An ophthalmic acumen

On symptoms and signs in early

Multiple Sclerosis

PhD Thesis by

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Oslo, the 24th of October 2020

Sigrid Aune de Rodez Benavent

To my patients

It has been such a privilege to examine (and follow) our Oslo-cohort of MS patients who were newly diagnosed back in 2012. I am forever grateful for your positive spirit towards research participation and your unlimited will to share your lives' ups and downs with me.

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Preface

« L'ophthalmoscopie, c'est l'anatomie pathologique faite sur le vivant. Mieux encore, c'est l'anatomie pathologique vivante. » Charcot

The ability to look into other human beeings brains and instantly be able to tell what is wrong, is why I became an ophthalmologist.

However, if the reality was always that simple this thesis should not have been carried out. But maybe our eyes tell us more than we apprehend ? Maybe even our pupils tell us more than we usually notice? This was what Gro O. Nygaard and I discussed over lunch outside building 36 at Ullevål on a sunny day. And she asked « do you have the possibility to do pupillometry at the eye department ? » Honestly, I didn't know and our quest began. The search led us to the University of Oslo and Prof. Bruno Laeng at the nueropsychologic department, to Prof. Emilia Kerty at the neurologic department at Rikshospitalet, our best specialist in neuro-ophthalmology, to Prof. Liv Drolsum at the eye department who kindly proposed to support all the ophthalmic testing together with the research optometrists and at this stage we understood that with even more testing involved than just pupillometry this tended to become a Phd-project and Prof. Elisabeth Celius who accepted without hesitation to support my project as main supervisor.

The departments of radiology and neurophysiology have been of tremendous support throughout all these years, as the co-authorships also prove. The MS-research group first led by Prof. Hanne F. Harbo and now by Elisabeth G. Celius, have been inspiring and given a touch of pure pleasure to these years by learning me that « research is fun ». Thank you !

First of all to all my patients and thereafter to my family, colleagues and friends, who have even taken part in this project as healthy controls ; I could not have done this without you, thank you

♥ Til Alva, Philip og Henri :

nå kommer jeg opp av doktorgraven !

Uten deres tålmodighet og klemmer ville ikke dette blitt noe av ♥

Abbreviations

ANS	Autonomic nervous system
BP	Blood pressure
CI	Cognitive impairment
CIS	Clinically isolated syndrome
CN I-XII	Cranial nerves I-XII
CNS	Central nervous system
FIS	Fatigue Impact Scale
FSS	Fatigue Severity Scale
GCL+IPL	Ganglion cell layer + inner plexiform layer
IMSVISUAL	The International Multiple Sclerosis Visual System Consortium
ISNT	Inferior>superior>nasal>temporal
INO	Internuclear ophthalmoplegia
ISCEV	International Society for Clinical Electrophysiology of Vision
LCS	Low cognitive score
LGN	Laterale geniculate nucleus
MFIS	Modified Fatigue Impact Scale
MRI	Magnetic resonance tomography
MS	Multiple sclerosis
NEDA	No evidence of disease activity
NONMS eyes	MS eyes without previous optic neuritis
OCT	Optical coherence tomography
ON	Optic neuritis

POTS	Postural orthostatic tachycardia syndrome
PLR	Pupillary light reflex
pRNFL	Peripapillar retinal nerve fiber layer thickness
PROM	Patient reported outcome measure
REK	Regional ethics committee
RRMS	Relapsing remitting multiple sclerosis
RSI	Restriction spectrum imaging
SDMT	Symbol Digits Modalities Test
SI-time	Saccadic initiation time
VEP	Visual evoked potentials

Publications included

Paper I Nygaard, G. O.*, de Rodez Benavent, S. A* et al. (2015). **"Eye and hand motor interactions with the Symbol Digit Modalities Test in early multiple sclerosis."** Multiple Sclerosis and Related Disorders 4(6): 585-589.

Paper II de Rodez Benavent, S. A.*, Nygaard, G. O.* et al. (2017). **"Fatigue and cognition: Pupillary responses to problem-solving in early multiple sclerosis patients."** Brain Behav 7(7): e00717.

Paper III de Rodez Benavent, S. A., et al. (2019). **"Neurodegenerative Interplay of Cardiovascular Autonomic Dysregulation and the Retina in Early Multiple Sclerosis."** Front Neurol 10: 507.

***shared first authorship**

Chapter 1 Introduction

Our eyes belong to the anterior visual pathways and the central nervous system (CNS). The direct access through the pupil enables us to observe the CNS in vivo with an ophthalmoscope, offering a unique possibility to study the relationship between structure and function in CNS diseases like multiple sclerosis (MS). It is debated whether ophthalmologic signs in MS precisely mirror the ongoing CNS pathology or simply represent localized signs of disease like in optic neuritis (ON).

MS is an inflammatory and neurodegenerative CNS disease. MS treatment has two primary aims: to prevent inflammatory relapses and to stop neuronal loss. Ophthalmologic exams (1), have together with the non-invasive retinal imaging technique called optical coherence tomography (OCT) (2), proven useful as outcome measures in clinical trials in MS as well as in studies of neuroprotection (3), unravelling subtle signs of functional and neuro-structural loss. Neuro-ophthalmic studies in MS, form a parabolic historical popularity timeline starting with Charcot sited in the preface of this thesis, then facing a downward curve with few new advances in diagnostics for some decades and then again gaining in interest with the possibilities provided by OCT.

MS' disease expression, the phenotype, has a multitude of manifestations. A diagnose is based upon fulfillment of a set of diagnostic criteria. The diagnostic criteria of MS (4), have evolved over time. There is a joint ongoing research effort to determine the diagnostic phenotypes at an early stage to initiate treatment in order to prevent future loss of function. The current diagnostic criteria involve the use of magnetic resonance imaging (MRI), which is both costly and time-consuming. OCT has gained great interest in MS as an imaging modality, but lacks scientific proof of diagnostic value to add to early phenotypic description (5), and needs further investigation.

