing a more accurate and reliable measure of the response.

Aim 5: Exploratory evaluation of the phenomena of contrast gain and the underlying excitatory and inhibitory processes with steady state VEP responses to isolated-check stimuli with application of the biophysical model to the contrast response functions (Zemon and Gordon, 2006).

Hypothesis 5: A biophysical model obtained by steady-state VEP responses to isolated-check stimuli to measure contrast gain applied to an MS population has not yet been reported in the literature. Contrast gain control is a mechanism found strictly in the magnocellular system, and given evidence of magnocellular deficits in MS populations, it is hypothesized that the MS group will demonstrate weaker shunting inhibition and decreased excitatory input compared to healthy controls (Thurtell et al., 2009).

Chapter II: Methods

Data were collected from the MS Center at Holy Name Medical Center (HNMC) in Teaneck, New Jersey. The facility serves approximately 1,800 patients each year, with approximately 3,000 total individuals registered in its patient database. The MS Center consists of a team of MS specialty neurologists, nurses, and support staff. Data were collected by graduate student researchers in the lab of Frederick W. Foley, Ph.D., the MS Center's director of clinical psychology. All students received appropriate training in the administration and interpretation of electrophysiological testing. An ethical review of the study protocol and procedures for obtaining informed consent was conducted by the Institutional Review Board of the Albert Einstein College of Medicine. The protocol was approved as IRB #2011-636.

Participants and Recruitment

MS participants were approached directly in the MS Center at HNMC for recruitment. Individuals with MS were established patients at this tertiary care clinic with MS diagnosis confirmed on interview and in chart review at the facility. A convenience sample was used for control participants and individuals were recruited through emails, word of mouth, and personal contacts of researchers. After being screened to ensure participants met inclusion and exclusion criteria, an appointment was arranged to come back to the MS Center for participation in the study.

Eligibility and Exclusion Criteria

Individuals between the ages of 20 and 40, who met 2010 McDonald Criteria for relapse-remitting MS were eligible to participate. This age range was chosen to reflect individuals earlier in their disease course, as the mean age of MS diagnosis is around 33 years old, with 70% of cases emerging between ages 21 and 40 (Kaufman et al., 2016). Additionally, limiting age of

participants enrolled in the study helped to minimize the impact of age-related declines in VEP responses, such as a loss of luminance contrast sensitivity (Fiorentini, Porciatti, Morrone, & Burr, 1996). Individuals diagnosed with clinically isolated syndrome, according to their medical records, were recruited as part of the larger parent study, however, they were not included in this study due to low sample size. Exclusion criteria included a diagnosis of an active seizure disorder, active MS exacerbation, corrected visual acuity less than 20/30, and visual diagnoses that impact the VEP response (e.g., glaucoma, diplopia). Best-corrected visual acuity, via contact lenses or eyeglasses, was measured behaviorally with an Early Treatment Diabetic Retinopathy Study (ETDRS) chart, with decimal scale used for measurement, from the viewing distance of 65 cm for each participant. Patients with optic neuritis were included with their status documented. Stratified sampling was used for gender in order to ensure comparable proportions of men and women in each group, as MS populations have a higher preponderance of females than males.

Informed consent procedures. Upon arrival for the appointment, all participants were oriented to the study then reviewed and signed an informed consent document which covered confidentiality and limits to confidentiality, risks and benefits of involvement, right to withdraw at any time, and contact information for the research coordinator and principal investigators.

Risks and benefits to participants. Minimal risk was involved for study participants. The physical contacts with the instruments were limited to the disposable EEG electrodes applied to the face and EEG electrodes applied with water-soluble electrode paste to the scalp. The displays were no more hazardous than a TV screen. The physical discomforts that may be involved include the slight abrasion of the scalp by the electrode paste and some fatigue resulting from sitting in one position for several minutes at a time. Participants were not provided any payment or other compensation for taking part in this study. While there were no direct benefits

from participation in the study, possible benefits might be obtained in the future if these experiments yield information useful on the basic functioning of the visual system and in the treatment or prevention of neural disorders.

Data security. Paper copies of study related materials (i.e. informed consent, demographics questionnaire, fatigue and depression measures, and EEG/electroretinography (ERG) protocol) were secured in a locked filing cabinet in a locked room with other research and clinical data within the MS Center at Holy Name Medical Center. Digital files of EEG/ERG data are stored, de-identified and encrypted, on the EvokeDx machine in a locked room in the MS Center.

Measures

VEP Equipment. All workspace and questionnaire supplies were provided by Holy Name Medical Center. An EvokeDx system (Konan Medical USA) was used for stimulus presentation, data collection, storage, and analysis. An organic light-emitting diode (OLED) display was used for stimulus presentations. An isolated differential amplifier, with a gain of 20K and a bandpass filter of 0.5-100 Hz, was used to protect against electrical shock. Visual acuities were collected monocularly from the viewing distance of stimulus presentation (65 cm) using the EvokeDx system. The EvokeDx system includes an infrared technology used to track eye movements to ensure participants had adequate fixation on the stimuli. Researchers closely monitored gaze of each participant during stimulus presentation to ensure a steady fixation was maintained. The EEG signal was recorded synchronized to the display's frame rate, amplified, digitized at ten samples per frame, and stored in the computer.

Stimuli. The isolated-check VEP (icVEP) is a steady-state, low-contrast to high-contrast response that is thought to target the magnocellular ON (bright) or OFF (dark) pathways at low

contrast (Zemon & Gordon, 2006). The dark and bright isolated-check stimuli are represented in Figures 1 and 2, respectively. The background luminance of the screen was approximately 50 cd/m^2, with a stimulus field size subtending 8.6^o x 8.6^o to 17^o x 17^o of visual angle depending on test condition with a screen resolution of 1920 x 1080 pixels. The frame rate was 60 Hz. An isolated differential amplifier, with a gain of 20K and a bandpass filter of 0.5-100 Hz, was used to protect against electrical shock. The luminance of the checks varies sinusoidally in time so that the pattern appears and disappears. Both the bright and dark isolated-check stimuli provide a depth of modulation (DOM) sweep from 1 to 32% DOM (peak contrast is double those values) with each stimulus step 1.6 seconds in duration. There is a brief, 1.6 second adaptation period before the first stimulus step is presented (with data collection commencing) with the pattern presented at half the contrast of the first stimulus step during adaptation. A 24 x 24 array of 16' checks in a 12.8^o x 12.8^o field size with background luminance of 50 nits was used.

Demographic Questionnaire. A demographics questionnaire was created for this study to collect additional participant information. The questionnaire gathered information related to the individual's gender, age, self-identified race, years of education, pertinent medical and psychiatric history, and current medications. Participants were asked about current visual symptoms including colorblindness, current or past ON, glasses/contacts, or other visual problems or hearing impairments.

Procedures

Testing occurred over a single session, lasting approximately 90 minutes, at the MS Center at HNMC. Participants were introduced to the study and informed consent was reviewed with the participant before allowing them ample time to review the consent and provide their signature. The participant was then then asked to fill out a brief questionnaire to gather basic

demographic information, including age, gender, self-identified race, contact information, history of visual/hearing impairment, current medical diagnoses, current medications, and current psychiatric diagnoses. Questionnaires that inquire about recent mood and fatigue symptoms (Beck Depression Inventory and the Fatigue Scale for Motor and Cognitive Functions) were included as part of the parent study and were not analyzed for this study. ERG and EEG testing followed completion of the forms and the participants were encouraged to take breaks as needed and throughout the testing process. ERG testing was included as part of the parent study and was not analyzed for this study. ERG testing was completed first, typically lasting approximately 20 minutes, followed by completion of EEG testing, which lasted approximately one hour. An EvokeDx system (Konan Medical USA) was used to record, store, and analyze retinal and brain responses. A 5-electrode lead (ERG) or 3-electrode lead (VEP) recording system was used to measure responses. For ERG data, collected binocularly, disposable electrodes were placed under each eye, directly centered beneath the retina, and approximately 2 cm from the outer canthus on either side of the head. For VEP data collection, two electrodes (*Oz*, active, and *Cz*, reference) were attached to the scalp with water-soluble electrode paste and a disposable ground electrode (*Pz*) was placed on the forehead, in accordance with the International 10-20 System (Jasper, 1958). During VEP data collection, participants wore an eye patch to cover the untested eye. Every other participant started testing with their right eye, as such, the other half of participants began testing with their left eye. While the parent study collected multiple stimuli for both VEP and ERG data, only isolated-check VEP stimuli for MS, CIS, and healthy controls were analyzed for this study. The parent study VEP battery consisted of eight test conditions. Each of the eight conditions were run until ten valid runs were collected per condition. An

auditory stimulus signaled the start of a test and the participant was asked to fixate on the crosshairs in the center of the screen.

Data Analysis Plan

The EvokeDx system was used to perform the Fourier transform and the calculations of SNR and T^2_{circ} statistic. A discrete Fourier transform was used to extract the frequency components of the responses. Stimuli were modulated with a temporal frequency of 10 Hz. The fundamental frequency component of the ssVEP elicited by the isolated-check stimulus is the dominant response. The results are represented in the EvokeDx system in a sine-cosine plot as well as amplitude and phase versus DOM. After ten complete runs for each stimulus condition are completed, the T^2_{circ} statistic was used to calculate the signal-to-noise ratio (SNR) for this component (Victor & Mast, 1991). Determination of a significant response at the .05 level is made when the SNR is greater than one. A signal-to-noise response lower than one indicates a deficit in low-contrast processing. Sine and cosine coefficients are used to derive amplitude and phase measures. The EvokeDx system displays sine-cosine plots with an error circle obtained with the T^2_{circ} statistic representing a 95% confidence region about the mean response. (Zemon & Gordon, 2006; Zemon et al., 1997).

The data were explored through descriptive analyses and scatterplots were used to detect bivariate outliers in the sample and to test for normality and linearity of the data. Then repeated measure ANOVAs were used to assess differences between the MS group and the healthy control group for Aims 1 and 2. For Aim 5, each condition for each eye was assessed and criteria were applied to determine whether data was appropriate for use in the biophysical model fit analysis. Data was removed from model fit analysis if there were less than 2 consecutive points out of the noise. After this initial standard analysis, the database was restructured to

perform linear mixed-effects modeling (LMM) to achieve Aims 1, 2, and 5. LMM permitted exploration of the intraclass correlation coefficients (ICCs) within individual observers as well as fixed interaction and main effects of group membership and the other factors within the study. ICC's computed for the null model in LMM were used to determine if data were correlated within an individual. Fixed effects were added to the models and to examine between-group differences in amplitude and SNR outcome measures.

The data are hierarchical and LMM accounts for variance at the different levels at the same time (Field, 2009; O'Dwyer & Parker, 2014; Woltman, Feldstain, MacKay, & Rocchi, 2012). LMM allowed for exploration of the variance components, for the assessment of differences within and across individuals, and how related data were within a unit.

LMM was used to analyze the critical measures of amplitude and SNR (Aims 1 and 2). The LMM was built up in several steps: Model 1 was the null model in which only participant (intercept) was treated as a random effect and there were no fixed effects other than the intercept. An ICC was calculated from the null model to determine whether LMM was a reasonable approach. There were just three parameters in Model 1. Terms and parameters were added systematically to develop the more complex models. In Model 2, group was added into the model as a main effect, which added one parameter, for a total of four parameters in this model. In Model 3, log depth of modulation (log_2DOM) was added as a covariate and treated as a fixed effect. This led to five parameters in Model 3. Model 4 contained a Group x Log_2Dom interaction term, leaving this model with six parameters. In Model 5, test condition (bright versus dark checks) was treated as a factor and added as a fixed effect, leaving the final model with seven parameters. The fit of each model was compared using chi-square differences. As the models were built up, the chi-square statistic for the *deviance*, change in the -2LL, was assessed,

and this number is expected to decrease significantly if the more complex hierarchical model provides a better fit to the data (Field, 2009).

LMM was also used in an exploratory analysis of the biophysical model of Zemon and Gordon (2006) (Aim 5). The model was built in several steps: Model 1 was the null model in which only participant (intercept) treated as a random effect and there were no fixed effects other than the intercept. An ICC was calculated from the null model to determine whether LMM was a reasonable approach. There were three parameters in Model 1. For Model 2, group was added as a main effect, adding one parameter, for a total of four parameters in this model. In Model 3, test condition (bright vs. dark checks) was added as a fixed effect, leaving this model with five parameters. Model 4, contained a Group x test condition interaction term, leaving the final model with six parameters.

Power Analysis. The current study aims to build upon the current diagnostic tools for MS diagnosis and monitoring of disease progression over time. Traditionally, VEP analysis has revolved around the characteristic P100 latency measure elicited through contrast-reversing VEP stimuli. Prior studies using a range of sample sizes have found significant differences and large effects between MS and control groups using the characteristic P100 latency measure (Chirapapaisan et al., 2015; Duwaer & Spekreijse, 1978; Halliday, McDonald, & Mushlin, 1973b; Hamurcu et al., 2017; Thurtell et al., 2009;). Thurtell et al. (2009) used a sample size of 15 MS patients and 15 controls and reported a significant difference in P100 latency ($p < .01$), with mean responses 20-30 ms greater for patients. Balnytė et al. (2011) found significantly increased P100 latencies in the MS group in both eyes, with mean latencies (ms) of 122.76 ± 14 for the right eye and 122.60 ± 12.52 for the left eye. Mean latencies (ms) of the control group were 103.94 ± 11.70 for the right eye and 14.53 ± 10.93 for the left eye. The same study

identified significantly decreased P100 amplitude in the MS group for both eyes, with mean amplitude (μV) of 3.68 ± 2.66 for the right eye and 3.65 ± 2.66 for the left eye. Mean amplitudes (μV) of the control group were 5.74 ± 2.44 for the right eye and 6.15 ± 2.55 or the left eye (Balnytė et al., 2011). Prior research that used the same isolated-check stimuli as this study has been done in different disease populations, such as autism spectrum disorder, demonstrating similar results. Weinger et al.'s (2014) study of children with ASD under the dark-check condition (SNR at 4% DOM, *M* = .765, *SD* = .539.751) and typically developing children (SNR at 4% DOM, *M* = 1.4, *SD* = .751) indicated that an alpha of .05 and a power of .80 would require 14 participants per group to detect an effect size (Cohen's *d*) of .97. The large effects observed in past research using similar techniques led us to expect that the current study will generate large effect sizes under monocular viewing conditions. To achieve a large effect size of *d* = 1.0 with α = .05 and power = .80 using the Mann-Whitney test, a sample size of 14 per group is needed. (G*Power v3.1.9.2). The proposed sample size accounted for unusable data.

Chapter III: Results

A number of the VEP measures were analyzed to satisfy the aims of the study: to compare signal-to-noise ratios of the steady-state isolated-check VEP in adults with MS relative to healthy controls (Aim 1); to compare amplitude measures of the steady-state isolated-check VEP between groups (Aim 2); to compare sine and cosine coefficients of the fundamental frequency component, which are used to compute the amplitude and phase measures, of the steady-state isolated-check VEP between groups (Aim 3); to estimate predictive accuracy for classification of individuals with MS and controls (Aim 4); and to examine application of the biophysical model to contrast-response functions (Aim 5). Scatterplots were used in the initial stages of analysis to detect bivariate outliers in the sample and to test for normality and linearity of the data.

A total of 40 adults were recruited for this study. Twenty individuals with a diagnosis of relapsing-remitting multiple sclerosis (MS) according to their medical records and twenty healthy control participants (CN) were recruited from Holy Name Medical Center and around the New York Tri-State area. Data from two control participants were removed from the study after waveform analysis revealed abnormal function that may represent a deficit in magnocellular functioning. It was suspected the abnormal waveforms presented in these two control participants may be related to age, as both of the participants were twenty years old. Removal of these two participants also made for better matched populations based on age range. The final sample comprised of 20 adults with MS and 18 healthy controls.

Table 1 summarizes the demographic information and clinical characteristics of the sample. A Mann-Whitney U test revealed no significant difference in age ($U = 136.5$, $p = .21$, two-tailed test) for control participants and MS participants. There was a difference between

groups for education, with the control group having more years of education on average (U = 87.5, p = .03, two-tailed test). A chi-square test for independence found there was no significant difference in gender between groups ($\chi^2(1, N = 38) = .07$, $p = .79$). Although 80.0% of the MS group and 83.0% of the control group were females, the difference reflects the greater proportion of females with MS in the general population (Koch-Henriksen, & Sorensen, 2010; Rotstein et al., 2018). All participants enrolled in the study were required to have a corrected visual acuity of at least 20/30 at a viewing distance of 65 cm for either eye. Acuity was measured by using the decimal form of acuity (e.g., 20/40 = 0.5). The MS group yielded a median acuity value of 1 (IQR = 0.2) for both eyes and the control group yielded a median value of 1 (IQR = 0) for both eyes. Mann-Whitney U test revealed no significant difference in acuity for the right eye (U = 144.0, p = .30) and the left eye (U = 125.5, p = .12) for the two groups. All but two participants in the patient group reported taking one or more medications at the time of testing. MS participants were on a variety of medications including Cyclobenzaprine, Lemtrada, Natalizumab, Copaxone, Abagio Topiramate, 54 Pilocarpine, Modafinil, Phenytoin, Ampyra, Baclofen, Ocrevus, Rituxan, Lexapro, Adderall, Nexplanon, Synthroid, Myrebetriq, Tizanidine, Acyclovir, and Levothyroxine.