The patients experience disease symptoms interpreted by the clinician in the phenotype description. MS patients often suffers from symptoms of cognitive changes and fatigue early in the disease course. Other symptoms are dizziness or heart palpitations when standing up, symptoms attributable to changes in the autonomic nervous system (ANS). Both cognitive changes (6), fatigue and ANS dysfunction relate to disease progression in MS (7), and probably neurodegeneration. Patient reported outcomes (PRO) gives us insight into the magnitude of these problems, but the mechanisms behind are yet to discover. There are several test possibilities measuring different aspects of ANS function. The pupillary light reflex (PLR) is an ANS reflex measurable with pupillometry acting as a proxy of central ANS function. We have used this traditional method for the first time in a combined ANS and neurodegenerative study in MS.

Our brain as illustrated in Figure 1, not only senses our entourage and accordingly executes motor control, it also performs higher order processing. Some of which is mirrored in the eyes, as exemplified by the fact that your pupils will widen if you are telling a lie, are in love with the person in front of you or is performing challenging mathematical tasks. This cognitive perspective of the pupillary reflex could prove to be a useful MS test as underlined by our study.

The scope of this thesis is to explore the relationship between symptoms in early MS and neuro-ophthalmic measurements by pin pointing signs of neurodegeneration and contribute to the understanding of how to prevent functional decline.

Figure 1. Photo from Pexels

1.1 The anterior visual pathways

The eyeball's inner surface, the retina, transforms photons to an electric input for the brain to see and apprehend the scenery of our physical surroundings. The optic nerves from each eye confluence in the optic chiasm and partly cross over to the contralateral side of the brain. This crossing of fibers is the basis of binocular vision. The axons then follow a defined route to the first relay in the visual pathways, the lateral geniculate nucleus (LGN). The LGN is defining the anatomical structures involved in vision as anterior if they are located before LGN and posterior after LGN when following the light's path to the occipital visual cortex.

Distantly related to this thesis, there is compelling imaging MS research on the LGN and the thalamus regarding vision, neurodegeneration and biorhythm (8-10).

The anterior visual pathways' major asset compared to the rest of the CNS, is the opportunity to appraise structure by simply looking into the eye and to relate this to function.

1.1.1 The pupil, the pupillary light reflex (PLR) and the autonomic nervous system (ANS)

Analogously to the aperture of a camera, the eye's pupils precisely doses the amount of light falling on the retina. The pupil size varies with age, refractive errors, state of sleepiness, degree of attention and arousal.

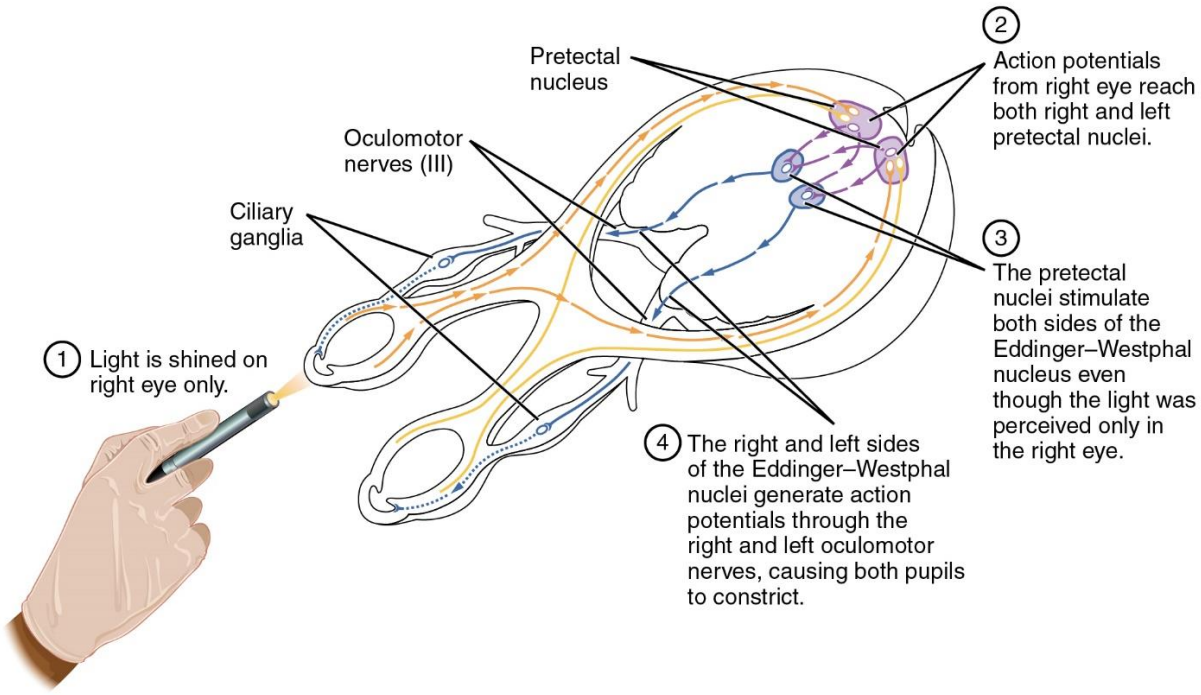
Reflex testing is the basis of all neurologic exams to ascertain the nervous system senses a stimulus and executes the appropriate motor response. In neuro-ophthalmology, one of these reflexes is the PLR (Figure 2).

Otto Lowenstein and Irene Loewenfeld developed infrared pupil measurements in 1947. Irene Loewenfeld continued making extensive contributions to the field of pupillometry (11), and her work has regained popularity with the newly won

knowledge about the different types of retinal ganglion cells discussed further in this thesis under the next subheading.

The PLR reveal whether the light transmission through the eye is successful. Photons follow a neurologic pathway from the retina, through the optic nerve, (the 2nd cranial nerve (CN II)), to the pretectal area in the midbrain, where the message is read and an executive message is send out through the oculomotor nerve (CN III) to the ciliary sphincter muscles in the pupils. These muscles contract and let less light into the eyes (11).

Figure 2. The pupillary light reflex (PLR). From openstax.org. Non commercial creative common license



The ANS (12) tunes the pupil constriction and then the re-dilatation that follows. These dynamics are interesting in MS and have been studied not only bedside, looking for a relative afferent pupillary defect following an ON, but also as ANS changes in relation to disease (13, 14).

The neuronal messages flow in the intercellular space, from one cell to another, by chemical signals called neurotransmitters. In the ANS, the main neurotransmitters sending the neuronal tuning message to the effector organs are

acetylcholine and noradrenaline. In this context "tuning" refers to the neuronal ANS tonus present at all times, resulting in an rigorous ability to tune up or down activity, not only turn it on or off, in a given organ. The ANS' parasympathetic branch dominates under resting conditions helping the body to store energy and maintain homeostasis, while the sympathetic branch commands under straining and physical activities preparing the use of energy as well as in stress and danger, it is our " fight or flight system".

There are cross-talks between the ANS and the immune system, this communication may be altered by disease. In MS, a disrupted communication leads to a dysfunctional stress-response contributing to disease progression (15).

The ANS is an incredibly intricate, but subtle system governing our bodies. Despite the ANS' importance, a relatively small amount of attention is devoted to it in the current neurological literature and research (16). There are however many MS patients complaining of ANS symptoms with functional constraints. The clinical signs of ANS dysfunction have been linked to MS neurodegeneration (7).

A study of both ANS function and neuronal integrity is an intriguing possibility by visualizing the eyes' pupil dynamics together with the physical condition of the retina and the optic nerve.

1.1.2 The retina, the ganglion cell layer, the retinal nerve fiber layer and the optic nerve

When light shines through the eye's pupil, the intraocular lens focuses it on the retina, like the film inside an analog camera. This fabulous curved camera (https://gearpatrol.com/2019/07/09/what-is-a-curved-camera-sensor/) captures the photons with the photoreceptor layer right on top of the light absorbing pigment epithelium, the dark side of the film. These cells, called rods and cones, then transforms the photons into an electric message and send it through bipolar cells to the receptive fields of the ganglion cells. The ganglion cells are the output cells of the retina (17). There are several types of ganglion cells and even

intrinsically photosensitive subtypes containing melanopsin (18, 19). These latter are involved in biorhythm (Figure 3) and PLR through the retinohypothlamic tract.

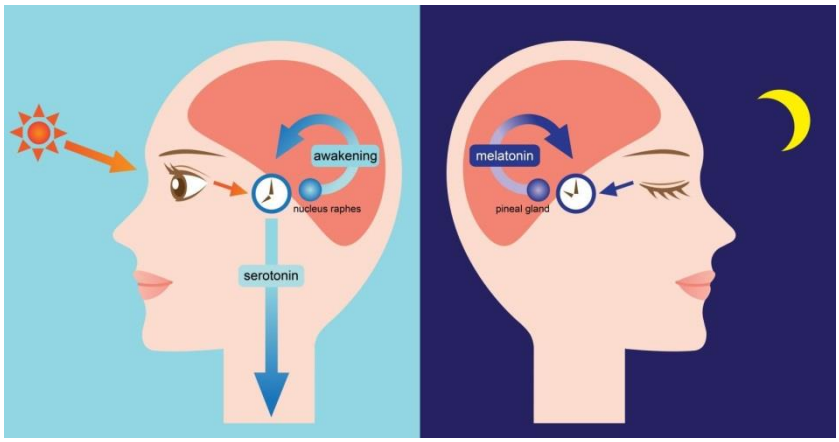


Figure 3. Biorhythm. Illustration from Bigstock.

If the PLR test is performed with certain light wavelengths, it is possible to ascertain if a pathology mainly affects either the photoreceptors or the retinal ganglion cells (20). Studies in MS have found correspondence between PLR and retinal OCT measurements (21). There are three possible retinal inputs for the PLR, the rods with their rhodopsin, the opsin containing cones and the intrinsically photosensitive melanopsin containing ganglion cells. These different routes are possible to test separately through different test paradigms (22).

The ganglion cell layer extends its long tails, the axons, out of the eye forming the optic nerve. Inside the eye, these axons line the inner surface of the retina, forming the retinal nerve fiber layer, observable with a red free filter in ophthalmic slit lamp and possible to photodocument as well (23, 24).

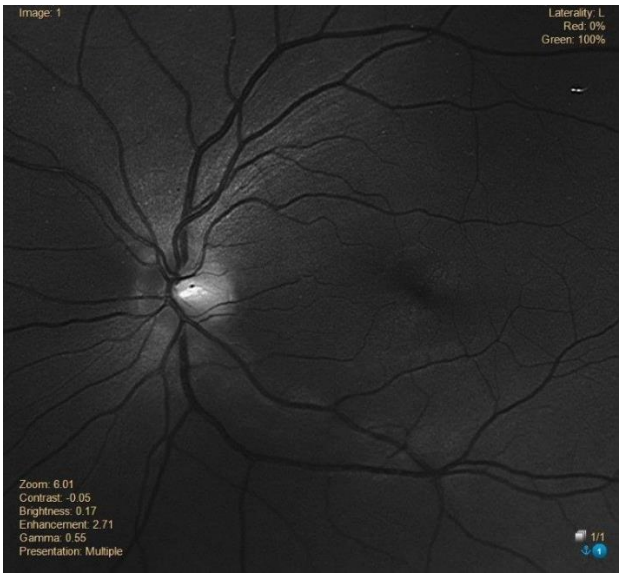


Figure 4. Monochromatic photodocumentation of the retina. (Authors' own photo)

To evaluate the integrity of the nerve fiber layer it is conceivable to study the concurrence of all the fibers before they exit the eye in the peripapillary area.

The optic nerve head (Figure 5) is inside the eye in front of the lamina cribrosa, while the rest of the nerve is behind the eye and need indirect study measures as MRI. The optic nerve head is the eye's blind spot by lacking photoreceptors. In ophthalmology, the slit lamp indirect biomicroscopy is still the golden standard when assessing the optic nerve head despite all technological advances. There are many aspects to consider such as size, shape, colour, cup/disk ratio, ISNT pattern (25), and signs of swollen tissue or bleedings. The pattern recognition of optic nerve head pathology points to the connective tissue meshwork in the lamina cribrosa as an important structure (26).

The post laminar portion of the orbital optic nerve is anatomically a nerve thread surrounded by its blood supply, the conduction-safe myelin insolation, as well as the brains' wrapping lamina.