Initial Analyses

Aim 1: Signal-to-noise ratio. Representative output from EvokeDx to bright isolated-check test condition for each cohort are illustrated in Figures 3A-B. After initial analysis of the data, it became clear that the participants' responses do not begin to rise above the noise until after 4% DOM. Mean SNRs fell clearly below 1 at 1% and 2% DOM, which indicates that the signal overall was not significant, as demonstrated in Figure 4 which aggregated bright and dark isolated-check conditions for each group. As such subsequent analyses were conducted on the

four highest levels of DOM. Because DOM increases in octave steps, doubling in DOM from 1% to 2% to 4% and so on, DOM was log transformed (base 2) ($\log_2$DOM). The highest levels of SNR are seen in the healthy control group. Signal-to-noise ratio (SNR) was calculated by dividing the mean amplitude by the radius of the 95% confidence circle. Figures 5A-D depict repeated measure ANOVA graphs of group differences in SNR for both stimulus conditions (bright and dark checks) and eyes among the four highest depths of modulation (Aim 1). There was no significant interaction effect in the bright condition for right eye, F (3,108) = 1.42, p = .25, η_p^2 = .04, or left eye F (3,108) = .56, p = .56, η_p^2 = .02. Similarly, there was no interaction effect in the dark condition for right eye, F (3,108) = 1.34, p = .26, η_p^2 = .04, or left eye, F (3,108) = .31, p = .73, η_p^2 = .01. Though this initial analysis did not reveal statistically significant differences between groups, on visual inspection, there were notable trends toward significance observed between cohorts, suggesting the possibility of higher SNR values in the control group relative to the MS group.

Aim 2: Amplitude. Amplitude was examined to assess the strength of the responses for each group (Aim 2). Analyses were completed with the four highest levels of depth of modulation, as it was determined that the response did not rise above noise level until after 4% DOM. Figures 6A-D depict repeated measure ANOVA graphs of group differences in amplitude for both stimulus conditions (bright and dark checks) and eyes among the four highest depths of modulation. There was no significant interaction effect in the bright condition for right eye, F (3,108) = 2.13, p = .10, η_p^2 = .06, or left eye F (3,108) = 1.20, p = .30, η_p^2 = .03. Similarly, there was no interaction effect in the dark condition for right eye, F (3,108) = 2.93, p = .08, η_p^2 = .08, or left eye, F (3,108) = 1.62, p = .21, η_p^2 = .04. Though this initial analysis did not reveal statistically significant differences between groups, on visual inspection there were notable

differences observed between cohorts, suggesting the possibility of stronger apmplitude values in the control group relative to the MS group.

Aim 3: Sine-cosine plot. Sine and cosine coefficients were analyzed for the fundamental frequency component, 8% DOM for bright and dark isolated-checks, as a measure of the combined effect of amplitude and phase (Aim 3). This critical condition contrast level generally provides the strongest separation as the rising point of the contrast function, as the earlier two contrast levels before this point are frequently clouded by noise. Sine vs. cosine coefficient scatterplots were generated, split for group. Linear regressions were plotted for both groups in the sine vs. cosine scatterplots for the fundamental frequency component of bright (Figure 7A) and dark (Figure 7B) check stimuli, with the different slopes representing the different phases of the responses for each group. For the bright check condition, the control group appeared to demonstrate a linear relation, with an r^2 value of .46. However, for the MS group, who yielded an r^2 value of .01, it appears that the slope is nearly zero which indicates phase in the cosine direction. The regression lines for each group in the dark check condition demonstrated a similar pattern, as seen in Figure 7B.

Analysis of covariance. To test for heterogeneity of regression coefficients, an ANCOVA was performed for both bright and dark isolated-check conditions for the fundamental frequency component (Aim 3). The factor was the group, either MS or control, and the outcome variable was the sine coefficient. Cosine coefficient was used as the covariate in this analysis. For the bright check condition, there was a significant interaction effect of Group x Cosine Coefficient, F (2, 72) = 23.14, $p < .001$, with a large effect size ($\eta_p^2 = .24$). There was a significant main effect for cosine, F (2, 72) = 18.57, $p < .001$, but not for group, F (2, 72) = 1.71, $p = .20$. A similar pattern was observed for the dark check condition at the same contrast level.

There was a significant interaction effect between group and cosine, F (2, 72) = 26.05, p < .001, with a large effect size (η_p^2 = .27). There was a significant main effect for cosine, F (2, 72) = 32.07, p < .001, but not for group, F (2, 72) = .20, p = .66. Results provide evidence supporting differential (deficient) VEP responses at the critical condition being examined in the MS population.

Linear Mixed-Effects Modeling

Following the initial inspection of SNR and amplitude through repeated measures ANOVA analysis, LMM was performed on the four highest levels of DOM, log transformed (base 2) ($\log_2$DOM), given the likely correlations among data within individuals. The aims of the study are to explore group differences in a novel response for this population. The standard statistical analysis assumes independent data, which is unlikely due to multiple measures per participant. LMM enables modeling of the data with explicit covariance structures while allowing exploration of effects as random as well as fixed. DOM was expected to be a critical covariate given the significant dependence of the response measure based on contrast level. Stimulus condition (bright vs. dark checks) was expected to be a key factor given the known differences between neurons in the ON and OFF Pathways.

The LMM models were built hierarchically. For Aims 1 and 2, Model 1 was the null model and only intercept (participant) was treated as a random effect. The intraclass correlation coefficient (ICC) was calculated with the variance components obtained from Model 1. Typically, an ICC > .05 is considered evidence for the need to use an LMM approach. Group was then added as a main effect in Model 2. In Model 3, $\log_2$DOM was treated as a covariate and added as a fixed effect. Model 4 incorporated the group by $\log_2$DOM interaction term, and it was added as a fixed effect. Finally, in Model 5, test condition (bright vs. dark checks) was treated as

a factor and added as a fixed effect. A summary of results for all four models for both response measures, amplitude and SNR, can be found in Tables 2 and 3.

For Aim 5, due to a limited sample size, an exploratory analysis with application of the biophysical model to the contrast response functions was conducted with LMM. Three primary parameters were examined: initial contrast gain (i.e., main determinant of the initial slope of the function), shunting coefficient (i.e., a measure of contrast gain as it determines the shunting conductance at a particular contrast level), and phase (i.e., initial phase of second harmonic response). First, the data were explored with non-parametric Mann-Whitney U Tests, which did not reveal significant results. Given the correlated data, it was determined that LMM would be the more sensitive and appropriate analysis for this data set. The LMM model was built hierarchically. Model 1 was the null model and only intercept (participant) was treated as a random effect. The intraclass correlation coefficient (ICC) was calculated with the variance components obtained from Model 1. For Model 2, group was added as a main effect. In Model 3, test condition (bright vs. dark checks) was added as a fixed effect. Finally, Model 4 contained a Group x Test Condition interaction term. Representative contrast-response functions for both the MS and control groups are represented in Figure 8, fitted with the biophysical model of Zemon and Gordon (2006).

Aim 1: Signal-to-noise ratio. A summary of the SNR results for the four models can be found in Table 2 (Aim 1). In Model 1, the null model, only participant was treated as a random effect, and the calculation of the ICC indicated correlated data (ICC = .310). In Model 2, group was added as a main effect and it was not statistically significant. Model 2 did not have a significantly better fit over all (Δ-2LL = 2.105, df = 1). In Model 3, $\log_2$DOM was added as a covariate and as a fixed effect and this model produced a significantly better fit to the data than

the previous model (Δ-2LL = 296.343, *df* = 1, $p < .01$). The covariate log_2DOM as a fixed effect was significant ($p < .01$). In Model 4, the Group x Log_2DOM interaction term was added as a fixed effect, which produced a significantly better fit than did Model 3 (Δ-2LL = 7.463, *df* = 1, $p < .01$). There was a significant Group x Log_2DOM interaction effect in this model ($p < .01$). Model 5 included test condition (bright vs. dark checks) as a fixed effect, which produced significantly better fit than Model 4 (Δ-2LL = 37.579, *df* = 1, $p < .01$). The effect of test condition (bright vs. dark checks) was significant ($p < .001$). There were no significant interaction effects noted for test condition and Model 5 fit the data best. Overall, analyses suggest that the MS population appeared to display significantly lower signal-to-noise ratios compared to the control group.

Aim 2: Amplitude. A summary of the amplitude results for the four models can be found in Table 3 (Aim 2). In Model 1, the null model, only participant was treated as a random effect, and the calculation of the ICC indicated correlated data (ICC = 0.416). In Model 2, group was added as a main effect. Model 2 did not have a significantly better fit over all (Δ-2LL = 2.492, *df* = 1). In Model 3, log_2DOM was added as a covariate and as a fixed effect, and this model produced a significantly better fit to the data than the previous model (Δ-2LL = 323.031, *df* = 1, $p < .01$). The covariate log_2DOM as a fixed effect was significant ($p < .001$). In Model 4, the Group x log_2DOM interaction term was added as a fixed effect, which produced a significantly better fit than did Model 3 (Δ-2LL = 18.917, *df* = 1, $p < .01$). There was a significant Group x Log_2DOM interaction effect in this model ($p < .001$). Model 5 included test condition (bright vs. dark checks) as a fixed effect, which produced significantly better fit than Model 4 (Δ-2LL = 31.022, *df* = 1, $p < .01$). The effect of test condition (bright vs. dark checks) was highly significant ($p < .001$). The data were analyzed for interaction effects and 1% and 2% DOM were

again left out of the analysis because the responses were not out of the noise at these levels. A three-way interaction Group x Log_2DOM x Test was considered with intercept as a random effect and log_2DOM as a covariate and a fixed effect, to mirror the linear mixed effects modeling completed above. The three-way interaction effect for Group x Test x Log_2DOM was not significant ($p = .37$). There was a significant two-way interaction present for Test x Log_2DOM ($p < .01$). Overall, analyses suggest that the MS population appeared to display significantly weaker amplitudes compared to the control group.

Aim 5: Contrast response functions fit with the biophysical model

Initial contrast gain. In Model 1, the null model, only participant was treated as a random effect and the calculation of the ICC indicated correlated data (ICC = 0.687). In Model 2, group was added as a main effect. Model 2 did not have a significantly better fit over all (Δ-2LL = 1.425, $df = 1$) and group as a fixed effect was not significant ($p = .24$). In Model 3, test condition (bright vs. dark checks) was added as a fixed effect, and this model produced a significantly better fit to the data than the previous model (Δ-2LL = 9.732, $df = 1$, $p < .01$). Test condition as a fixed effect was significant ($p < .01$). In Model 4, the Group x Test Condition interaction term was added as a fixed effect, which did not produce a significantly better fit than Model 3 (Δ-2LL = 0.952, $df = 1$, $p > .05$). The interaction effect for Group x Test Condition was not significant ($p = .33$).

Shunting coefficient. In Model 1, the null model, only participant was treated as a random effect, and the calculation of the ICC indicated correlated data (ICC = 0.260). In Model 2, group was added as a main effect. Model 2 had a significantly better fit over all (Δ-2LL = 4.906, $df = 1$, $p < .05$) and group as a fixed effect was significant ($p = .03$). In Model 3, test condition (bright vs. dark checks) was added as a fixed effect, and this model did not produce a

significantly better fit to the data than the previous model (Δ-2LL = 0.618, *df* = 1, *p* > .05). Test condition as a fixed effect was not significant (*p* = .43). In Model 4, the Group x Test Condition interaction term was added as a fixed effect, which did not produce a significantly better fit than Model 3 (Δ-2LL = 1.777, *df* = 1, *p* > .05). The interaction effect for Group x Test Condition was not significant (*p* = .18).

Phase. Notably, the phase values were adjusted by 2π radian for a cutoff value of > -13 for all eyes. In Model 1, the null model, only participant was treated as a random effect, and the calculation of the ICC indicated correlated data (ICC = 0.703). In Model 2, group was added as a main effect. Model 2 did not have a significantly better fit over all (Δ-2LL = 0.213, *df* = 1, *p* > .05) and group as a fixed effect was not significant (*p* = .65). In Model 3, test condition (bright vs. dark checks) was added as a fixed effect, and this model produced a significantly better fit to the data than the previous model (Δ-2LL = 7.642, *df* = 1, *p* < .01). Test condition as a fixed effect was significant (*p* < .01). In Model 4, the Group x Test Condition interaction term was added as a fixed effect, which did not produce a significantly better fit than Model 3 (Δ-2LL = 0.063, *df* = 1, *p* > .05). The interaction effect for Group x Test Condition was not significant (*p* = .80).

Aim 4: Classification Accuracy

ROC Curve Analysis. ROC curve analyses were used to estimate predictive accuracy for classification of individuals with MS and controls (Aim 4). Predictive power for group membership was assessed using signal-to-noise and amplitude measures obtained from steady-state VEP responses to bright and dark isolated-check stimuli for the fundamental frequency component. Results are graphically presented in Figure 9. SNR responses to bright checks at 8% DOM did not provide significant results (AUC = .59, 95% CI [0.46, 0.72], *p* = .18). However, at 8% DOM, amplitude responses to bright checks provided significant classification between

groups (AUC = .63, 95% CI [0.51, 0.76], p = .05). Among SNR and amplitude responses to dark checks at 8% DOM, both SNR (AUC = .66, 95% CI [0.54, 0.79], p = .01) and amplitude (AUC = .70, 95% CI [0.58, 0.82] p < .01) provided significant classification between groups. Overall, amplitude slightly outperformed SNR in classification accuracy, with modest effects.

Logistic Regression. A hierarchical logistic regression was performed for the bright and dark isolated check conditions to assess the predictive ability of each level of contrast (Aim 4). At the fundamental frequency condition, 8% DOM, for bright checks, the model (-2 log likelihood = 92.989, Nagelkerke R^2 = .197) classified correctly 67% of the overall population, correctly classifying 58% of controls and 75% of individuals with MS and misclassified 15 controls and 10 patients. The sine coefficient made a uniquely statistically significant contribution to the model (p < .01), with an odds ratio of 2.21, which is a moderate effect. There was no significance improvement when the interaction term was added to the model.

At 8% DOM for the dark checks, the model (-2 log likelihood = 95.82, Nagelkerke R^2 = .154) classified correctly 67% of the overall population, correctly classifying 61% of controls and 73% of individuals with MS and misclassified 11 patients and 14 controls. The sine coefficient again made a uniquely statistically significant contribution to the model (p < .01), however the odds ratio, 1.74, was lower in comparison to the bright check condition. The addition of the Cosine x Sine interaction term was significant and increased the overall classification to 68% (-2 log likelihood = 87.88, Nagelkerke R^2 = .271), correctly classifying 58% of the controls and 78% of the patients, while misclassifying 9 patients and 15 controls, with moderate effect. Overall, the logistic regression supported classification accuracy with modest effects.

Chapter IV: Discussion

This study is an exploration of group differences in isolated-check VEP responses for adults with multiple sclerosis and healthy control adults. The bright and dark isolated-check stimuli have not yet been applied to an MS population, however, there has been substantial research validating their use in various clinical populations, including schizophrenia, autism, glaucoma, headaches, epilepsy, and retinitis pigmentosa (Alexander et al., 2005; Butler et al., 2001; Coppola et al., 2013; Conte & Victor, 2009; Greenstein et al., 1998; Siper et al., 2016; Weinger et al., 2014).

In order to tap into activity of ON and OFF cells, bright and dark isolated-check stimuli were used to collect VEP responses monocularly. A high temporal frequency of modulation was used to sweep from low to high contrasts in order to emphasize contributions from the magnocellular pathway. Signal-to-noise ratio, amplitude, sine/cosine coefficient measures, and contrast-response measures (initial contrast gain, shunting coefficient, and phase) were analyzed, and comparisons were made between the MS and healthy control groups. Multivariate statistics were run to test for group differences and LMM was applied as well to account for the correlated data within individuals. The models were built hierarchically to account for specific factors and covariates. To most accurately assess aims 1 and 2 of the present study, and to assess the unique contribution of group on each outcome measure the variable DOM (depth of modulation) was treated as a covariate and test condition (bright vs. dark checks) were added as fixed factors. LMM allowed for the multiple contrast levels (4% through 32% DOM) to be analyzed at one time, rather than comparing each DOM step separately. Aim 5 of the present study incorporated LMM models build hierarchically to account for the unique contribution of group as a main

effect and test condition as a fixed effect. Predictive accuracy for classification of individuals with MS and healthy controls was completed with ROC curve analysis and logistic regression.

Interpretation

Aim 1: Signal-to-noise Ratio. SNR is calculated by dividing the mean amplitude by the radius of the 95% confidence circle, and this measure adjusts for the noise in the responses. SNR is a crucial measure, due to the magnitude of the VEP responses relative to the noise level, as it is more representative of the actual strength of the response. When considering a clinical population as heterogenous as those with multiple sclerosis, sizable amplitude differences across individuals is expected, but the adjustment for noise in the calculation of the SNR normalizes the data. Analysis of group differences using repeated measure ANOVAs for both bright and dark check conditions did not reveal significant results for either eye tested. Though these analyses did not meet the level of statistical significance, visual inspection of the data clearly demonstrated separation of the groups and these initial analyses assumed independence of residuals. However, these data are sampled from similar contexts, therefore independence is unlikely to be true, and residuals are likely to be correlated due to the influence of the contextual elements. LMM factors contextual variables into the analysis, overcoming the problem of non-independent observations.

When taking into account the variance within and across individuals simultaneously, statistically significant differences were identified between groups. After building the model hierarchically for Aim 1, Model 5 demonstrated significant differences among MS and healthy control groups for the fixed effect of depth of modulation as a covariate, the effect of test condition (bright vs. dark checks), and the interaction effect between group and depth of modulation, supporting Hypothesis 1. The LMM for SNR revealed that the overall group

differences were not significant, which is not surprising given inter-individual variability of the SNR data.

The MS group demonstrated weaker SNR responses overall, which may be due to magnocellular pathway deficits within that population (Thurtell et al., 2009). These finding can help shed more light on visual dysfunction experiences in those with MS, which is of particular relevance to this population when considering most individuals with MS endorse some form of visual disturbance along the course of their disease, with 90% having identified lesions along their visual pathway, irrespective of clinical history (Ikuta & Zimmerman, 1976; Toussaint, Périer, Verstappen, & Bervoets, 1983). These novel VEP methods allow the analysis of the earliest stages of visual processing in order to isolate whether there might be a specific pathway deficit, even without clinical manifestations of the disease.

Aim 2: Amplitude. Amplitude data were considered to assess group differences in the magnitude of the responses. Similar to analyses of SNR, repeated measures ANOVAs did not find significant differences between groups for bright or dark isolated-checks in either eye. Again, LMM analysis takes into account contextual factors, while examining the variance within and across individuals simultaneously. After building the model hierarchically, Model 5 demonstrated significant differences among MS and healthy control groups for the fixed effects of depth of modulation as a covariate and the effect of test condition (bright vs. dark checks). There were also significant interaction effects of Group x Depth of Modulation, as well as Test Condition x Depth of Modulation. Significant findings for amplitude provide support for Hypothesis 2. There were no overall group differences in the LMM for amplitude, which like SNR, is not surprising given inter-individual variability of the amplitude data. These findings

also support the hypothesis of deficient functioning along the magnocellular pathway among individuals with MS.

Aim 3: Sine and cosine coefficients as measures of the combined effect of amplitude and phase. Linear regressions conducted for both groups demonstrated a clear difference in slopes, representing the phase of the response. Similar trends for both groups were seen in the bright and dark isolated check conditions. Tests for heterogeneity of the regression coefficients, for both bright and dark check conditions, revealed significant findings. For the bright check condition, there was a significant interaction effect of Group x Cosine which was used as the covariate. A similar pattern was observed for the dark check condition, with a significant interaction effect of Group x Cosine. These findings support Hypothesis 3 and suggest a phase shift for the MS group. The complexities of the visual system complicate the interpretation of these findings; however, it is possible that a transmission delay or demyelination may influence a shift in phase. Conventional VEP methods have identified increased response latency to low contrast stimuli as a characteristic VEP response in MS, yet the answer as to why there is an apparent time delay remains unclear (Blacer et al., 2015; Galetta & Balcer, 2013). However, these finding support the differences in specific mechanisms and neural pathways between MS compared to controls, particularly as it relates to the magnocellular pathway.

Aim 4: Classification accuracy of signal-to-noise and amplitude measures. Both ROC curve analyses and logistic regression were used to estimate predictive accuracy for classification of individuals with MS and controls. ROC curve analyses demonstrated modest predictions with the fundamental frequency component, for 8% DOM. For the bright check condition, SNR did not provide significant results, however, response amplitude did provide significant and

moderate classification between groups. For the dark check condition, both SNR and response amplitude provided significant classification between groups, again with modest effects.

Similar results were observed when a hierarchical logistic regression was performed for the bright and dark isolated-check conditions. When analyzed at 8% DOM for bright checks, the model classified correctly 67% of the overall population. The sine coefficient made a uniquely significant contribution to the model, of moderate effect. For the dark check condition, at the same DOM, the model classified correctly 67% of the overall population, with sine coefficient making a uniquely significant contribution, of moderate effect. For the dark check condition, the addition of the Cosine x Sine interaction significantly increased the overall classification to 68%, again with moderate effect. Even in this small sample, moderate predictive accuracy for group membership was obtained, supporting Hypothesis 4.

Aim 5: Exploratory analysis of contrast response measures (initial contrast gain, shunting coefficient, and phase) with the biophysical model. A preliminary analysis of the data via non-parametric Mann-Whitney U analysis did not reveal significant differences between groups. However, when taking into consideration the variance within and across individuals simultaneously, statistically significant differences were identified between groups with linear mixed-effects modeling. When initial contrast gain was considered in the assessment of group differences, the LMM Model 4 demonstrated significant differences for the fixed effect of test condition (bright vs. dark checks), supporting hypothesis 5. However, there was no significant effect for group, nor was there a significant interaction effect of Group x Test Condition. In consideration of the shunting coefficient parameter, Model 4 demonstrated significant differences for group as a fixed effect. Test condition as a fixed effect and the interaction effect of Group x Test Condition were not significant in this model. When the parameter phase was

considered, the LMM Model 4 demonstrated significant differences for test condition as a fixed effect, yet group as a fixed effect and the interaction effect of Group x Test Condition were not significant. Notably, these exploratory analyses in Aim 5 are completed with a small data set and results show promise for the possibility of additional significant findings in the context of a larger study. Even in this small sample, significant effects for group (shunting coefficient parameter) and test condition (phase and contrast gain parameters) were obtained, supporting Hypothesis 5.

Clinical Implications

The study findings indicate that the steady-state isolated-check VEP response is a valid means by which to detect the presence of the MS disease process within the visual system. Results are consistent with prior MS literature that suggested increases in response latency to low contrast stimuli and decreases in VEP amplitude attributed to axonal loss (Balcer, Miller, Reingold, & Cohen, 2015; Galetta & Balcer, 2013; Hamurcu, Orhan, Sarıcaoğlu, Mungan & Duru, 2017; Sakai et al., 2011; Thurtell et al., 2009). Even in a small sample, results of this study suggest the magnocellular pathways of individuals with MS may be differentially affected when compared to healthy controls. This study further validates the extant literature supporting the use of VEPs as a paraclinical tool in the assessment of MS. While predictive classification accuracy of icVEP measures analyzed in this study were modest, they demonstrate exciting promise, warranting additional research into these techniques. These results only begin to scratch the surface of the knowledge that can be obtained through VEP techniques, as continued research with larger sample sizes will allow for analyses that we were not able to complete in this study. Evidence from this study provides additional validation of the use of steady-state, short-duration VEPs in clinical settings, reproducing results that are characteristic of the MS disease process

(e.g. diminished amplitudes, phase shifts). Notably,. When considering the implication for improved quality of life, VEP measures are non-invasive, reliable, repeatable, and relatively less expensive when compared to current assessment tools of brain dysfunction. The results of this study provide additional support for the use of VEPs in the MS population, introducing the potential examination of the magnocellular pathway through novel icVEP techniques.

Limitations and Future Directions

While analyses are appropriately powered for the aims of the current study, this study serves as a preliminary examination of novel VEP techniques in an MS population, and due to the relatively small sample, generalizability of the results to the larger clinical population is limited. The significant findings presented in this study confirm hypothesized differences between MS and control groups, warranting additional research of these measures on a larger scale. A more robust study could be useful in confirming these preliminary analyses and allow for additional analyses that require larger samples. With a large enough sample size, further analyses could be done to examine the possible influences of cultural, racial, and environmental factors, given that literature has suggested that the MS disease process differs across racial, ethnic, and geographic groups (Al-Kawaz et al., 2017; Johnson et al., 2010; Koch-Henriksen, & Sorensen, 2010; Lichtman-Mikol et al., 2019; Rotstein et al., 2018; Weinshenker et al., 1989). The cross-sectional design of this study limited our interpretations regarding reproducibility of the results over time. Knowing the unpredictable nature and heterogenous course of MS within and between individuals, a longitudinal study can provide tremendously informative data about disease course and progression for the same individual over time. Of interest would be the inclusion of individuals diagnosed with CIS, to assess differences between those with CIS who eventually convert to MS and those who do not. While this study did not collect information on

years since MS diagnosis, such information will be helpful to target disease processes that occur at the onset of the disease, as this is a critical point in the diagnostic timeline. In the MS population, it is not uncommon for one eye to be preferentially affected by the disease, as frequently seen in cases of optic neuritis. While a preliminary analysis of amplitude values did not reveal significant differences in eyes between groups for this study, it is possible that separating eyes by weaker and stronger eye may yield significant differences and this may be of value to examine in future research. All but two of the MS participants were taking disease modifying medications at the time of testing, and it is unknown whether these medications have an impact on their VEP responses. This preliminary examination of icVEPs in an MS population, completed as part of a larger, parent study, contained a lengthy VEP battery. Given that fatigue is a common symptom with MS individuals, shortening the battery may help reduce the impact of confounding factors, like fatigue, potentially improving classification accuracy.

While work remains to be done on the development and application of these novel VEP techniques as a sensitive and reliable measure of neural dysfunction, findings of this study support the argument for continued research on a larger scale. Future studies may continue to target the utility of the VEP as a critical paraclinical tool in the assessment and diagnosis of MS. The current study is part of a larger, ongoing study examining the neurological correlates and interrelationships between EEG, ERG, mood, and neuropsychological measures in individuals with MS, CIS, and healthy controls with the goal of collecting enough data to further analyze these relationships.

References

Alexander, K. R., Rajagopalan, A. S., Seiple, W., Zemon, V. M., & Fishman, G. A. (2005). Contrast response properties of magnocellular and parvocellular pathways in retinitis pigmentosa assessed by the visual evoked potential. *Invest Ophthalmol Vis Sci, 46*(8), 2967-2973. doi:10.1167/iovs.05-0231

Al-Kawaz, M., Monohan, E., Morris, E., Perumal, J. S., Nealon, N., Vartanian, T., & Gauthier, S. A. (2017). Differential impact of multiple sclerosis on cortical and deep gray matter structures in African Americans and Caucasian Americans. *Journal of Neuroimaging*, *27*(3), 333-338.

Alonso, R., Gonzalez-Moron, D., & Garcea, O. (2018). Optical coherence tomography as a biomarker of neurodegeneration in multiple sclerosis: A review. *Mult Scler Relat Disord, 22*, 77-82. doi:10.1016/j.msard.2018.03.007

Andersson, M., Alvarez-Cermeño, J., Bernardi, G., Cogato, I., Fredman, P., Frederiksen, J., Fredrikson, S., Gallo, P., Grimaldi, L. M., & Grønning, M. (1994). Cerebrospinal fluid in the diagnosis of multiple sclerosis: a consensus report. *Journal of neurology, neurosurgery, and psychiatry, 57*(8), 897–902. https://doi.org/10.1136/jnnp.57.8.897

Andersen, O., Hildeman, A., Longfils, M., Tedeholm, H., Skoog, B., Tian, W., Zhong, J., Ekholm, S., Novakova, L., Runmarker, B., Nerman, O., & Maier, S. E. (2018). Diffusion tensor imaging in multiple sclerosis at different final outcomes. *Acta neurologica Scandinavica*, *137*(2), 165–173. https://doi.org/10.1111/ane.12797

Arrambide, G., Tintore, M., Espejo, C., Auger, C., Castillo, M., Río, J., Castilló, J., Vidal-Jordana, A., Galán, I., Nos, C., Mitjana, R., Mulero, P., de Barros, A., Rodríguez-Acevedo, B., Midaglia, L., Sastre-Garriga, J., Rovira, A., Comabella, M., & Montalban,

X. (2018). The value of oligoclonal bands in the multiple sclerosis diagnostic criteria. *Brain, 141*(4), 1075–1084. https://doi.org/10.1093/brain/awy006

Baier, M. L., Cutter, G. R., Rudick, R. A., Miller, D., Cohen, J. A., Weinstock-Guttman, B., Mass, M., & Balcer, L. J. (2005). Low contrast letter acuity testing captures visual dysfunction in patients with multiple sclerosis. *Neurology, 64*(6), 992–995. https://doi.org/10.1212/01.WNL.0000154521.40686.63

Bakshi, R., Thompson, A. J., Rocca, M. A., Pelletier, D., Dousset, V., Barkhof, F., Inglese, M., Guttmann, C. R., Horsfield, M. A., & Filippi, M. (2008). MRI in multiple sclerosis: current status and future prospects. *The Lancet. Neurology, 7*(7), 615–625. https://doi.org/10.1016/S1474-4422(08)70137-6

Balcer, L. J., Miller, D. H., Reingold, S. C., & Cohen, J. A. (2015). Vision and vision-related outcome measures in multiple sclerosis. *Brain*, *138*(1), 11-27.

Balnytė, R., Ulozienė, I., Rastenytė, D., Vaitkus, A., Malcienė, L., & Laučkaitė, K. (2011). Diagnostic value of conventional visual evoked potentials applied to patients with multiple sclerosis. *Medicina (Kaunas, Lithuania), 47*(5), 263–269.

Bates, D. (2011). Treatment effects of immunomodulatory therapies at different stages of multiple sclerosis in short-term trials. *Neurology, 76*(1 Suppl 1), S14-25. doi:10.1212/WNL.0b013e3182050388

Beck A.T, Steer R.A., & Brown G.A. (1996) *Beck Depression Inventory-II (BDI-II) Manual.* Oxford, England: Pearson.

Beck, R., Ruchman, M., Savino, P., & Schatz, N. (1984). Contrast sensitivity measurements in acute and resolved optic neuritis. *The British journal of ophthalmology, 68*(10), 756–759. https://doi.org/10.1136/bjo.68.10.756

Beh, S. C., Frohman, T. C., & Frohman, E. M. (2016). Monitoring Treatment in Multiple Sclerosis. In *Optical Coherence Tomography in Multiple Sclerosis* (pp. 161-169). Springer, Cham.

Bennett, J. L., De Seze, J., Lana-Peixoto, M., Palace, J., Waldman, A., Schippling, S., ... & Fujihara, K. (2015). Neuromyelitis optica and multiple sclerosis: seeing differences through optical coherence tomography. *Multiple Sclerosis Journal*, *21*(6), 678-688.

Borg-Graham, L.J., Monier, C. & Frégnac,Y. (1998). Visual input evokes transient and strong shunting inhibition in visual cortical neurons. Nature, 393, 369-373.

Boster, A., Caon, C., Perumal, J., Hreha, S., Zabad, R., Zak, I., Tselis, A., Khan, O. (2008). Failure to develop multiple sclerosis in patients with neurologic symptoms without objective evidence. *Multiple Sclerosis Journal*, *14*(6), 804–808.

Branas, P., Jordan, R., Fry-Smith, A., Burls, A., Hyde, C. (2000). Treatments for fatigue in multiple sclerosis: a rapid and systematic review. Health Technol Assess, 4, 1–61.

Britze, J., & Frederiksen, J. L. (2018). Optical coherence tomography in multiple sclerosis. *Eye (London, England)*, *32*(5), 884–888. https://doi.org/10.1038/s41433-017-0010-2

Brown, J., Coles, A., Horakova, D., Havrdova, E., Izquierdo, G., Prat, A., Girard, M., Duquette, P., Trojano, M., Lugaresi, A., Bergamaschi, R., Grammond, P., Alroughani, R., Hupperts, R., McCombe, P., Van Pesch, V., Sola, P., Ferraro, D., Grand'Maison, F., Terzi, M., … MSBase Study Group (2019). Association of initial disease-modifying therapy with later conversion to secondary progressive multiple sclerosis. *JAMA*, *321*(2), 175–187. https://doi.org/10.1001/jama.2018.20588

Browne, P., Chandraratna, D., Angood, C., Tremlett, H., Baker, C., Taylor, B. V., & Thompson, A. J. (2014). Atlas of Multiple Sclerosis 2013: A growing global problem with

widespread inequity. *Neurology*, *83*(11), 1022–1024. http://doi.org/10.1212/WNL.0000000000000768

Brownlee, W. J., Hardy, T. A., Fazekas, F., & Miller, D. H. (2017). Diagnosis of multiple sclerosis: progress and challenges. *Lancet, 389*(10076), 1336-1346. doi:10.1016/s0140-6736(16)30959-x

Butler, P. D., Martinez, A., Foxe, J. J., Kim, D., Zemon, V., Silipo, G., . . . Javitt, D. C. (2007). Subcortical visual dysfunction in schizophrenia drives secondary cortical impairments. *Brain, 130*(2), 417-430. doi:10.1093/brain/awl233

Butler, P. D., Schechter, I., Zemon, V., Schwartz, S. G., Greenstein, V. C., Gordon, J., . . . Javitt, D. C. (2001). Dysfunction of early-stage visual processing in schizophrenia. *Am J Psychiatry, 158*(7), 1126-1133. doi:10.1176/appi.ajp.158.7.1126

Butler, P. D., Zemon, V., Schechter, I., Saperstein, A. M., Hoptman, M. J., Lim, K. O., . . . Javitt, D. C. (2005). Early-stage visual processing and cortical amplification deficits in schizophrenia. *Arch Gen Psychiatry, 62*(5), 495-504. doi:10.1001/archpsyc.62.5.495

Calabresi, P. A., & Newsome, S. D. (2010). Multiple sclerosis. In Neurology for the non-neurologist (Sixth Edition, pp. 192–221). Walters Kluwer Lippincott Williams & Wilkins.

Calderone, D. J., Martinez, A., Zemon, V., Hoptman, M. J., Hu, G., Watkins, J. E., . . . Butler, P. D. (2013). Comparison of psychophysical, electrophysiological, and fMRI assessment of visual contrast responses in patients with schizophrenia. *Neuroimage, 67*, 153-162. doi:10.1016/j.neuroimage.2012.11.019

Carlson, N. R. (2013). Physiology of Behavior (11th ed.). New Jersey: Pearson.

Chirapapaisan, N., Laotaweerungsawat, S., Chuenkongkaew, W., Samsen, P., Ruangvaravate, N., Thuangtong, A., & Chanvarapha, N. (2015). Diagnostic value of visual evoked potentials for clinical diagnosis of multiple sclerosis. *Documenta Ophthalmologica, 130*(1), 25-30.

Confavreux, C., Aimard, G., & Devic, M. (1980). Course and prognosis of multiple sclerosis assessed by the computerized data processing of 349 patients. *Brain, 103*(2), 281–300. https://doi.org/10.1093/brain/103.2.281

Conte, M. M., & Victor, J. D. (2009). VEP indices of cortical lateral interactions in epilepsy treatment. *Vision Res, 49*(9), 898-906. doi:10.1016/j.visres.2008.04.030

Coppola, G., Parisi, V., Di Lorenzo, C., Serrao, M., Magis, D., Schoenen, J., & Pierelli, F. (2013). Lateral inhibition in visual cortex of migraine patients between attacks. *J Headache Pain, 14*, 20. doi:10.1186/1129-2377-14-20

Costello F. (2013). The afferent visual pathway: designing a structural-functional paradigm of multiple sclerosis. *ISRN Neurology, 2013* 134858. https://doi.org/10.1155/2013/134858

Costello F. (2016). Vision Disturbances in Multiple Sclerosis. *Seminars in neurology, 36*(2), 185–195. https://doi.org/10.1055/s-0036-1579692

Coyle, P. K. (2008). Early treatment of multiple sclerosis to prevent neurologic damage. *Neurology, 71*(24 Suppl 3), S3-7. doi:10.1212/WNL.0b013e31818f3d6f

Creutzfeldt O. D.& Kuhnt, U. (1973) Electrophysiology and topographical distribution of visual evoked potentials in animals. In *The handbook of sensory physiology* (pp. 595-646) Heidelberg Germany: Springer-Verlag.