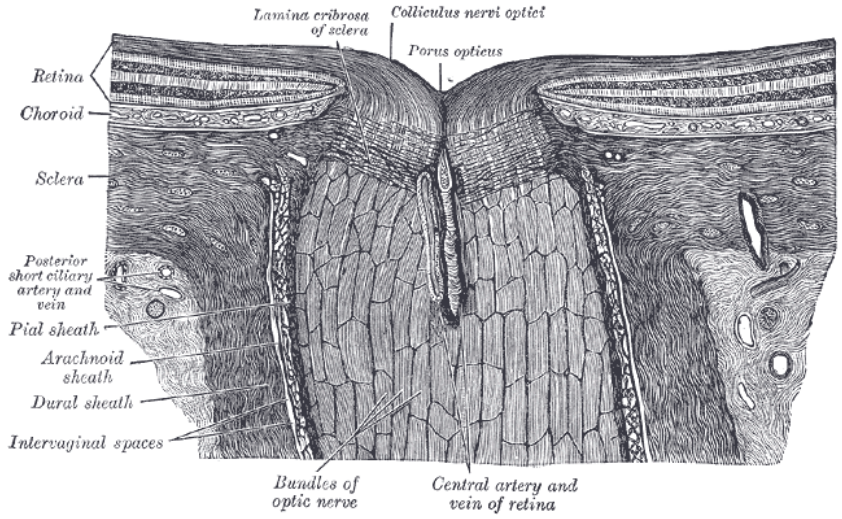


Figure 5. The optic nerve head. By Henry Vandyke Carter - Henry Gray (1918) Anatomy of the Human Body Bartleby.com: Gray's Anatomy, Plate 880, Public Domain

The optic nerve is challenging to image properly with MRI (27), because of its relatively small size, the surrounding soft tissue structures, the bony orbit, the air filled adjacent sinuses and the skull base. Other options are therefore to measure their electrical conduction capacities as with visual evoked potentials (VEP) (Figure 6) and to compare this with functional measures. This research field in MS (28, 29), has gained interest the last decade. Another advantage of VEPs are their ability to detect subclinical ON changes, adding to the understanding of diffuse visual problems in MS.

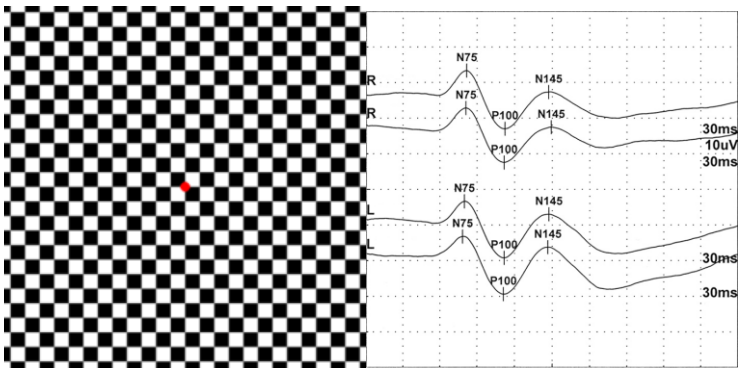


Figure 6. Visual evoked potentials (VEP). By Medicus of Borg - Own work, CC BY-SA 4.0, https://commons.wikimedia.org

1.1.3 Vision

The sense of seeing is a marvellous ability to apprehend our surroundings, a photographic and cognitive integrating skill. In popular culture, valiantly personalized by the comic book hero, Vision (https://www.marvel.com/characters/vision). Historically this sense is often associated with supernatural explanatory models as for St. Lidwina the patron of ice-skating (https://en.wikipedia.org/wiki/Lidwina). She was a young Dutch woman living in the 14th century, who was declared a Saint; when she as a 15 year old fell on the ice while skating and thereafter developed unilateral optic neuritis and a complex symptom description fitting what we today call MS.

Greek mythology is rich in medical connotations and the ancient myths actually cover several aspects of vision such as the visual field, ocular mobility and ocular trauma. The medical person, the healer, had a mythical status and the first ophthalmologist was Apollo himself (Figure 7) (30).

Figure 7. Apollon, image by Pixabay

Vision is not only a Snellen equivalent often, but highly erroneous, translated into percentage of visual acuity. The Snellen equivalent is a test of high contrast macular vision (Figure 8), something you only use when reading black optotypes on a white background in a lighted room.

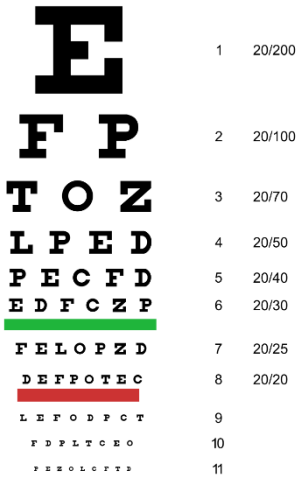


Figure 8. Snellen chart.

Vision encompasses this by far, and contains visual field, night vision, colour vision, contrast vision, binocular stereoscopic vision, fluent conjugated gaze and higher order processing of vision like visual pattern recognition, imagery and vivid dreams, and the ability to understand and recall complex visual scenery in order to anticipate and react accordingly. Opposed to this, a "perfectly seeing eye" may not have an adequate higher order interpretation of the photographic image transmitted to the brain exemplified by the condition called visual agnosia (31).

In the clinic, we often choose to use the Snellen equivalent to quantify a patient's vision. However, in MS, low contrast vision has proven to be a valuable marker of residual deficits after ON (32), and serving as a marker of disease progression and an outcome measure in clinical MS research (1, 33). Contrast vision is what we use in order to recognise a familiar face (34), and to know where the edge of the sidewalk is. Skiing down a white hill on a day with flat light you might have noticed how difficult it is to anticipate unevenness. This it is the lack of contrast making you go "blind".

An emphasis on the plurality of vision help us understand the complexity of the many cases of fluctuating neuro-ophthalmic MS complaints (35, 36). Screening tools help the clinician, like the 10-item neuro-ophthalmic supplement to the NEI-VFQ-25 (37), to select appropriate supplementary tests supporting the clinical neuro-ophthalmic exam. An MS patient complaining of oscillopsia may present with a suspicion of internuclear ophthalmoplegia and we may need to monitor eye movement to determine where to look for our patients' brain stem lesions (38).