Deisenhammer, F., Zetterberg, H., Fitzner, B., & Zettl, U. (2019). The Cerebrospinal Fluid in Multiple Sclerosis. *Frontiers in immunology*, *10*, 726. https://doi.org/10.3389/fimmu.2019.00726

Di Russo, F., Teder-Sälejärvi, W., & Hillyard, S. (2002). Steady-state VEP and attentional visual processing. In A. Zani & A. M. Proverbio (Eds.), *The Cognitive Electrophysiology of Mind and Brain* (pp. 259-274). New York: Academic Press.

Dixon, C., & Robertson, D. (2018). To diagnose or not to diagnose? Timing is the question: balancing early diagnosis of multiple sclerosis with misdiagnosis. *Expert review of neurotherapeutics*, *18*(5), 355–357. https://doi.org/10.1080/14737175.2018.1464392

Ducati, A., Fava, E. & Motti, E.D. (1988) Neuronal generators of the visual evoked potentials: intracerebral recording in awake humans. *Electroencephalogr Clin Neurophysiol*, **71**, 89-99.

Duwaer, A.L. & Spekreijse, H. (1978) Latency of luminance and contrast evoked potentials in multiple sclerosis patients. *Electroencephalogr Clin Neurophysiol*, **45**, 244-258.

Ebers, G. (2001). Natural history of multiple sclerosis. Journal of Neurology, Neurosurgery, and Psychiatry, 71(Suppl 2), ii16–ii19. http://doi.org/10.1136/jnnp.71.suppl_2.ii16

Eccles, J. C. (1951). Interpretation of action potentials evoked in the cerebral cortex. *Electroencephalogr Clin Neurophysiol*, *3*(4), 449-464.

Evangelou, N., Konz, D., Esiri, M. M., Smith, S., Palace, J., & Matthews, P. M. (2001). Size-selective neuronal changes in the anterior optic pathways suggest a differential susceptibility to injury in multiple sclerosis. Brain, 124(9), 1813-1820.

Field, A. (2009). *Discovering statistics using SPSS:(and sex and drugs and rock'n'roll)*. Sage.

Filippi, M., Absinta, M., & Rocca, M. A. (2013). Future MRI tools in multiple sclerosis. *Journal of the neurological sciences*, *331*(1-2), 14–18. https://doi.org/10.1016/j.jns.2013.04.025

Filippi, M., Bar-Or, A., Piehl, F., Preziosa, P., Solari, A., Vukusic, S., & Rocca, M. A. (2018). Multiple sclerosis. Nature Reviews. *Disease Primers*, *4*(1), 43. doi: 10.1038/s41572-018-0041-4

Filippi, M., Rocca, M. A., Ciccarelli, O., De Stefano, N., Evangelou, N., Kappos, L., Rovira, A., Sastre-Garriga, J., Tintorè, M., Frederiksen, J. L., Gasperini, C., Palace, J., Reich, D. S., Banwell, B., Montalban, X., Barkhof, F., & MAGNIMS Study Group (2016). MRI criteria for the diagnosis of multiple sclerosis: MAGNIMS consensus guidelines. *Neurology*, *15*(3), 292–303. https://doi.org/10.1016/S1474-4422(15)00393-2

Filippi M., Rocca, M.A., Martino, G., Horsfield, M.A., Comi, G. (1998). Magnetization transfer changes in the normal appearing white matter precede the appearance of enhancing lesions in patients with multiple sclerosis. *Ann Neurol. 43*(6), 809-814.

Fiorentini, A., Baumgartner, G., Magnussen, S., Schiller, P. H., & Thomas, J. P. (1990). The perception of brightness and darkness: Relations to neuronal receptive fields. In L. Spillmann & J. S. Werner (Eds.), Visual perception: The neurophysiological foundations (p. 129–161). Academic Press.

Fiorentini, A., Porciatti, V., Morrone, M. C., & Burr, D. C. (1996). Visual ageing: unspecific decline of the responses to luminance and colour. *Vision research*, *36*(21), 3557–3566. https://doi.org/10.1016/0042-6989(96)00032-6

Fischer, A., Fischer, M., Nicholls, R. A., Lau, S., Poettgen, J., Patas, K., . . . Gold, S. M. (2015). Diagnostic accuracy for major depression in multiple sclerosis using self-report questionnaires. *Brain Behav, 5*(9), e00365. doi:10.1002/brb3.365

Fisher, J. B., Jacobs, D. A., Markowitz, C. E., Galetta, S. L., Volpe, N. J., Nano-Schiavi, M. L., . . . Balcer, L. J. (2006). Relation of visual function to retinal nerve fiber layer thickness in multiple sclerosis. *Ophthalmology, 113*(2), 324-332. doi:10.1016/j.ophtha.2005.10.040

Foster, R. E., Whalen, C. C., & Waxman, S. G. (1980). Reorganization of the axon membrane in demyelinated peripheral nerve fibers: morphological evidence. *Science, 210*(4470), 661-663.

Frohman, E. M., Racke, M. K., & Raine, C. S. (2006). Multiple sclerosis--the plaque and its pathogenesis. *The New England journal of medicine*, *354*(9), 942–955. https://doi.org/10.1056/NEJMra052130

Fujimoto, J. G., Pitris, C., Boppart, S. A., & Brezinski, M. E. (2000). Optical coherence tomography: an emerging technology for biomedical imaging and optical biopsy. *Neoplasia*, *2*(1-2), 9–25. https://doi.org/10.1038/sj.neo.7900071

Galetta, K. M., & Balcer, L. J. (2013). Measures of visual pathway structure and function in MS: clinical usefulness and role for MS trials. *Multiple Sclerosis and Related Disorders, 2*(3), 172-182.

Galetta, K. M., Calabresi, P. A., Frohman, E. M., & Balcer, L. J. (2011). Optical Coherence Tomography (OCT): Imaging the Visual Pathway as a Model for Neurodegeneration. *NeuroTherapeutics*, *8*(1), 117–132. http://doi.org/10.1007/s13311-010-0005-1

Giesser, B. S. (2011). *Primer on multiple sclerosis* (2nd Edition). Oxford University Press.

Gordon-Lipkin, E., Chodkowski, B., Reich, D. S., Smith, S. A., Pulicken, M., Balcer, L. J., . . . Calabresi, P. A. (2007). Retinal nerve fiber layer is associated with brain atrophy in

multiple sclerosis. *Neurology, 69*(16), 1603-1609. doi:10.1212/01.wnl.0000295995.46586.ae

Granberg, T., Martola, J., Aspelin, P., Kristoffersen-Wiberg, M., & Fredrikson, S. (2013). Radiologically isolated syndrome: an uncommon finding at a university clinic in a high-prevalence region for multiple sclerosis. *BMJ, 3*(11), e003531. https://doi.org/10.1136/bmjopen-2013-003531

Granberg, T., Martola, J., Kristoffersen-Wiberg, M., Aspelin, P., & Fredrikson, S. (2013). Radiologically isolated syndrome–incidental magnetic resonance imaging findings suggestive of multiple sclerosis, a systematic review. *Multiple Sclerosis Journal, 19*(3), 271-280.

Greenstein, V., Seliger, S., Zemon, V., & Ritch, R. (1998). Visual evoked potential assessment of the effects of glaucoma on visual subsystems. *Vision Res, 38*(12), 1901-1911.

Gutowitz, H., Zemon, V., Victor, J., & Knight, B. W. (1986). Source geometry and dynamics of the visual evoked potential. *Electroencephalogr Clin Neurophysiol, 64*(4), 308-327.

Halliday, A. M., McDonald, W. I., & Mushin, J. (1973). Visual evoked response in diagnosis of multiple sclerosis. *British medical journal*, *4*(5893), 661–664. https://doi.org/10.1136/bmj.4.5893.661

Hamurcu, M., Orhan, G., Sarıcaoğlu, M. S., Mungan, S., & Duru, Z. (2017). Analysis of multiple sclerosis patients with electrophysiological and structural tests. *International Ophthalmology*, *37*(3), 649–653. doi:10.1007/s10792-016-0324-2

Hartung, D. M., Bourdette, D. N., Ahmed, S. M., & Whitham, R. H. (2015). The cost of multiple sclerosis drugs in the US and the pharmaceutical industry: Too big to fail? *Neurology, 84(*21), 2185–2192. http://doi.org/10.1212/WNL.0000000000001608

Henze, T., Rieckmann, P., & Toyka, K. V. (2006). Symptomatic treatment of multiple sclerosis. *European neurology, 56*(2), 78-105.

Hou, Y., Jia, Y., & Hou, J. (2018). Natural Course of Clinically Isolated Syndrome: A Longitudinal Analysis Using a Markov Model. *Scientific reports, 8*(1), 10857. https://doi.org/10.1038/s41598-018-29206-y

Hubel, D. & Wiesel, T. (1961). Integrative action in the cat's lateral geniculate body. *The Journal of physiology, 155*(2), 385–398. https://doi.org/10.1113/jphysiol.1961.sp006635

Hurwitz, B. J. (2009). The diagnosis of multiple sclerosis and the clinical subtypes. *Annals of Indian Academy of Neurology, 12*(4), 226–230. http://doi.org/10.4103/0972-2327.58276

Ikuta, F., & Zimmerman, H. M. (1976). Distribution of plaques in seventy autopsy cases of multiple sclerosis in the United States. *Neurology*, *26*(6 PT 2), 26-28. https://doi.org/10.1212/wnl.26.6_part_2.26

Jasper, H. H. (1958). The ten twenty electrode system of the International Federation. *Electroencephalography Journal, 10*, 371-375.

Jasse, L., Vukusic, S., Durand-Dubief, F., Vartin, C., Piras, C., Bernard, M., ... & Tilikete, C. (2013). Persistent visual impairment in multiple sclerosis: prevalence, mechanisms and resulting disability. *Multiple Sclerosis Journal*, *19*(12), 1618-1626.

Johnson, B. A., Wang, J., Taylor, E. M., Caillier, S. J., Herbert, J., Khan, O. A., ... & Hauser, S. L. (2010). Multiple sclerosis susceptibility alleles in African Americans. *Genes & Immunity*, *11*(4), 343-350.

Kaplan, E. (1991). The receptive field structure of retinal ganglion cells in cat and monkey. In A. Leventhal (Ed.), Vision and Visual Dysfunction (pp. 10-40). Boston: CRC Press.

Kaplan, E. (2004). The M, P, and K pathways of the primate visual system. In L. Chalupa

& J. Werner (Ed.), The Visual Neurosciences (pp. 481–493). Cambridge, MA: MIT Press.

Kaplan, E. (2013). The M, P and K pathways of the primate visual system revisited. In L. Chalupa & J. Werner (Eds.), The New Visual Neuroscience (pp. 215–226). Cambridge, MA: MIT Press

Kaplan, E., & Shapley, R. M. (1986). The primate retina contains two types of ganglion cells, with high and low contrast sensitivity. *Proc Natl Acad Sci U S A, 83*(8), 2755-2757.

Kappos, L., Edan, G., Freedman, M. S., Montalban, X., Hartung, H. P., Hemmer, B., ... & Pleimes, D. (2016). The 11-year long-term follow-up study from the randomized BENEFIT CIS trial. *Neurology, 87*(10), 978-987.

Katzan, I., & Rudick, R. (1996). Guidelines to avoid errors in the diagnosis of multiple sclerosis. *Annals of Neurology, 40* (3), 246-246.

Kaufman, D. M., Geyer, H. L., & Milstein, M. J. (2016). Kaufman's Clinical Neurology for Psychiatrists E-Book. Elsevier Health Sciences.

Kaunzner, U. W., & Gauthier, S. A. (2017). MRI in the assessment and monitoring of multiple sclerosis: an update on best practice. *Therapeutic Advances in Neurological Disorders, 10*(6), 247–261. http://doi.org/10.1177/1756285617708911

Khanifar, A. A., Parlitsis, G. J., Ehrlich, J. R., Aaker, G. D., D'Amico, D. J., Gauthier, S. A., & Kiss, S. (2010). Retinal nerve fiber layer evaluation in multiple sclerosis with spectral domain optical coherence tomography. *Clin Ophthalmol, 4*, 1007-1013.

Kim, D., Zemon, V., Saperstein, A., Butler, P. D., & Javitt, D. C. (2005). Dysfunction of early-stage visual processing in schizophrenia: harmonic analysis. *Schizophr Res, 76*(1), 55-65. doi:10.1016/j.schres.2004.10.011

Klem, G. H., Luders, H. O., Jasper, H. H., & Elger, C. (1999). The ten-twenty electrode system of the International Federation. The International Federation of Clinical Neurophysiology. *Electroencephalogr Clin Neurophysiol Suppl, 52,* 3-6.

Kilstorner, A., Garrick, R., Barnett, M. H., Graham, S. L., Arvind, H., Sriram, P., & Yiannikas, C. (2013). Axonal loss in non-optic neuritis eyes of patients with multiple sclerosis linked to delayed visual evoked potential. *Neurology, 80*(3), 242-245. doi:10.1212/WNL.0b013e31827deb39

Koch-Henriksen, N., & Sorensen, P. S. (2010). The changing demographic pattern of multiple sclerosis epidemiology. *Lancet Neurol, 9*(5), 520-532. doi:10.1016/s1474-4422(10)70064-8

Kolasa, M., Hakulinen, U., Brander, A., Hagman, S., Dastidar, P., Elovaara, I., & Sumelahti, M. L. (2019). Diffusion tensor imaging and disability progression in multiple sclerosis: A 4-year follow-up study. *Brain and behavior*, *9*(1), e01194. https://doi.org/10.1002/brb3.1194

Kroenke, K., Spitzer, R. L., & Williams, J. B. W. (2001). The PHQ-9: Validity of a brief depression severity measure. *Journal of General Internal Medicine*, *16*, 606–613.

Kruit, M. C., van Buchem, M. A., Launer, L. J., Terwindt, G. M., & Ferrari, M. D. (2010). Migraine is associated with an increased risk of deep white matter lesions, subclinical posterior circulation infarcts and brain iron accumulation: the population-based MRI CAMERA study. *Cephalalgia*, *30*(2), 129–136. https://doi.org/10.1111/j.1468-2982.2009.01904.x

Kuffler, S. W. (1953). Discharge patterns and functional organization of mammalian retina. *Journal of neurophysiology*, *16*(1), 37-68.

Kuhle, J., Disanto, G., Dobson, R., Adiutori, R., Bianchi, L., Topping, J., ... & Runia, T. (2015). Conversion from clinically isolated syndrome to multiple sclerosis: a large multicentre study. *Multiple Sclerosis Journal*, *21*(8), 1013-1024.

Lebrun, C., Bensa, C., Debouverie, M., De Seze, J., Wiertlievski, S., Brochet, B., . . . Roullet, E. (2008). Unexpected multiple sclerosis: follow-up of 30 patients with magnetic resonance imaging and clinical conversion profile. *J Neurol Neurosurg Psychiatry, 79*(2), 195-198. doi:10.1136/jnnp.2006.108274

Lee, B., Pokorny, J., Smith, V., Martin, P., & Valberg, A. (1990). Luminance and chromatic modulation sensitivity of macaque ganglion cells and human observers. *J Opt Soc Am A, 7*(12), 2223-2236.

Lichtman-Mikol, S., Razmjou, S., Yarraguntla, K., Bao, F., Santiago-Martinez, C., Seraji-Bozorgzad, N., & Bernitsas, E. (2019). Racial differences in retinal neurodegeneration as a surrogate marker for cortical atrophy in multiple sclerosis. *Multiple sclerosis and related disorders*, *31*, 141-147.

Lublin, F. D., & Reingold, S. C. (1996). Defining the clinical course of multiple sclerosis: results of an international survey. *Neurology*, *46*(4), 907-911.

Lublin, F. D., Reingold, S. C., Cohen, J. A., Cutter, G. R., Sørensen, P. S., Thompson, A. J., . . . Polman, C. H. (2014). Defining the clinical course of multiple sclerosis: The 2013 revisions. *Neurology, 83*(3), 278-286. doi:10.1212/WNL.0000000000000560

Mahajan, K. R., & Ontaneda, D. (2017). The Role of Advanced Magnetic Resonance Imaging Techniques in Multiple Sclerosis Clinical Trials. *Neurotherapeutics, 14*(4), 905–923. http://doi.org/10.1007/s13311-017-0561-8

McDonald W.I., & Sears, T.A. (1969). Effect of demyelination on conduction in the central nervous system. *Nature. 221*(5176), 182-183.

McDonald, W. I., Compston, A., Edan, G., Goodkin, D., Hartung, H. P., Lublin, F. D., . . . Wolinsky, J. S. (2001). Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol, 50*(1), 121-127.

Medaer R. (1979). Does the history of multiple sclerosis go back as far as the 14th century?.*Acta neurologica Scandinavica, 60*(3), 189–192. https://doi.org/10.1111/j.1600-0404.1979.tb02968.x

Miller D.H., Barkhof, F., Nauta, J.J. (1993). Gadolinium enhancement increases the sensitivity of MRI in detecting disease activity in multiple sclerosis. *Brain. 116*(5), 1077-1094

Miller, D. H., Chard, D. T., & Ciccarelli, O. (2012). Clinically isolated syndromes. *The Lancet. Neurology, 11*(2), 157–169. https://doi.org/10.1016/S1474-4422(11)70274-5

Miller, D. H., Grossman, R. I., Reingold, S. C., & McFarland, H. F. (1998). The role of magnetic resonance techniques in understanding and managing multiple sclerosis. *Brain, 121*(1), 3-24.

Miller, D. H., Weinshenker, B. G., Filippi, M., Banwell, B. L., Cohen, J. A., Freedman, M. S., Galetta, S. L., Hutchinson, M., Johnson, R. T., Kappos, L., Kira, J., Lublin, F. D., McFarland, H. F., Montalban, X., Panitch, H., Richert, J. R., Reingold, S. C., & Polman, C. H. (2008). Differential diagnosis of suspected multiple sclerosis: a consensus approach. *Multiple sclerosis, 14*(9), 1157–1174. https://doi.org/10.1177/1352458508096878

Morris, Z., Whiteley, W. N., Longstreth, W. T., Jr, Weber, F., Lee, Y. C., Tsushima, Y., Alphs, H., Ladd, S. C., Warlow, C., Wardlaw, J. M., & Al-Shahi Salman, R. (2009). Incidental findings on brain magnetic resonance imaging: systematic review and meta-analysis. *BMJ (Clinical research ed.), 339*, b3016. https://doi.org/10.1136/bmj.b3016

Naismith, R. T., Tutlam, N. T., Xu, J., Shepherd, J. B., Klawiter, E. C., Song, S. K., & Cross, A. H. (2009). Optical coherence tomography is less sensitive than visual evoked potentials in optic neuritis. *Neurology, 73*(1), 46-52. doi:10.1212/WNL.0b013e3181aaea32

Nakamura, M., Kakigi, R., Okusa, T., Hoshiyama, M. & Watanabe, K. (2000) Effects of check size on pattern reversal visual evoked magnetic field and potential. *Brain research*, 872, 77-86.

Noseworthy, J. H., Lucchinetti, C., Rodriguez, M., & Weinshenker, B. G. (2000). Multiple sclerosis. *The New England journal of medicine, 343*(13), 938–952. https://doi.org/10.1056/NEJM200009283431307

Odom, J. V., Bach, M., Brigell, M., Holder, G. E., McCulloch, D. L., Mizota, A., ... & International Society for Clinical Electrophysiology of Vision. (2016). ISCEV standard for clinical visual evoked potentials:(2016 update). *Documenta Ophthalmologica*, *133*(1), 1-9.

O'Dwyer, L. M., & Parker, C. E. (2014). A Primer for Analyzing Nested Data: Multilevel Modeling in SPSS Using an Example from a REL Study. REL 2015-046. *Regional Educational Laboratory Northeast & Islands*.

O'Riordan, J. I., Losseff, N. A., Phatouros, C., Thompson, A. J., Moseley, I. F., MacManus, D. G., . . . Miller, D. H. (1998). Asymptomatic spinal cord lesions in clinically isolated optic

nerve, brain stem, and spinal cord syndromes suggestive of demyelination. *J Neurol Neurosurg Psychiatry, 64*(3), 353-357.

Penner, I. K., Raselli, C., Stocklin, M., Opwis, K., Kappos, L., & Calabrese, P. (2009). The Fatigue Scale for Motor and Cognitive Functions (FSMC): validation of a new instrument to assess multiple sclerosis-related fatigue. *Mult Scler, 15*(12), 1509-1517. doi:10.1177/1352458509348519

Polman, C. H., O'Connor, P. W., Havrdova, E., Hutchinson, M., Kappos, L., Miller, D. H., Phillips, J. T., Lublin, F. D., Giovannoni, G., Wajgt, A., Toal, M., Lynn, F., Panzara, M. A., Sandrock, A. W., & AFFIRM Investigators (2006). A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. *The New England journal of medicine, 354*(9), 899–910. https://doi.org/10.1056/NEJMoa044397

Polman, C. H., Reingold, S. C., Edan, G., Filippi, M., Hartung, H. P., Kappos, L., . . . Wolinsky, J. S. (2005). Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". *Ann Neurol, 58*(6), 840-846. doi:10.1002/ana.20703

Polman, C. H., Reingold, S. C., Banwell, B., Clanet, M., Cohen, J. A., Filippi, M., . . . Wolinsky, J. S. (2011). Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol, 69*(2), 292-302. doi:10.1002/ana.22366

Porciatti, V., & Sartucci, F. (1996). Retinal and cortical evoked responses to chromatic contrast stimuli. Specific losses in both eyes of patients with multiple sclerosis and unilateral optic neuritis. Brain : a journal of neurology, 119 (Pt 3), 723–740. https://doi.org/10.1093/brain/119.3.723

Poser, C. M., Paty, D. W., Scheinberg, L., McDonald, W. I., Davis, F. A., Ebers, G. C., Johnson, K. P., Sibley, W. A., Silberberg, D. H., & Tourtellotte, W. W. (1983). New diagnostic

criteria for multiple sclerosis: guidelines for research protocols. *Annals of neurology*, *13*(3), 227–231. https://doi.org/10.1002/ana.410130302

Pulicken, M., Gordon-Lipkin, E., Balcer, L. J., Frohman, E., Cutter, G., & Calabresi, P. A. (2007). Optical coherence tomography and disease subtype in multiple sclerosis. *Neurology, 69*(22), 2085-2092. doi:10.1212/01.wnl.0000294876.49861.dc

Purpura, D.P. (1959) Nature of electrocortical potentials and synaptic organizations in cerebral and cerebellar cortex. *Int. Rev. Neurobiol, 1*, 47-163.

Quaranta, D., Marra, C., Zinno, M., Patanella, A. K., Messina, M. J., Piccininni, C., . . . Gainotti, G. (2012). Presentation and validation of the multiple sclerosis depression rating scale: a test specifically devised to investigate affective disorders in multiple sclerosis patients. *Clin Neuropsychol, 26*(4), 571-587. doi:10.1080/13854046.2012.668220

Rae-Grant, A., Day, G. S., Marrie, R. A., Rabinstein, A., Cree, B., Gronseth, G. S., Haboubi, M., Halper, J., Hosey, J. P., Jones, D. E., Lisak, R., Pelletier, D., Potrebic, S., Sitcov, C., Sommers, R., Stachowiak, J., Getchius, T., Merillat, S. A., & Pringsheim, T. (2018). Comprehensive systematic review summary: Disease-modifying therapies for adults with multiple sclerosis: Report of the guideline development, dissemination, and implementation subcommittee of the American academy of neurology. *Neurology*, *90*(17), 789–800. https://doi.org/10.1212/WNL.0000000000005345

Ratliff, F., & Zemon, V. (1984). Visual evoked potentials elicited in normal subjects and in epileptic patients by windmill-dartboard stimuli. In R. H. N. a. C. Barber (Ed.), *Evoked Potentials II* (251-259). Stoneham, Mass: Butterworth.

Regan, D. (1966) Some characteristics of average steady-state and transient responses evoked by modulated light. *Electroencephalography and Clinical Neurophysiology*, *20*, 238-248.

Regan, D. (1989). *Human brain electrophysiology. Evoked potentials and evoked magnetic fields in science and medicine*. New York: Elsevier.

Ringelstein, M., Albrecht, P., Kleffner, I., Buhn, B., Harmel, J., Muller, A. K., . . . Dorr, J. (2015). Retinal pathology in Susac syndrome detected by spectral-domain optical coherence tomography. *Neurology, 85*(7), 610-618. doi:10.1212/wnl.0000000000001852

Rot, U., Ledinek, A. H., & Jazbec, S. Š. (2008). Clinical, magnetic resonance imaging, cerebrospinal fluid and electrophysiological characteristics of the earliest multiple sclerosis. *Clinical neurology and neurosurgery*, *110*(3), 233-238.

Rotstein, D. L., Chen, H., Wilton, A. S., Kwong, J. C., Marrie, R. A., Gozdyra, P., . . . Tu, K. (2018). Temporal trends in multiple sclerosis prevalence and incidence in a large population. *Neurology, 90*(16), e1435-e1441. doi:10.1212/wnl.0000000000005331

Rovira, À., Wattjes, M. P., Tintoré, M., Tur, C., Yousry, T. A., Sormani, M. P., De Stefano, N., Filippi, M., Auger, C., Rocca, M. A., Barkhof, F., Fazekas, F., Kappos, L., Polman, C., Miller, D., Montalban, X., & MAGNIMS study group (2015). Evidence-based guidelines: MAGNIMS consensus guidelines on the use of MRI in multiple sclerosis-clinical implementation in the diagnostic process. *Nature reviews. Neurology*, *11*(8), 471–482. https://doi.org/10.1038/nrneurol.2015.106

Rudick, R. A., Stuart, W. H., Calabresi, P. A., Confavreux, C., Galetta, S. L., Radue, E. W., Lublin, F. D., Weinstock-Guttman, B., Wynn, D. R., Lynn, F., Panzara, M. A., Sandrock, A. W., & SENTINEL Investigators (2006). Natalizumab plus interferon beta-1a for relapsing multiple sclerosis. *The New England journal of medicine, 354*(9), 911–923. https://doi.org/10.1056/NEJMoa044396

Sakai, R. E., Feller, D. J., Galetta, K. M., Galetta, S. L., & Balcer, L. J. (2011). Vision in multiple sclerosis: the story, structure-function correlations, and models for neuroprotection. *Journal of neuro-ophthalmology, 31*(4), 362.

Scalfari, A., Neuhaus, A., Daumer, M., Muraro, P. A., & Ebers, G. C. (2014). Onset of secondary progressive phase and long-term evolution of multiple sclerosis. *Journal of neurology, neurosurgery, and psychiatry, 85*(1), 67–75. https://doi.org/10.1136/jnnp-2012-304333

Schiller P. H. (1982). Central connections of the retinal ON and OFF pathways. Nature, 297 (5867), 580–583. https://doi.org/10.1038/297580a0

Schiller P. H. (2010). Parallel information processing channels created in the retina. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(40), 17087–17094. https://doi.org/10.1073/pnas.1011782107

Schroeder, C., Tenke, C., Givre, S., Arezzo, J. & Vaughan, H. (1991) Striate cortical contribution to the surface-recorded pattern-reversal VEP in the alert monkey. *Vision research*, *31*, 1143-1157.

Siegel, Kasey B. (2019). *Electrophysiological indices of disease status in multiple sclerosis* (Publication No. 27794087) [Doctoral dissertation, Yeshiva University]. ProQuest Dissertations Publishing.

Siper, P. M., Zemon, V., Gordon, J., George-Jones, J., Lurie, S., Zweifach, J., . . . Kolevzon, A. (2016). Rapid and Objective Assessment of Neural Function in Autism Spectrum Disorder Using Transient Visual Evoked Potentials. *PLoS One, 11*(10), e0164422. doi:10.1371/journal.pone.0164422

Shapley, R., & Victor, J. D. (1978). The effect of contrast on the transfer properties of cat retinal ganglion cells. *J Physiol, 285*, 275-298.

Shapley, R., & Victor, J. D. (1979). The contrast gain control of the cat retina. *Vision Res, 19*(4), 431-434.

Shigeto, H., Tobimatsu, S., Yamamoto, T., Kobayashi, T. & Kato, M. (1998) Visual evoked cortical magnetic responses to checkerboard pattern reversal stimulation: a study on the neural generators of N75, P100 and N145. *Journal of the neurological sciences*, *156*, 186-194.

Sjonnesen, K., Berzins, S., Fiest, K. M. M. Bulloch, A. G., Metz, L. M., Thombs, B. D., & Patten, S. B. (2012). Evaluation of the 9-item Patient Health Questionnaire (PHQ-9) as an assessment instrument for symptoms of depression in patients with multiple sclerosis. *Postgraduate Medicine*, *124*, 69–77. http://dx.doi.org.elibrary.einstein.yu.edu/10.3810/pgm.2012.09.2595

Siper, P. M., Zemon, V., Gordon, J., George-Jones, J., Lurie, S., Zweifach, J., . . . Kolevzon, A. (2016). Rapid and Objective Assessment of Neural Function in Autism Spectrum Disorder Using Transient Visual Evoked Potentials. *PLoS One*, *11*(10). doi:10.1371/journal.pone.0164422

Smith, K.J., & McDonald, W.I. (1999). The pathophysiology of multiple sclerosis: the mechanisms underlying the production of symptoms and the natural history of the disease. *Philos Trans R Soc Lond B Biol Sci*, *354*(1390), 1649-1673.

Solomon, A. J., Bourdette, D. N., Cross, A. H., Applebee, A., Skidd, P. M., Howard, D. B., … Weinshenker, B. G. (2016). The contemporary spectrum of multiple sclerosis

misdiagnosis: A multicenter study. *Neurology*, *87*(13), 1393–1399. http://doi.org/10.1212/WNL.0000000000003152

Solomon, A. J., Bourdette, D. N., Cross, A. H., Applebee, A., Skidd, P. M., Howard, D. B., ... & Yadav, V. (2016). The contemporary spectrum of multiple sclerosis misdiagnosis: a multicenter study. *Neurology*, *87*(13), 1393-1399.

Solomon, A. J., Klein, E. P., & Bourdette, D. (2012). "Undiagnosing" multiple sclerosis: The challenge of misdiagnosis in MS. *Neurology*, *78*(24), 1986–1991. http://doi.org/10.1212/WNL.0b013e318259e1b2

Solomon, A. J., Naismith, R. T., & Cross, A. H. (2019). Misdiagnosis of multiple sclerosis: Impact of the 2017 McDonald criteria on clinical practice. *Neurology, 92*(1), 26–33. https://doi.org/10.1212/WNL.0000000000006583

Sullivan, M. J., Weinshenker, B., Mikail, S., & Bishop, S. R. (1995). Screening for major depression in the early stages of multiple sclerosis. *Can J Neurol Sci, 22*(3), 228-231.

Tennakoon, D. K., Mehta, R. S., Ortega, S. B., Bhoj, V., Racke, M. K., & Karandikar, N. J. (2006). Therapeutic induction of regulatory, cytotoxic CD8+ T cells in multiple sclerosis. *Journal of immunology*, *176* (11), 7119–7129. https://doi.org/10.4049/jimmunol.176.11.7119

Thrower B. W. (2009). Relapse management in multiple sclerosis. *The neurologist, 15*(1), 1–5. https://doi.org/10.1097/NRL.0b013e31817acf1a

Thompson, A. J., Banwell, B. L., Barkhof, F., Carroll, W. M., Coetzee, T., Comi, G., . . . Cohen, J. A. (2018). Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *The Lancet Neurology*, *17*(2), 162-173. doi:10.1016/S1474-4422(17)30470-2

Thurtell, M. J., Bala, E., Yaniglos, S. S., Rucker, J. C., Peachey, N. S., & Leigh, R. J. (2009). Evaluation of optic neuropathy in multiple sclerosis using low contrast visual evoked potentials. *Neurology, 73*(22), 1849–1857. https://doi.org/10.1212/WNL.0b013 e3181c3fd43

Tintore, M., Rovira, À., Río, J., Otero-Romero, S., Arrambide, G., Tur, C., ... & Galán, I. (2015). Defining high, medium and low impact prognostic factors for developing multiple sclerosis. *Brain*, *138*(7), 1863-1874.

Toussaint, D., Périer, O., Verstappen, A., & Bervoets, S. (1983). Clinicopathological study of the visual pathways, eyes, and cerebral hemispheres in 32 cases of disseminated sclerosis. *Journal of clinical neuro-ophthalmology*, *3*(3), 211–220.

Traboulsee, A., Simon, J. H., Stone, L., Fisher, E., Jones, D. E., Malhotra, A., Newsome, S. D., Oh, J., Reich, D. S., Richert, N., Rammohan, K., Khan, O., Radue, E. W., Ford, C., Halper, J., & Li, D. (2016). Revised Recommendations of the Consortium of MS Centers Task Force for a Standardized MRI Protocol and Clinical Guidelines for the Diagnosis and Follow-Up of Multiple Sclerosis. *AJNR. American journal of neuroradiology*, *37*(3), 394–401. https://doi.org/10.3174/ajnr.A4539

Tremlett, H., Zhao, Y., Joseph, J., Devonshire, V., & UBCMS Clinic Neurologists (2008). Relapses in multiple sclerosis are age- and time-dependent. *Journal of neurology, neurosurgery, and psychiatry*, *79*(12), 1368-1374. https://doi.org/10.1136/jnnp.2008.145805

Vernooij, M. W., Ikram, M. A., Tanghe, H. L., Vincent, A. J., Hofman, A., Krestin, G. P., Niessen, W. J., Breteler, M. M., & van der Lugt, A. (2007). Incidental findings on brain

MRI in the general population. *The New England Journal of Medicine*, *357*(18), 1821–1828.

Victor, J. D., & Mast, J. (1991). A new statistic for steady-state evoked potentials. *Electroencephalogr Clin Neurophysiol, 78*(5), 378-388.

Wallin, M. T., Culpepper, W. J., Campbell, J. D., Nelson, L. M., Langer-Gould, A., Marrie, R. A., Cutter, G. R., Kaye, W. E., Wagner, L., Tremlett, H., Buka, S. L., Dilokthornsakul, P., Topol, B., Chen, L. H., LaRocca, N. G., & US Multiple Sclerosis Prevalence Workgroup (2019). The prevalence of MS in the United States: A population-based estimate using health claims data. *Neurology, 92*(10), e1029–e1040. https://doi.org/10.1212/WNL.0000000000007035

Watson, T. M., Ford, E., Worthington, E., & Lincoln, N. B. (2014). Validation of mood measures for people with multiple sclerosis. *Int J MS Care, 16*(2), 105-109. doi:10.7224/1537-2073.2013-013

Weinger, P. M., Zemon, V., Soorya, L., & Gordon, J. (2014). Low contrast response deficits and increased neural noise in children with autism spectrum disorder. *Neuropsychologia, 63*, 10-18. doi:10.1016/j.neuropsychologia.2014.07.031

Weinshenker, B. G., Bass, B., Rice, G. P., Noseworthy, J., Carriere, W., Baskerville, J., & Ebers, G. C. (1989). The natural history of multiple sclerosis: a geographically based study. I. Clinical course and disability. *Brain, 112*(1), 133–146. https://doi.org/10.1093/brain/112.1.133

Williams, R. M., Turner, A. P., Hatzakis, M., Jr., Bowen, J. D., Rodriquez, A. A., & Haselkorn, J. K. (2005). Prevalence and correlates of depression among veterans with multiple sclerosis. *Neurology*, *64*, 75-80.

http://dx.doi.org.elibrary.einstein.yu.edu/10.1212/01.WNL.0000148480.31424.2A

Woltman, H., Feldstain, A., MacKay, J. C., & Rocchi, M. (2012). An introduction to hierarchical linear modeling. *Tutorials in quantitative methods for psychology*, *8*(1), 52-69.