1.2 Multiple Sclerosis (MS)

The Norwegian MS population currently counts more than 10 000 individuals (39). MS is a multifactorial immune mediated inflammatory and neurodegenerative chronic CNS disease with onset usually in young adulthood. Why and how they have developed MS is unknown and the individual disease course is highly variable. However, a combination of genetic susceptibility (40) and environmental factors (41, 42) play a role in the disease development, a disease characterized by episodic neurologic deficits from which the patient may or may not fully recover. After two to three decades, the disease typically takes on a progressive course without the episodic events. About 5-15% of MS patients suffer from a progressive disease from onset (43). Each patient present with a unique immune-effector mechanism (44), and this explains why several different immune treatments shows efficacy in delaying disease development in MS.

1.2.1 MS and signs of disease in the anterior visual pathways

About 20% of MS patients have their first encounter with this chronic condition as a loss of visual function in the form of an inflammation in the optic nerve (an ON) (45). Up to 80% of patients will experience some kind of eye problems throughout their life with MS. ON causes the nerve's insolating myelin wrapping to crumble and thereby leaving the nerve threads, the axons, bare (Figure 9).

Depending on the severity of the inflammatory process, the relapse may transect axons causing a back-dying effect on the neuronal ganglion cell body in the retina. As an uttermost consequence, the ganglion cell dies (46). Depending on the severity of ON, the sequelae vary accordingly, from none (full recovery) to complete loss of vision.

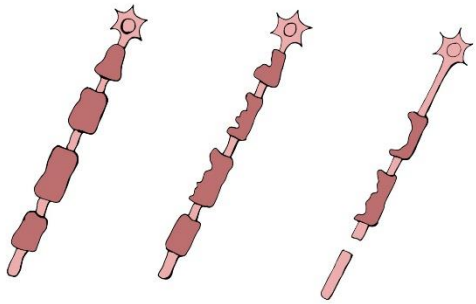


Figure 9. Demyelination.
Illustration by MS-research group at Oslo University Hospital and the University of Oslo.

Colour vision deficiencies are often one of the first visual symptoms from an ON (47). Colours seem faint seen with the affected eye compared to the other eye. Traditionally the type of colour vision loss may guide us to the etiology of the eye disease according to Köllner's rule (48). In MS there is no specific acquired dyschromatopsia known as pathognomonic for the disease. This is probably partly due to the inhomogeneous nature of the disease itself with various possible demyelinating and axonal damaging sites. Macular volume loss, loss of fibers in the papillomacular bundle and colour vision deficiencies are associated with disease severity and subclinical progression of disease in MS (49-52).

High contrast vision loss is the most obvious and easiest to quantify when our patients present with their visual complaints. However, it does not tell us where in the visual pathways the problem is, unless we observe an optic nerve head change, an OCT measurement change or there is pain upon eye movement as in

an ON. It is important in such cases to remember, "it is the brain that sees", and cortical demyelination does in rare MS cases lead to blindness (53). Visual acuity may vary throughout the day or in relation to activity, infection and stress due to Uhthoff's phenomenon. Sometimes it is difficult to know if the patient is having a breakthrough exacerbation of disease or simply a neuronal conduction problem due to Uhthoff's phenomenon (54).

Another cause of blurry vision is loss of contrast, a residual defect even after good visual recovery following an ON. MS patients report problems in daily life in relation to contrast vision loss emphasizing the need to test for and discover these changes in order to optimize ON treatment (55). Both monocular and binocular measurements are important since there is binocular summation of contrast vision (56), mostly acting as a promoter of better results with both eyes open. Loss of vision in one eye does however in some cases, lead to inhibition of contrast vision in the better eye under binocular conditions. There are correlations between inter-eye differences in retinal axonal and ganglion cell OCT measures and degree of binocular summation in MS (57). This is why certain patients experience less blurring when they occlude the affected eye, even in the absence of diplopia (Figure 10) or squint.

Figure 10. Diplopia.

By Jonathan Trobe, M.D. - University of Michigan Kellogg Eye Center, creative commons.org.

Ocular motor disturbances contribute to double vision (diplopia) or unsteady and moving surroundings (oscillopsia). In a recent state-of-the-art review on MS and eye movement disorders (58), internuclear ophthalmoplegia (INO) is described as the most recurrent eye motor difficulty, further discussing other entities such as bilateral INO, exotropia and loss of convergence, and the so called one-and-a-half syndrome. Other possibilities are different forms of non-volontary spontaneous movement disturbances as in nystagmus and saccadic dysmetrias or intrusion phenomena. Early and asymptomatic ocular motor changes are briefly touched upon in the same review. Studies of saccadic eye movements in MS are anew gaining importance with the application of non-invasive eye tracker interfaces (59, 60).

Visual field defects occur in ON, but also from lesions throughout the visual pathways. Perimetry is not part of the diagnostic battery in MS due to the large variety of possible defects. As a functional test, it is a sensitive and reliable test, which allows quantification of even subclinical changes in the case of normal VEP metrics. Subclinical visual field alterations underlines the fact that MS eyes seldom may be considered as "healthy control eyes" even in the absence of subclinical ON and normal VEP findings (61).

Inflammation is also present in MS eyes. Retinal inflammation, as often the case in the rest of the brain, exists in the perivascular space, presenting as periphlebitis on eye exams. Another inflammatory MS related eye entity is uveitis, which most often presents as an intermediate uveitis and more seldom as an anterior uveitis. Uveitis occurs in relatively older female patients than those with idiopatic intermediate uveitis and has generally a good visual prognosis (62).

1.2.2 The anterior visual pathways as a reflection of CNS pathology in MS

Even in the absence of evident MS relapses and from early on in the disease course, there is a diffuse ongoing loss of neuronal tissue in the CNS (63), eventually seen as brain atrophy on conventional MRI. It is crucial to pin-point

the rate at which this loss goes on in order to pace it down and hopefully stop it (3, 64), before the atrophy as a sign of disease progression is measurable on MRI.