Xu, L. J., Zhang, L., Li, S. L., Zemon, V., Virgili, G., & Liang, Y. B. (2017). Accuracy of isolated-check visual evoked potential technique for diagnosing primary open-angle glaucoma. *Documenta Ophthalmologica*, *135*(2), 107-119.

Yamout, B. I., Khoury, S. J., Ayyoubi, N., Doumiati, H., Fakhreddine, M., Ahmed, S. F., Tamim, H., Al-Hashel, J. Y., Behbehani, R., & Alroughani, R. (2017). Alternative diagnoses in patients referred to specialized centers for suspected MS. *Multiple sclerosis and related disorders, 18,* 85–89. https://doi.org/10.1016/j.msard.2017.09.016

Zalc B. (2018). One hundred and fifty years ago Charcot reported multiple sclerosis as a new neurological disease. *Brain, 141*(12), 3482–3488. https://doi.org/10.1093/brain/awy287

Zemon, V. (1984). *The VEP: Analysis of functional subsystems in the brain.* Paper presented at the Sixth Annual Conference IEEE Engineering in Medicine and Biology Society.

Zemon, V., Eisner, W., Gordon, J., Grose-Fifer, J., Tenedios, F., & Shoup, H. (1995). Contrast-dependent responses in the human visual system: childhood through adulthood. *Int J Neurosci*, *80*(1-4), 181-201.

Zemon, V., & Gordon, J. (2006). Luminance-contrast mechanisms in humans: visual evoked potentials and a nonlinear model. *Vision Res*, *46*(24), 4163-4180. doi:10.1016/j.visres.2006.07.007

Zemon, V., & Gordon, J. (2018). Quantification and statistical analysis of the transient visual evoked potential to a contrast-reversing pattern: A frequency-domain approach. *Eur J Neurosci*, *48*(2), 1765-1788. doi:10.1111/ejn.14049

Zemon, V., Gordon, J., & Welch, J. (1988). Asymmetries in ON and OFF visual pathways of humans revealed using contrast-evoked cortical potentials. *Vis Neurosci, 1*(1), 145-150.

Zemon, V., Gutowski, W., & Horton, T. (1983). Orientational anisotropy in the human visual system: an evoked potential and psychophysical study. *Int J Neurosci, 19*(1-4), 259-286.

Zemon, V., Hartmann, E. E., Gordon, J., & Prunte-Glowazki, A. (1997). An electrophysiological technique for assessment of the development of spatial vision. *Optom Vis Sci, 74*(9), 708-716.

Zemon, V., Kaplan, E., & Ratliff, F. (1980). Bicuculline enhances a negative component and diminishes a positive component of the visual evoked cortical potential in the cat. *Proc Natl Acad Sci U S A, 77*(12), 7476-7478.

Zemon, V., Kaplan, E., & Ratliff, F. (1986). The role of GABA-mediated intracortical inhibition in the generation of visual evoked potentials. In R. Cracco & I. Bodis-Wollner (Eds.), *Evoked Potentials, Frontiers of Clinical Neuroscience v. 3* (pp. 287-295). New York: Alan R. Liss, Inc.

Zemon, V., Pinkhasov, E., & Gordon, J. (1993). Electrophysiological tests of neural models: evidence for nonlinear binocular interactions in humans. *Proc Natl Acad Sci U S A, 90*(7), 2975-2978.

Zemon, V., Kaplan, E. & Ratliff, F. (1980) Bicuculline enhances a negative component and diminishes a positive component of the visual evoked cortical potential in the cat. *Proc Natl Acad Sci U S A*, **77**, 7476-7478.

Zemon, V., & Ratliff, F. (1982). Visual evoked potentials: evidence for lateral interactions. *Proc Natl Acad Sci U S A, 79*(18), 5723-5726.

Zemon, V., & Ratliff, F. (1984). Intermodulation components of the visual evoked potential: responses to lateral and superimposed stimuli. *Biol Cybern, 50*(6), 401-408.

Zemon, V., Tsai, J. C., Forbes, M., Al-Aswad, L. A., Chen, C. M., Gordon, J., . . . Jindra, L. F. (2008). Novel electrophysiological instrument for rapid and objective assessment of magnocellular deficits associated with glaucoma. *Doc Ophthalmol, 117*(3), 233-243. doi:10.1007/s10633-008-9129-6

Zemon, V., Victor, J. D., & Ratliff, F. (1986). Functional subsystems in the visual pathways of humans characterized using evoked potentials. In R. Q. Cracco & I. Bodis-Wollner (Eds.), *Frontiers of Clinical Neuroscience, Evoked Potentials* (Vol. 3, pp. 203-210). New York: Alan R. Liss, Inc.

Zemon, V. M., Weinger, P. M., Harewood, A., Nunez, V., Michel, J.-P., Azizgolshani, S., . . . Gordon, J. (2012). A Short-Duration Visual Evoked Potential (VEP) Test Protocol. *Investigative Ophthalmology & Visual Science, 53*(14), 5719-5719.

Zimmern, R. L., Campbell, F. W., & Wilkinson, I. M. (1979). Subtle disturbances of vision after optic neuritis elicited by studying contrast sensitivity. *Journal of neurology, neurosurgery, and psychiatry*, *42*(5), 407–412. https://doi.org/10.1136/jnnp.42.5.407

Table 1.

Demographic and Clinical Characteristics for Controls and MS Cohorts

Variable	*Controls* (*n* = 18)	*MS* (*n* = 20)	*p*
Age (yrs), *Mdn* (Range)	30.5 (24-40)	32.4 (24-39)	.206
Education (yrs), Mean (Range)	15.8 (12-19)	14.1 (12-18)	.029*
ETDRS Acuity, *Mdn* (IQR)			
Right eye (OD)	1 (0)	1 (0.2)	.303
Left eye (OS)	1 (0)	1 (0.2)	.112
Gender, *n* (%)			.791
Male	3 (16.7)	4 (20.0)	
Female	15 (83.3)	16 (80.0)	
Self-Identified Race, *n* (%)			.049*
Caucasian	17 (94.4)	11 (55.0)	
African American	0 (0)	1 (5.0)	
Hispanic	1 (5.6)	5 (25.0)	
Caucasian/Hispanic	0 (0)	3 (15.0)	

Note. Number of participants per group is noted for missing data. Decimal scale used for measurement of acuity. Listed p values represent comparison of subsamples using Mann-Whitney U test (age, education, acuity) and a chi-square test for independence (gender, race). *p < .05.

Table 2.
Multilevel Linear Models – Signal-to-Noise Ratio

	Regression coefficients (estimates of fixed effects)				
Effect	Model 1 (null) Estimate (*SE*)	Model 2 Estimate (*SE*)	Model 3 Estimate (*SE*)	Model 4 Estimate (*SE*)	Model 5 Estimate (*SE*)
Intercept	2.131 (0.182) ***	1.88 (0.244) ***	-1.123 (0.289)***	-0.748 (0.322)*	-0.454 (0.321)
Main effects					
Group					
CN	—	0.825 (0.633)	0.522 (0.355)	-0.316 (0.468)	-0.316 (0.462)
MS	—	0[a]	0[a]	0[a]	0[a]
Log2DOM	—	—	0.865 (0.044)***	0.752 (0.060)***	0.752 (0.058)***
Group x Log2DOM					
CN	—	—	—	0.239 (0.087)**	0.239 (0.085)**
MS	—	—	—	0[a]	0[a]
Test Condition					
Bright	—	—	—	—	-0.588 (0.094)***
Dark	—	—	—	—	0[a]
Variance components (random effects)					
Residual (σ^2)	2.463 (0.146) ***	2.463 (0.146)***	1.464 (0.087) ***	1.446 (0.086)***	1.353 (0.080)***
Intercept (τ_{00})	1.108 (0.290) ***	1.040 (0.274)***	1.102 (0.274) ***	1.104 (0.274)***	1.109 (0.274)***
Model summary					
Deviance (-2 LL)	2352.488	2351.383	2055.040	2047.677	2009.998
Parameters	3	4	5	6	7

Note: Standard errors for parameter estimates are listed in parentheses.
[a]The parameter is set to zero because it is redundant
[b]Log base 2 depth of modulation (DOM)
[c]Deviance (-2 LL) is a measure of how well the model fits the data; smaller numbers reflect a better fit.
* $p < .05$; ** $p < .01$; *** $p < .001$

Table 3.
Multilevel Linear Models – Amplitude

	Regression coefficients (estimates of fixed effects)				
Effect	Model 1 (null) Estimate (*SE*)	Model 2 Estimate (*SE*)	Model 3 Estimate (*SE*)	Model 4 Estimate (*SE*)	Model 5 Estimate (*SE*)
Intercept	2.854 (0.318) ***	2.385 (0.424)***	-2.047 (0.474)***	-1.177 (0.513)*	-0.810 (0.512)
Main effects					
Group					
CN	—	0.988 (0.615)	0.988 (0.615)	-0.848 (0.745)	-0.848 (0.738)
MS	—	0[a]	0[a]	0[a]	0[a]
Log2DOM	—	—	1.266 (0.061)***	1.018 (0.082)***	1.018 (0.080)***
Group x Log2DOM					
CN	—	—	—	0.524 (0.120)***	0.525 (0.116)***
MS	—	—	—	0[a]	0[a]
Test Condition					
Bright	—	—	—	—	-0.734 (0.130)***
Dark	—	—	—	—	0[a]
Variance components (random effects)					
Residual (σ^2)	4.942 (0.293) ***	4.942 (0.293)***	2.804 (0.166) ***	2.712 (0.161)***	2.567 (0.152)***
Intercept (τ_{00})	3.527 (0.880) ***	3.283 (0.824)***	3.417 (0.824) ***	3.422 (0.824)***	3.432 (0.824)***
Model summary					
Deviance (-2 LL)	2792.607	2790.115	2467.084	2448.167	2417.145
Parameters	3	4	5	6	7

Note: Standard errors for parameter estimates are listed in parentheses.
[a]The parameter is set to zero because it is redundant
[b]Log base 2 depth of modulation (DOM)
[c]Deviance (-2 LL) is a measure of how well the model fits the data; smaller numbers reflect a better fit.
* $p < .05$; ** $p < .01$; *** $p < .001$

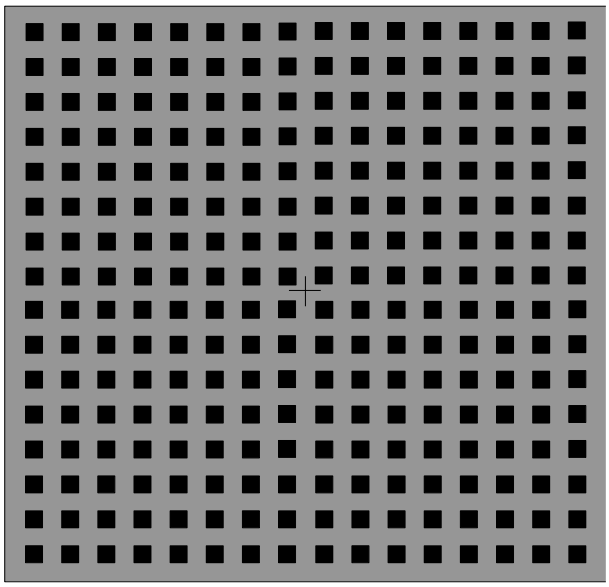

Figure 1. Dark isolated-check pattern. Image for demonstration purposes a 24 x 24 array of 16' checks in a 12.8°x12.8° field size with background luminance of 50 nits was used for this study.

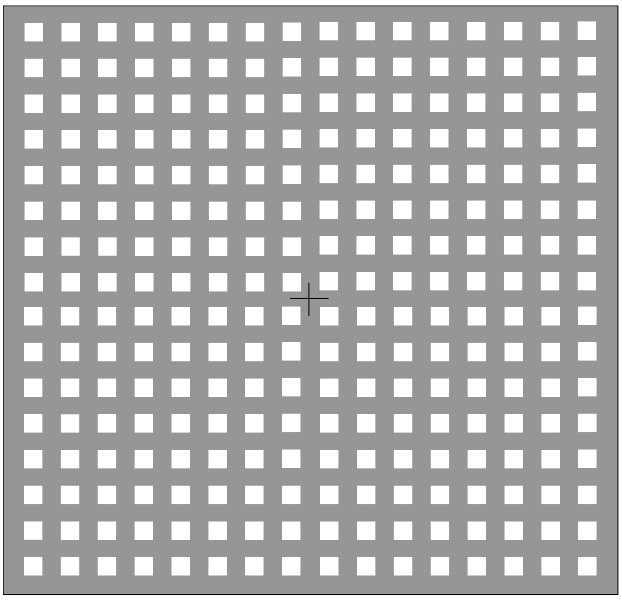

Figure 2. Bright isolated-check pattern. Image for demonstration purposes a 24 x 24 array of 16' checks in a 12.8°x12.8° field size with background luminance of 50 nits was used for this study.

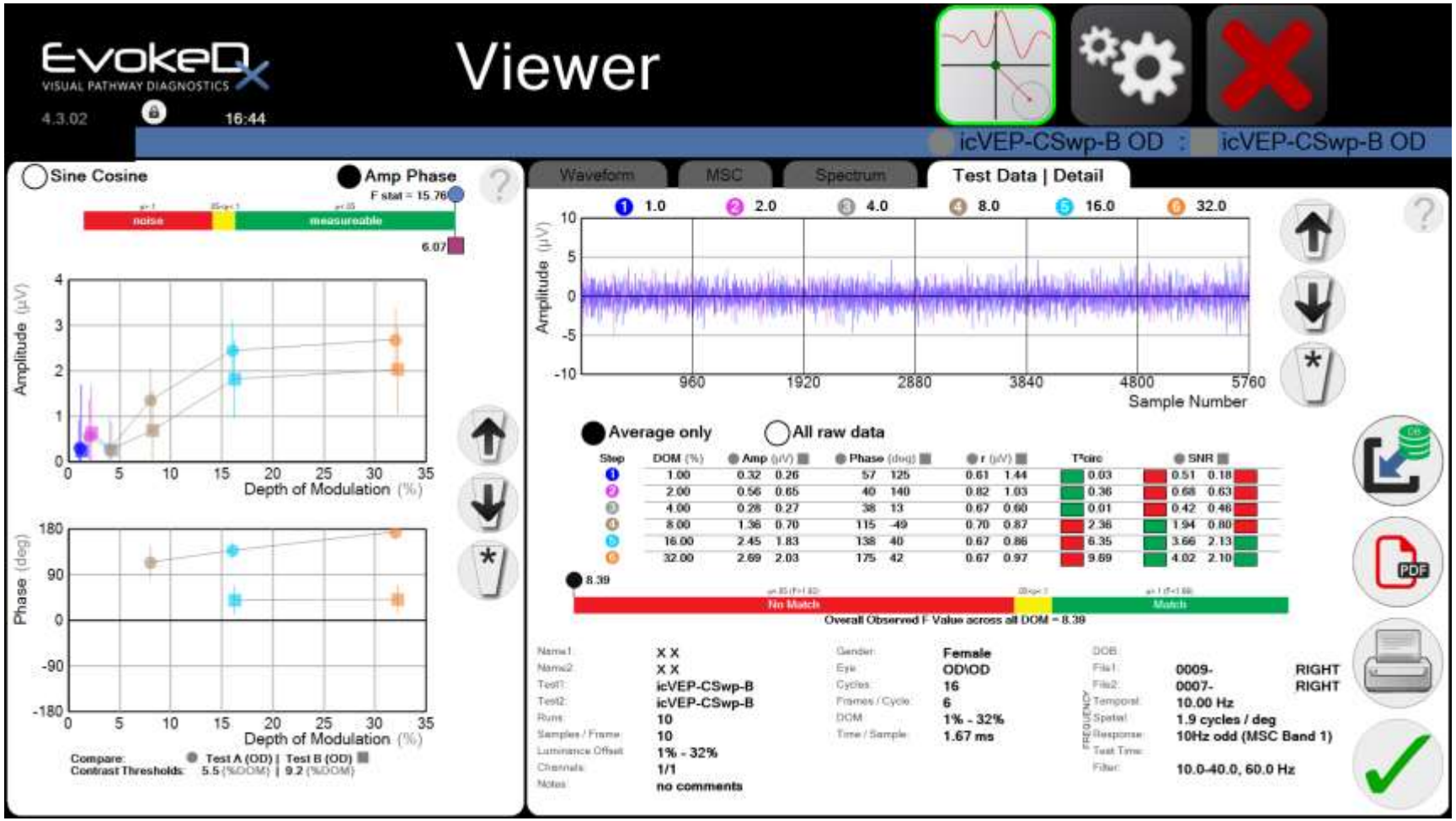

Figure 3A. Representative output from EvokeDx for the bright isolated-check condition for a control and MS participant, superimposed.