It is possible to measure neurodegeneration in the anterior visual pathways with OCT technology (65-67). Correlation between loss of retinal neuronal tissue and CNS pathology in MS (68-71), is under continuous investigation. A four-year follow-up study puts forward OCT as a clinical monitoring tool for CNS neurodegeneration and brain atrophy (72). In addition to pure neuronal loss, retinal changes also contribute to the understanding of neuronal inflammation in MS. Inner nuclear layer volume and thickness, correlate with disease activity whereas microcystic edema parallels disability (73-75). A relatively small study on MS eyes without previous ON (NONMS eyes) has also pointed out a relation between retinal OCT measures, cognitive and physical disability in early MS (76). The International Multiple Sclerosis Visual System Consortium (IMSVISUAL, founded in November 2014) has recently published a study on OCT tresholds reflecting past unilateral ON (57). The same study also pointed out clinically relevant OCT findings correlated to contrast vision changes in eyes with preserved high contrast visual acuity.

1.3 Symptoms in early MS

MS is a multifactorial illness and has several phenotypic expressions as reflected in the complex and variable symptomatology containing a multitude of sensory, motor, cognitive and ANS troubles even present at diagnosis.

1.3.1 Cognitive impairment

Cognitive impairment (CI) occurs early in MS (77), and may be present before there is a definite MS diagnosis as in clinically isolated syndrome (CIS), studies have uncovered signs of cognitive affection years before the first clinical event (78). The most prevalent reported difficulties are decreased information processing speed, attention, episodic verbal and visuo-spatial memory deficits.

Our understanding of cognitive impairment derive from the diagnostic detection methods, and several studies in the field does not clearly define the cognitive concept in question (79). Adding to the complexity, the pathophysiologic substrates causing these deficits are not equivalent to clearly defined anatomical structures since cognition relies on cerebral networks (Figure 11) (80). Studies have shown that different networks work together as a unity with visual networks as a central part in understanding cognitive impairment in MS (81).

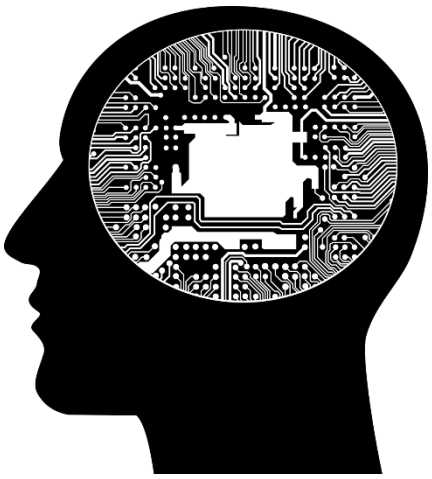


Figure 11. Cerebral networks. Image from Pixabay

1.3.2 Fatigue

MS patients often describe a variable, but overwhelming sense of "energy lack" without apparent connection to sadness, muscle tiredness or lack of sleep. This description fits into either one of two fatigue categories, mental or physical fatigue. Fatigue is highly disabling (82), and patients suffer from this throughout the course of the disease. In order to define fatigue in a medical framework it must endorse both a recognizable description of the symptom from a patient's perspective and provide a meaningful medical framework as Mills et al did in 2008 (83). To assess fatigue from a patient's perspective the administration of the Fatigue Severity Scale (FSS) (84), is a validated and often used instrument. This nine item-self report scale is easy to fill in and is well-established for rating fatigue in medical and neurological disorders. It is translated to, and validated in Norwegian (85). Another instrument, the Fatigue Scale for Motor and Cognitive

Function (FSMC) graduates and quantifies motor and cognitive fatigue and aims to reliably uncover the core symptoms of fatigue based upon item validation and administration to patients across all education levels with or without cognitive impairment (86). Contrarily, it is difficult to measure fatigue objectively (87), as we don't know the cause of this symptom. Studies point towards an association between gray matter pathology and fatigue (88), as measured with MRI. Inflammation is a hypothetic pathogenic link to fatigue as in viral and bacterial infections, with cytokines as fatigue mediators. There are some association between markers of systemic inflammation and fatigue (89-91). MS-related fatigue tend to worsen in conjunction with MS relapses and in some cases, fatigue is the only marker of a relapse (92). Fatigue is possibly the subjective sickness behavior of inflammation (93), mediated by cytokines in brain areas such as the hypothalamus and the insula. As with cognitive impairment, there may be a complex and multifaceted pathological explanation to this symptom. (94)

1.3.3 Autonomic dysfunction

ANS symptoms are frequent in MS, and up to fifty percent complain about postural dizziness (95). Alterations of cardiovascular orthostatic reflexes cause the dizziness when the affected individual changes posture from either supine or sitting position, to standing. Other common symptoms of orthostatic intolerance are lightheadedness, blurry vision, heart palpitations and near syncope caused by cerebral hypoperfusion. Orthostatic intolerance (96), encompasses several terms such as orthostatic hypo-or hypertension and postural orthostatic tachycardia syndrome (POTS) (97), and affects the patient either by a sustained change in blood pressure or in heart beat frequency for more than 3 minutes upon posture change. A recent study shows that even transient orthostatic loss of blood pressure may have long-term health implications (98), such as syncope or fall with alarming consequences like fractures and even death. Orthostatic intolerance is recurrent in MS (99), and there is a relationship between distinct orthostatic features (100), and MS such as POTS (101). Physical activity and systematic

training of sustained upright positioning is important for patients suffering from POTS (102), but the condition manifests especially under physical activity leading to a vicious circle. Females are more often affected than males. The menstrual cycle may worsen the symptoms at specific time points in accordance with bodily fluid shifts caused by hormonal changes (103). Cyclic changes may even occur at rest due to blood pressure and heart rate changes. There are several possible explanatory POTS mechanisms such as autonomic neuropathy, excessive sympathetic activity, volume dysregulation, impaired cerebral autoregulation and hypervigilance. These are not mutually exclusive. Comorbidities like insomnia, fatigue and visceral abdominal symptoms with dysmotility and pain, are not negligible.

Chapter 2 Aims

Pathology is 'pattern recognition in vivo', defining diseases as recognisable changes compared to the normal population. How to measure functional and structural changes is constantly evolving and the phenotypic disease descriptions do accordingly. This is particularly challenging in MS with a multitude of disease expressions.

This Thesis aim to explore early MS symptoms' relationship with clinically measurable signs in the anterior visual pathways to pin point signs of neurodegeneration and thereby contribute to the prevention of functional decline.

- Cognitive changes and motor disturbances are frequent early symptoms in MS. Several cognitive test batteries used in MS include the Symbol Digits Modalities Test (SDMT). Fine motor disturbances may be a confounder of the SDMT. We conducted a case-control study between relapsing remitting MS (RRMS) patients and healthy controls to explore whether the written version of the SDMT could be confounded by hand and eye motor function (Paper I).

- Theoretically, there is a link between fatigue and cognition in MS due to possible alterations in central neuropeptides and possibly the ANS. We examined the possibility to measure early functional brain changes by employing a test of increasing cognitive load while measuring the participants' pupil sizes with pupillometry together with self-reports of fatigue level (Paper II).

- ANS dysfunction is troublesome and related to neurodegeneration in MS, but difficult to test for at an early stage of the disease. We aimed at exploring this with a bedside test battery together with PLR-measurements and OCT (Paper III).

Chapter 3 Material and methods

This chapter is a comment on the materials and methods used to test our research hypothesis, i.e. the suitability of our study population, the relevance and preciseness of the test measures and the choice of statistical methods. For a complete description of the methods, the study outcomes and the tests performers, please refer to the respective papers.

Ill 1.Image by Peggy and Marco Lachmann-Anke at Pixabay

3.1 Ethics

We conducted our studies according to the Helsinki declaration, and the Universal Declaration of Human Rights underling the equality of MS patients and healthy controls as study participants and their right to withdraw from the study at any moment without the need of any explanation. All participants gave written informed consent. The regional ethics committee (REK) of South Eastern Norway approved the study protocols (REK 2011/1846 A) as well as the Oslo University Hospital.

3.2 Study population

3.2.1 Sample recruitment

The Oslo region is the most densely populated one in Norway, and all MS patients in the region are treated at Oslo University Hospital. To avoid selection bias (104), we aimed at inviting all newly diagnosed MS patients at Oslo University Hospital, Ullevål, during the inclusion period fulfilling the study criterions (Figure 12).

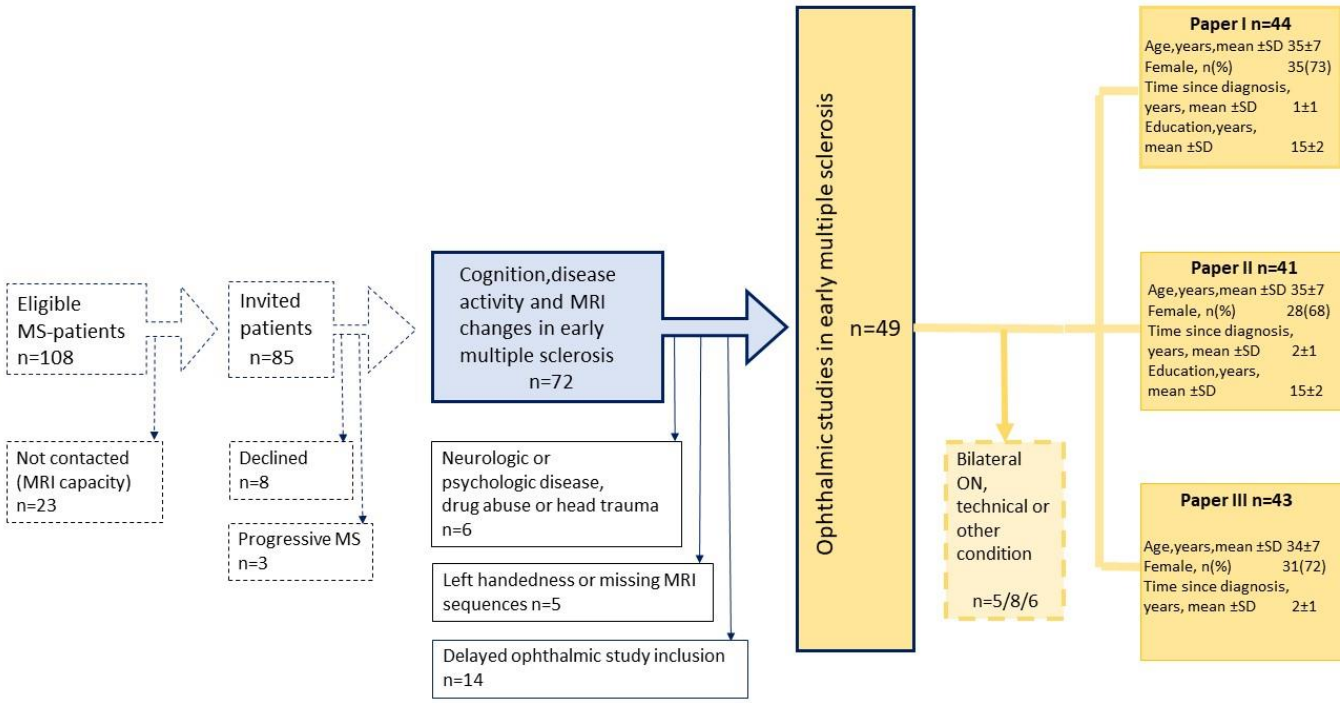


Figure 12. Patient inclusion

Our sample size was primarily limited by the availability of participants from an ongoing study of cognition and MRI changes and by test capacity. Out of 108 eligible patients, 23 were not invited, due to limited MRI capacity. This is however a possible non intended selection bias in our sample. Only 8 declined our invitation, we therefore assume the both non-response and selection bias to be ignorable. The present neuro-ophthalmic study population of 49 was an overlap between primary inclusion and follow-up of the main study due to later study start. Exclusion criteria as stated below, proper to this sub-study, gave the final participant number of 49.

The exclusion criteria in the main study was uncertain diagnosis, non-fluency in Norwegian, neurological or psychiatric disease, age under 18 years or older than 50 years, drug abuse, previous head trauma, ongoing pregnancy, previous adverse gadolinium reaction and patients not responding upon telephone contact or moved to an address outside Oslo. Added to this, the neuro-ophthalmic sub-

study excluded those who had prior known ophthalmologic disease, a refractive error with a spherical equivalent of more than ±6 diopters or known MRI-lesions in the visual pathways.

These measures regarding inclusion and exclusion assured a homogenous study population reducing possible bias in our effect analyses, by minimizing confounding factors (105).

Based on the above assumptions, we believe our results are at least generalizable to the newly diagnosed MS-population in affluent, highly educated, societies (106).