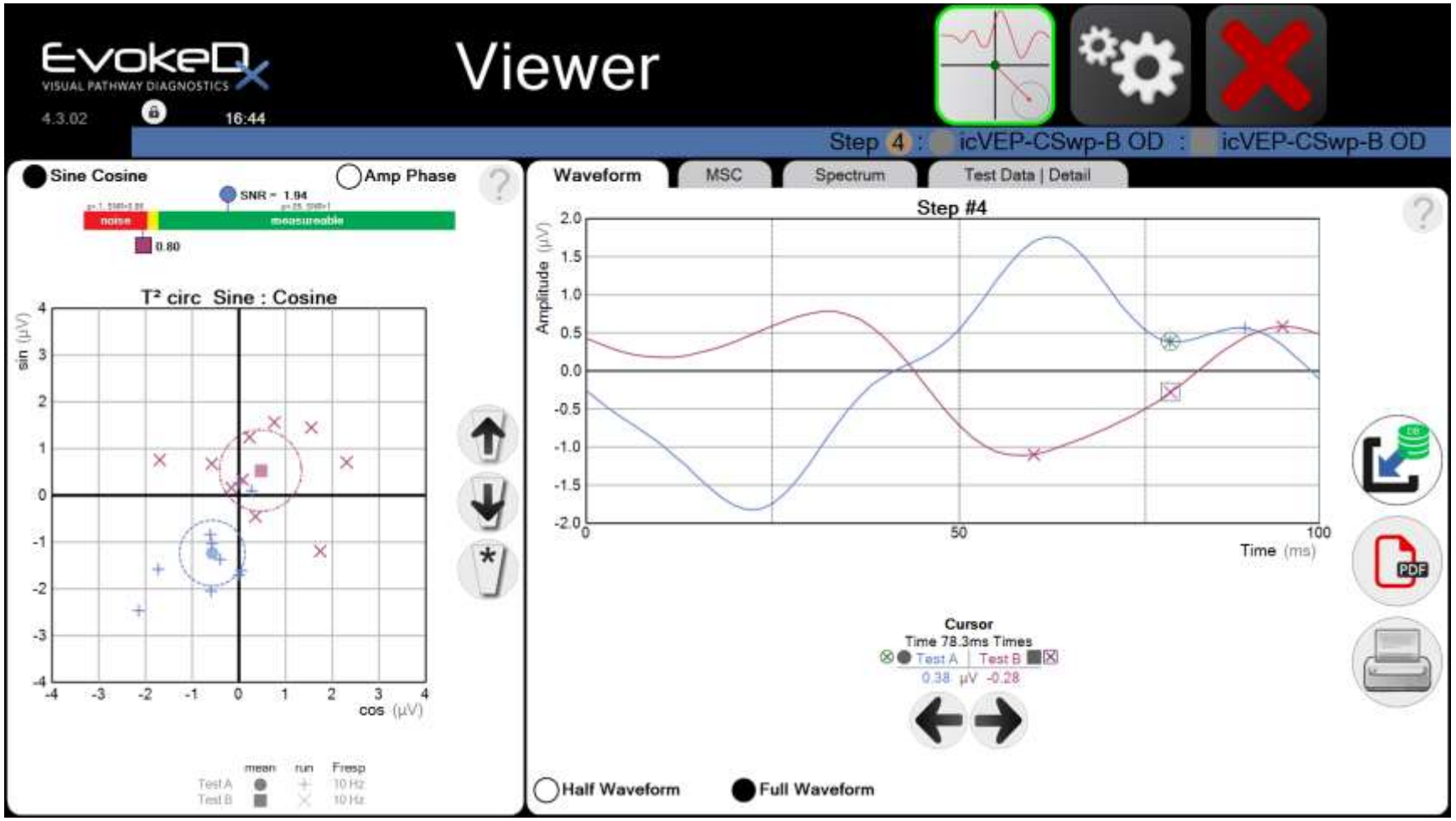

Figure 3B. Representative waveform from EvokeDx for the bright isolated-check condition for a control and MS participant, superimposed.

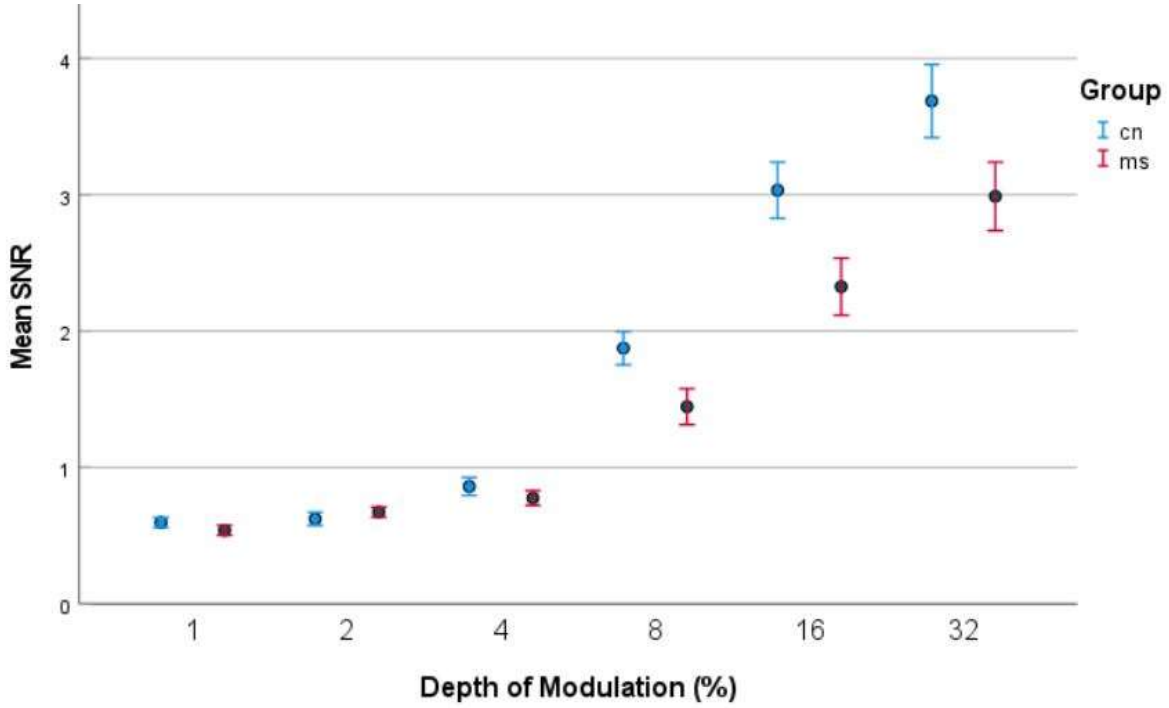

Figure 4. Mean signal-to-noise ratio (SNR) plotted as a function of depth of Modulation (DOM) aggregated for bright- and dark-checks and eye condition. Depth of modulation increased in octave 1.6-s steps (swept) from 1-32% (peak contrast 2-64%). Note that DOM is logarithmically spaced. Error bars are ± 1 *SE*.

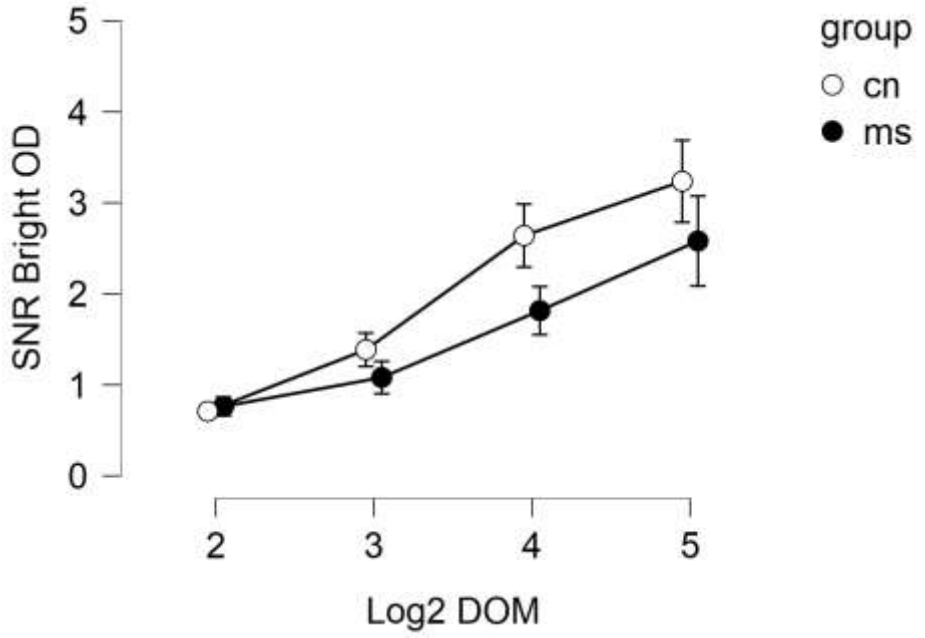

Figure 5A. Group differences in signal-to-noise ratio for the four highest depths of modulation for the bright check condition, right eye. Error bars are ± 1 *SE*.

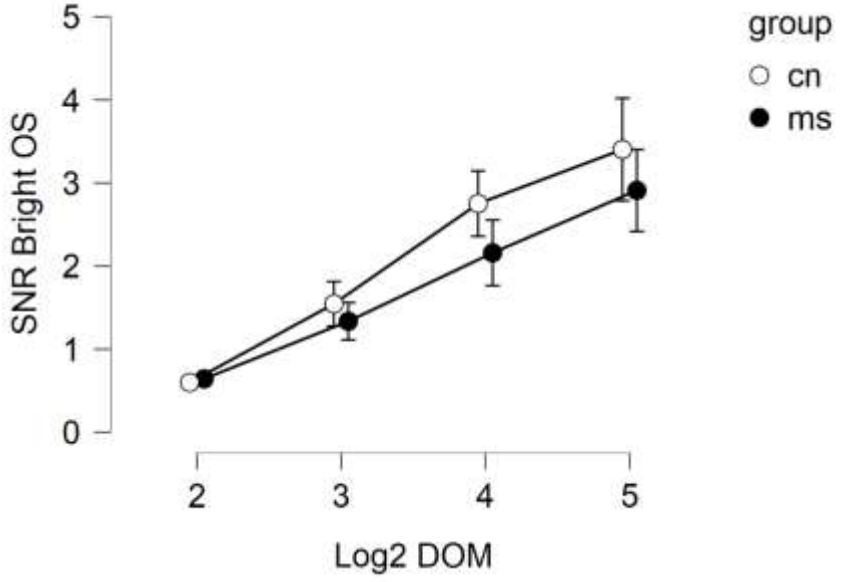

Figure 5B. Group differences in signal-to-noise ratio for the four highest depths of modulation for the bright check condition, left eye. Error bars are ± 1 *SE*.

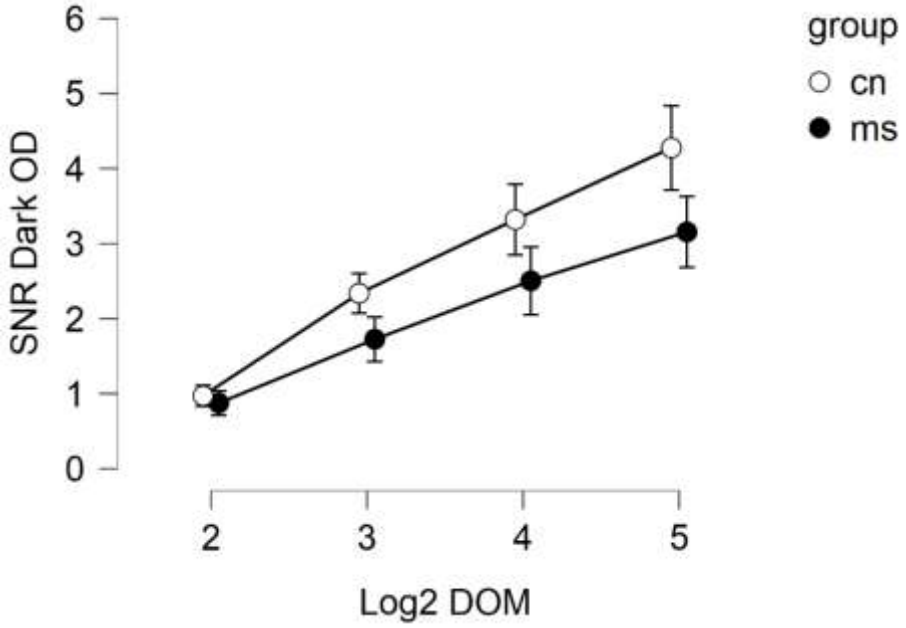

Figure 5C. Group differences in signal-to-noise ratio for the four highest depths of modulation for of the dark check condition, right eye. Error bars are ± 1 *SE*.

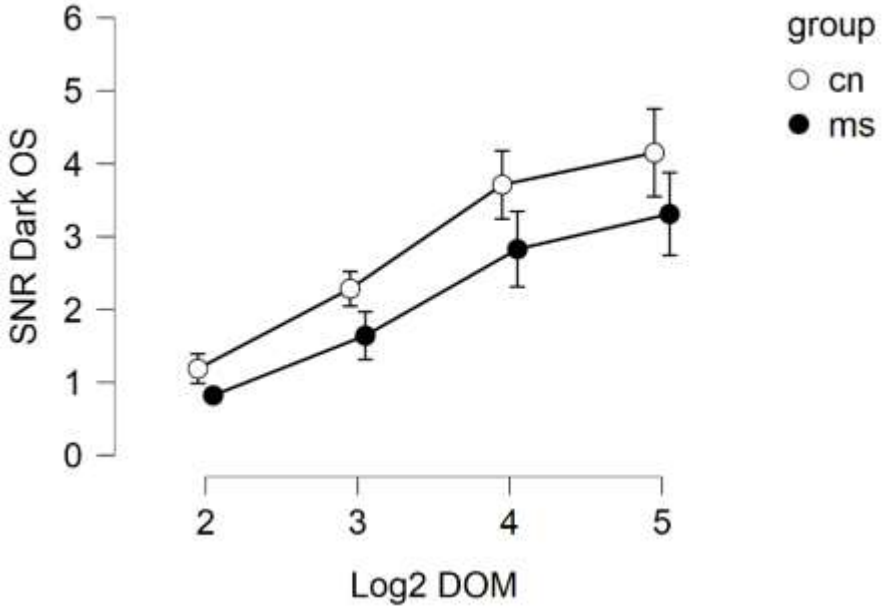

Figure 5D. Group differences in signal-to-noise ratio for the four highest depths of modulation for the dark check condition, left eye. Error bars are ± 1 *SE*.

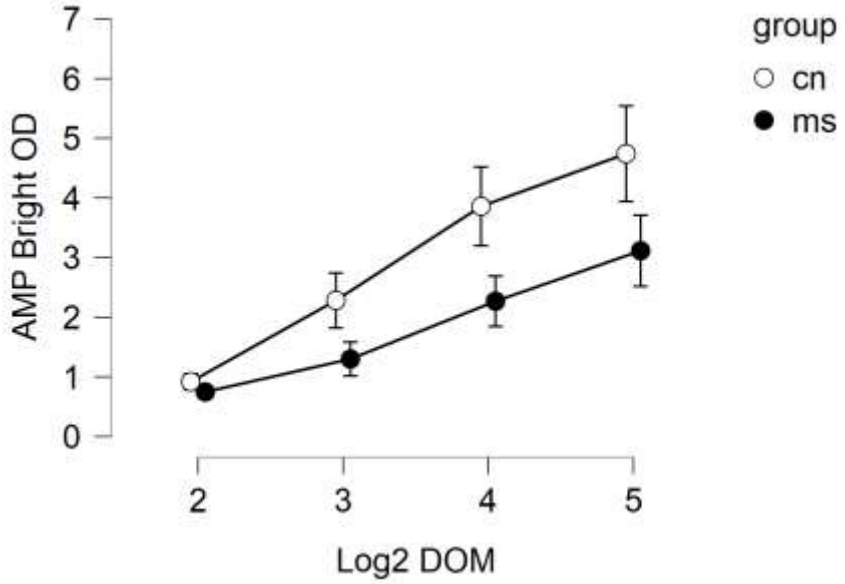

Figure 6A. Group differences in amplitude for the four highest depths of modulation for the bright check condition, right eye. Error bars are ± 1 *SE.*

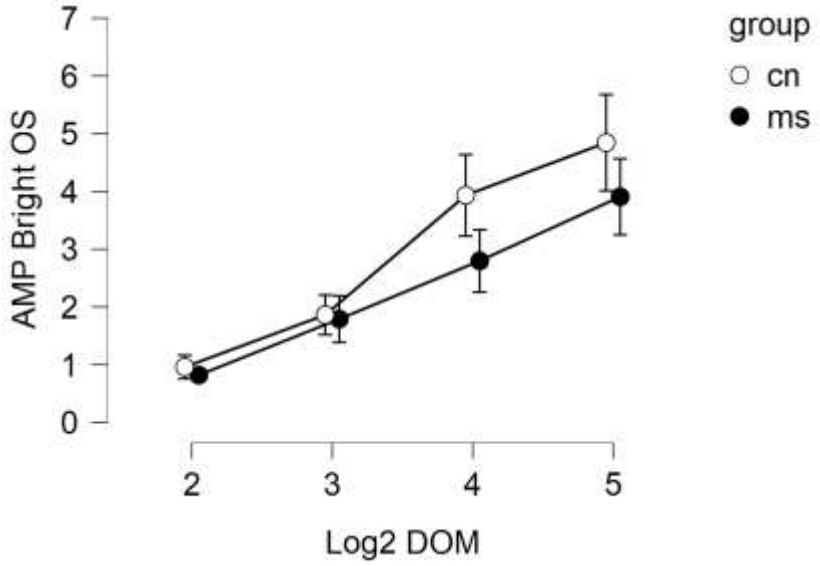

Figure 6B. Group differences in amplitude for the four highest depths of modulation for the bright check condition, left eye. Error bars are ± 1 *SE.*

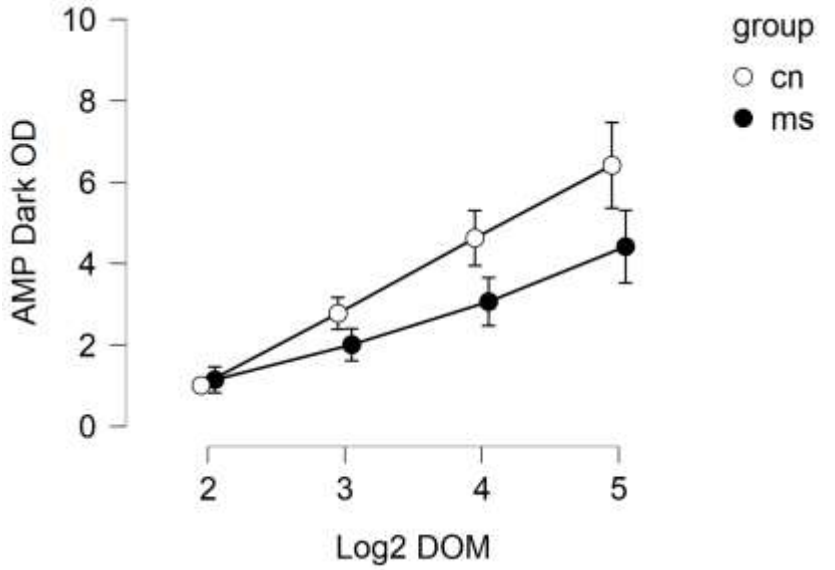

Figure 6C. Group differences in amplitude for the four highest depths of modulation for the dark check condition, right eye. Error bars are ± 1 *SE*.

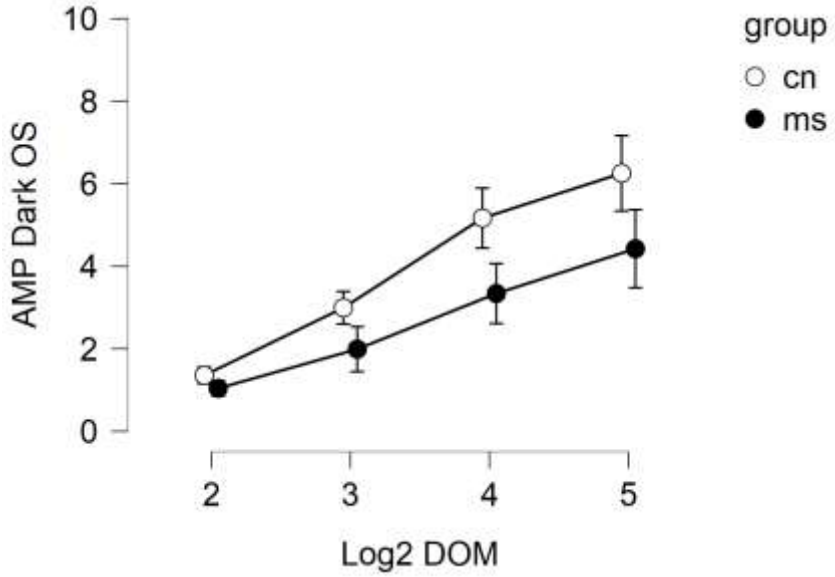

Figure 6D. Group differences in amplitude for the four highest depths of modulation for the dark check condition, left eye. Error bars are ± 1 *SE*.

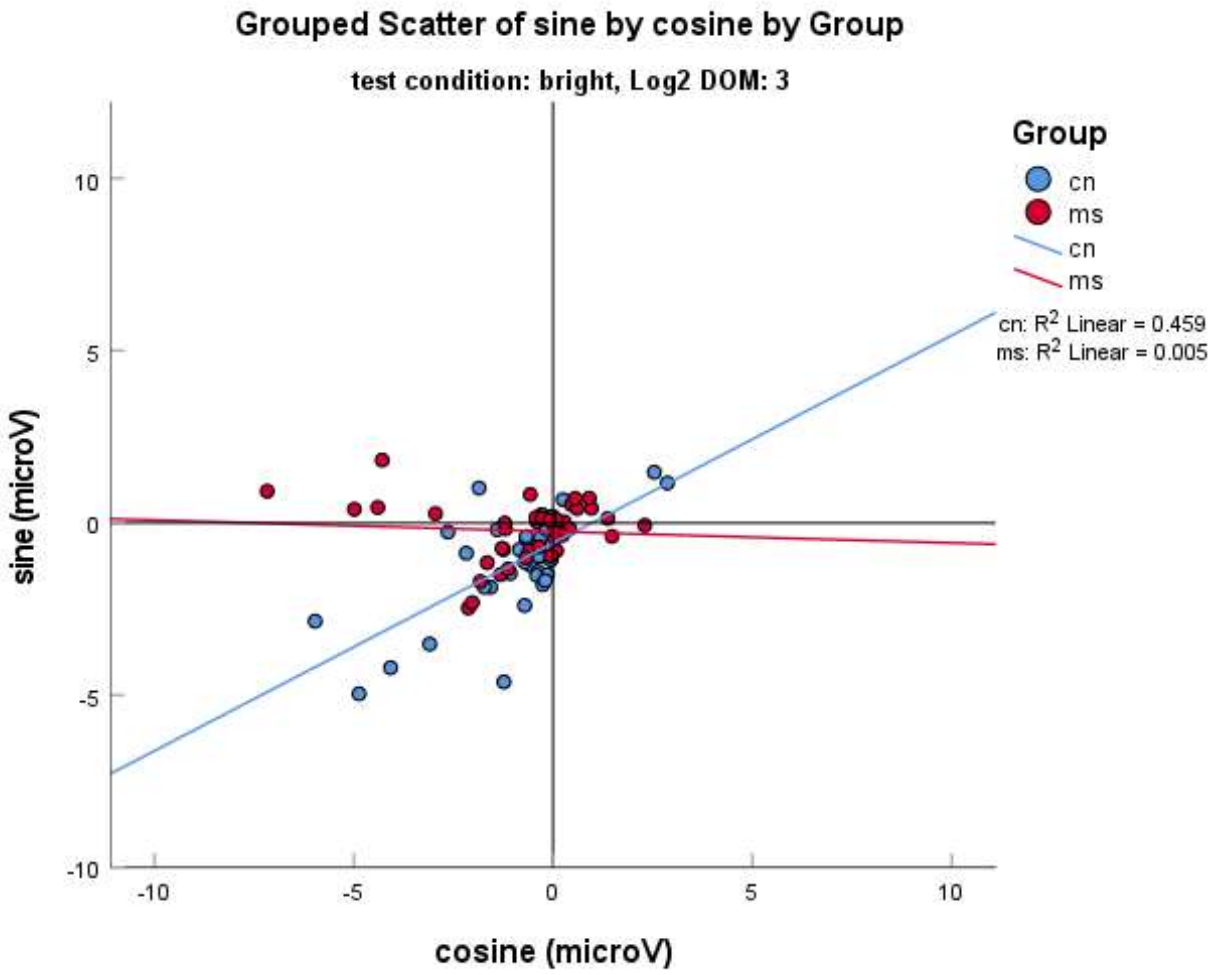

Figure 7A: Grouped scatterplot of averaged sine vs. cosine coefficients at the critical condition, 8% DOM, for the bright check condition. Linear regressions were fit for the multiple sclerosis (ms) and control (cn) groups.

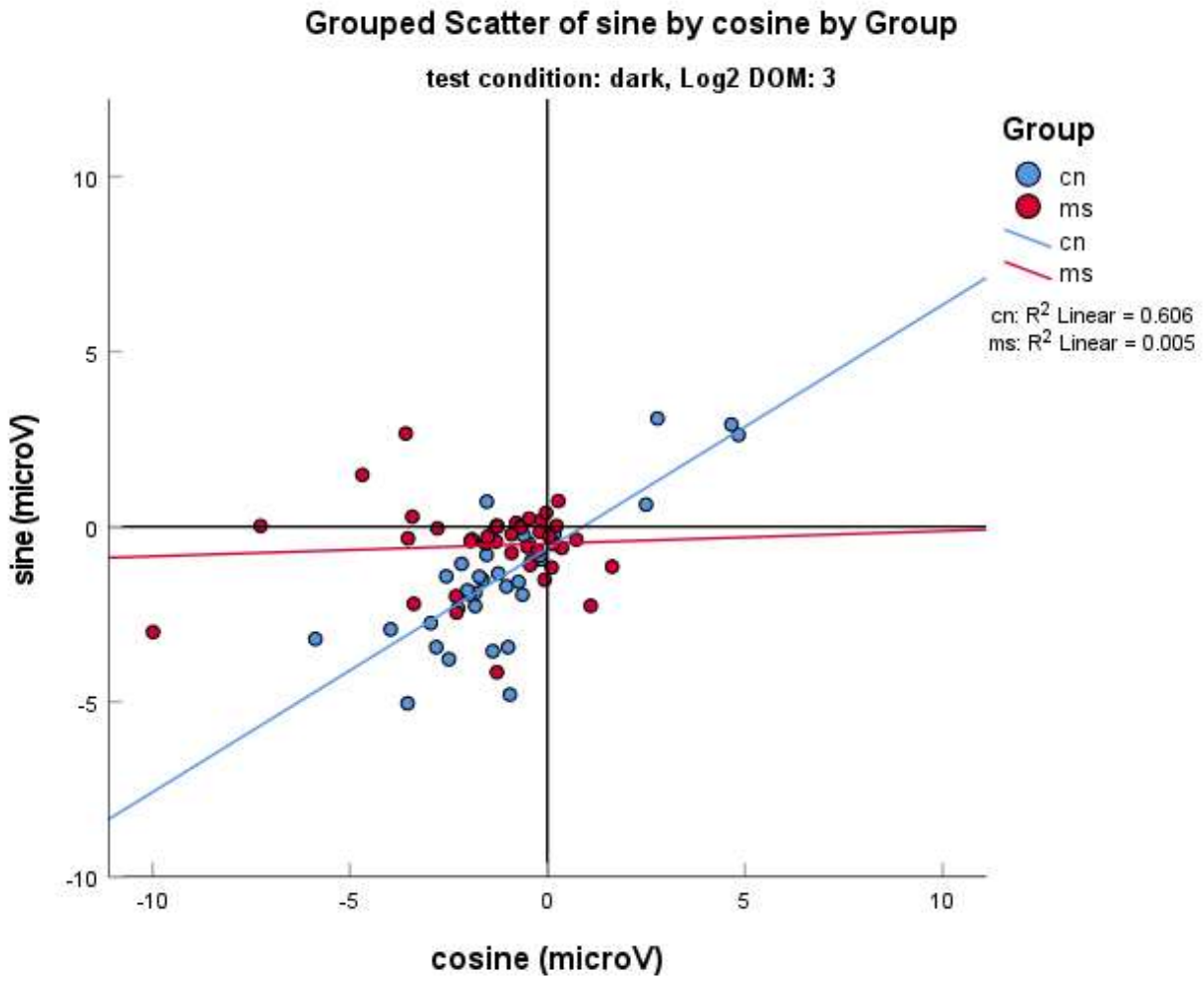

Figure 7B: Grouped scatterplot averaged sine vs. cosine coefficients at the critical condition, 8% DOM, for the dark check condition. Linear regressions were fit for the multiple sclerosis (ms) and control (cn) groups.

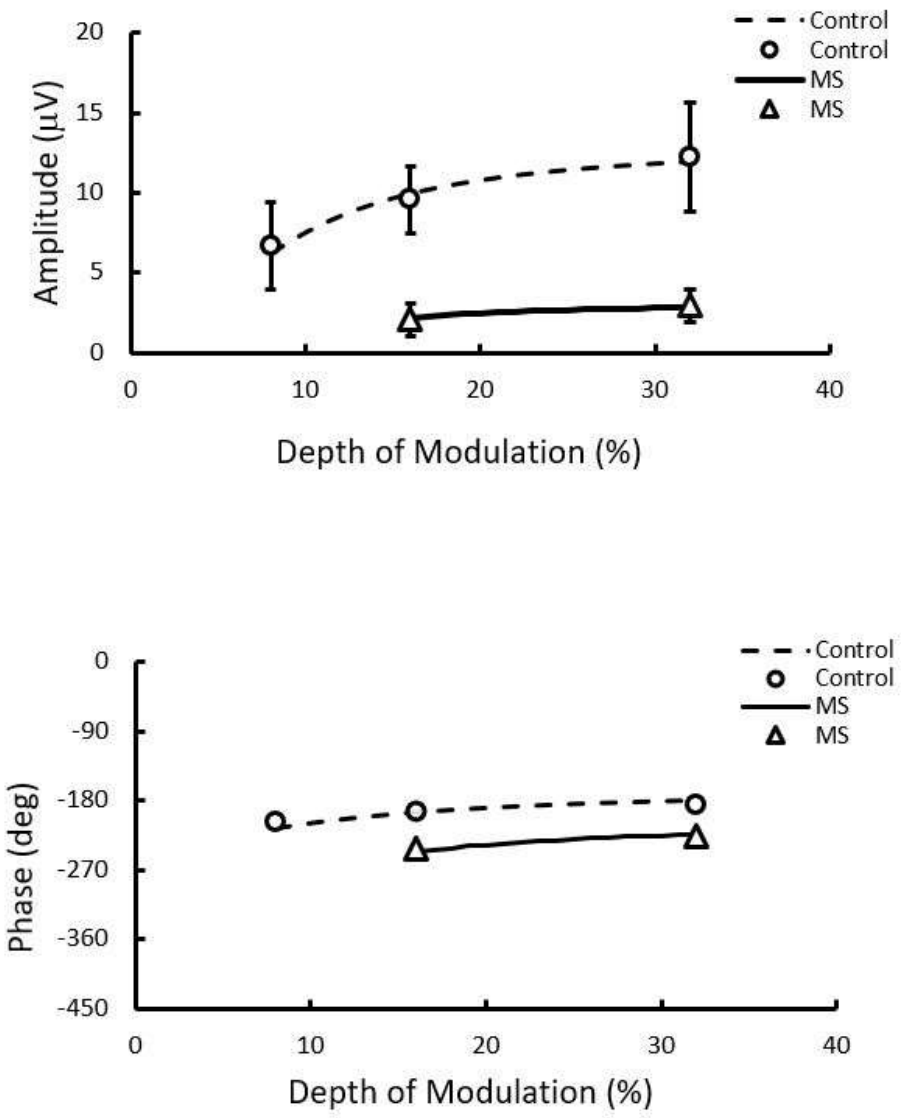

Figure 8: Monocular amplitude and phase waveforms for representative control and MS participants. Curves through the amplitude and phase data are fits with the biophysical model of Zemon and Gordon (2006).

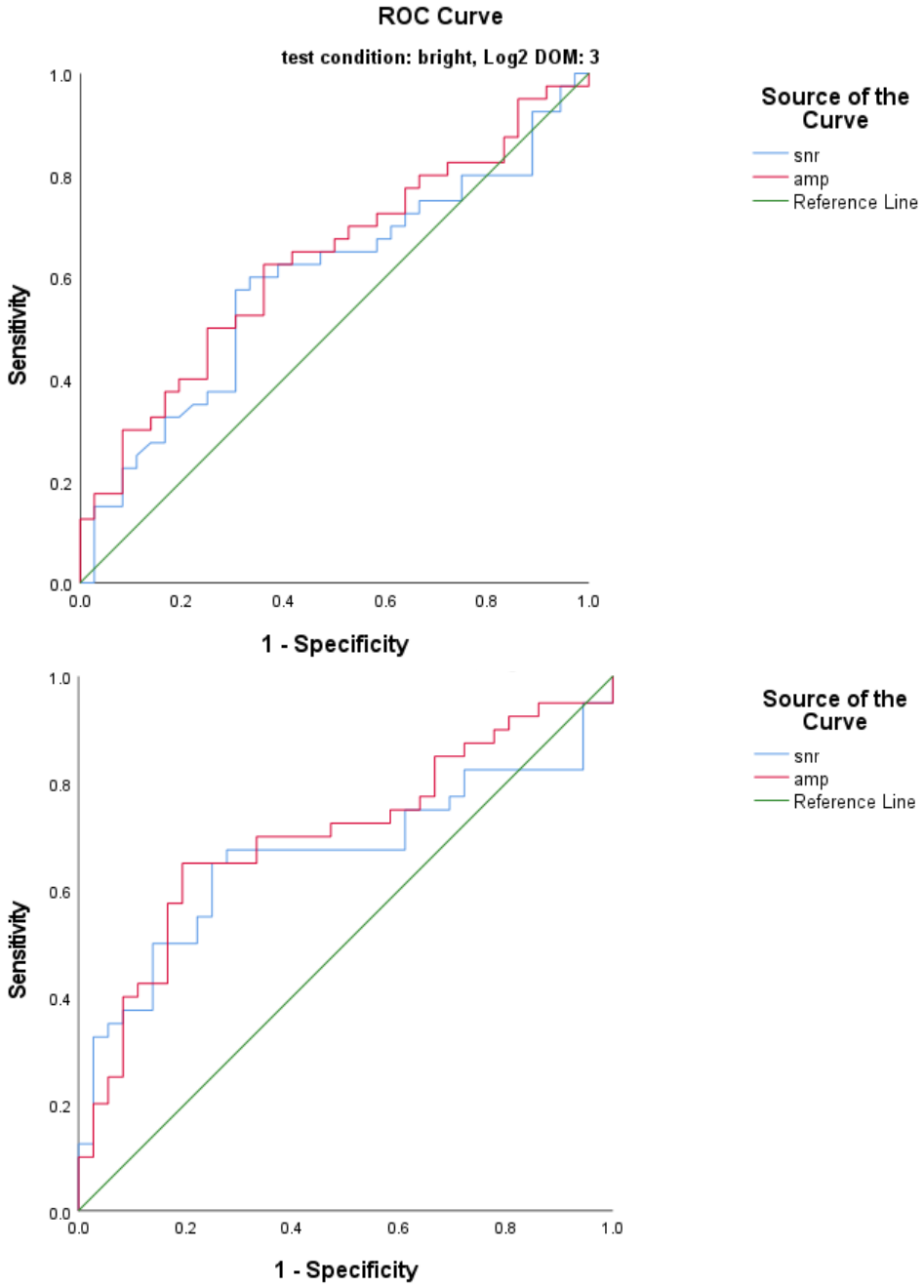

Figure 9: Receiver-operating-characteristic (ROC) curves for steady-state VEP responses to bright (top) and dark (bottom) isolated-checks for classification of healthy controls and individuals with MS at the critical condition, 8% DOM.