In Paper II and III, the sub group numbers were small and our results warrant replication in larger cohorts, but represent nonetheless interesting pilots in the field of cognition, fatigue and dysautonomy in newly diagnosed MS patients.

3.2.2 Recruitment of healthy controls

Matched healthy controls based on sex, age and educational level, were recruited in the hospital and university environment (Figure 13). They were invited by direct e-mail enquiry. The same exclusion criteria as described above were applied.

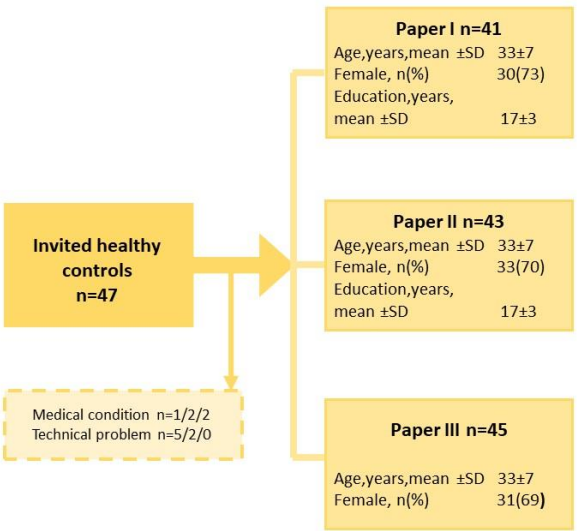


Figure 13. Healthy controls.

Our choice of recruitment method might have contributed to a selection bias with participants of better general health and higher educational level compared to our patient cohort. However the strict exclusion criteria and the comparable number of participants with low cognitive score (Paper II) in both groups, support our assumption that the groups are comparable for our study purposes.

3.3 Study design, interventions and assessments.

The papers included in this thesis use a cross sectional design (107), with all the participants examined at one time point. Our studies were observational, per se without interventions, containing only recording of a given set of findings. However, with more time available for each participant than we usually have in a clinical out-patient setting, our study environment could represent a non-intendent psychological intervention.

3.3.1 Clinical neurological and ophthalmological assessments

The MS-patients took part in thorough neurologic and ophthalmologic examinations. Clinical exams are prone to non-intended interpretation bias, also known as ascertainment bias, on behalf of the examiner. In our studies, this was kept at a minimum by employing few examiners. However, on a group level, a systematic non-intended bias may have created a skewed group profile for our participants affecting the impact of our research when compared to other studies.

Depending on accessible equipment, presumed identical tests may be slightly altered, when performed in different clinical departments. As highlighted in our studies where both the neurologist and the ophthalmologist performed a high contrast visual acuity test on the same day for each MS patient. Some of our participants had not had their best corrected visual acuity tested before entering the study and had their neurologic assessment before the ophthalmologic one. We observed a difference in visual acuity when first tested without spectacles/or

old ones, by the neurologist, and then at the ophthalmologic department with new subjective refraction performed by a trained research optometrist. This underlines the necessity of a multidisciplinary approach to ascertain correct acquisition of clinical parameters.

3.3.2 Neuropsychological testing

In neuropsychological MS test batteries, the symbol digits modality test (SDMT) has emerged as one of the preferred screening instruments. The test performance is likely altered by slowed motor function in people with MS. The oral SDMT is the most commonly used version of the test in MS, as hand and general motor function is noticeably impaired in this group. Oral motor impairment has however been shown to affect the SDMT test results. Our study on saccadic eye movements and the SDMT used the written version of the test, as it is easily manageable in the clinic, where the test may be performed in an e-format before the doctors' appointment. In order to make a qualified choice between the oral and the written version of the SDMT, a study employing both in the same patient population compared to healthy controls would have been a good way to point out the impact of hand and oral motor function on the test.

In general, an additional strength to our studies would be to perform all the tests in both patients and healthy controls.

The non-blinded neuropsychological testing in our studies, could have contributed to an ascertainment bias, but our tests were easily quantifiable giving no possible freedom of interpretation depending on participant group.

3.3.3 Patient reported out-come measurements (PROMs)

PROMs are assessments of self-perceived health status involving the patient actively in the research process to coproduce data. PROMs are subjective measures and patients might over rate their symptomatic burden (108). Patients may also be prone to recall-bias when we are asking about the duration of symptoms. The last question in our ANS form regarding orthostatism, may be

perceived as socially distressful because we are asking about sexual difficulties and prone to produce response bias.

To correct for non-MS related fatigue and occurrence of ANS symptoms in the general population, our healthy controls should also have been asked to take part in these questionnaires.

Considering fatigue in Paper II, FSS is a general measure of fatigue, which does not distinguish its cognitive and physical component. Sleepiness/alertness differences cannot be fully excluded as a confounding factor because the subjects had not been interviewed on sleep problems.

There are few tests and publications that cover ANS symptoms in early MS, and future patient involvement in designing meaningful ANS PROs would be of great value. Future studies would benefit from implementation of health-related quality of life scales to see whether ANS symptoms affect the patients' life in general. As for the choices made in the setting of our studies, the participants faced a demanding test platform and we had to make strict choices as to how many tests each participant was to complete. The percentage of non-responders was at the 1% level for the PROMs.

3.3.4 Retinal imaging with Optical coherence tomography (OCT)

From the methods section in Paper III "Optohistologic slicing of the retina was carried out with the spectral domain RS-3000 OCT Retina Scan (Nidek Inc., CA, USA) with a scanning speed of 53000 A-scans/sec and 4μm digital resolution. The examinations were performed by the first author (SADRB). Peripapillary retinal nerve fiber layer thickness (pRNFL) data were obtained with the Disc Circle protocol with a scan width of 3.45 mm centered on the optic nerve head without crossing of the two inner scan circles. The ganglion cell layer (GCL+IPL) thickness measurements were automatically generated from the 9x9mm macula map scan glaucoma segmentation in the included software. All scans included had a signal strength of 8/10 or better." This is in line with the OSCAR-IB criteria (109, 110), with minor adjustments according to the Nidek

settings and software. The actual reference population in the provided software was incomplete at the time of the study and did not fully match our patients'cohort. We preferred using a matched healthy control cohort for comparison. There are several different OCT manufacturers and measurements are not directly comparable between different machines. Studies have focused on both inter-rater reliability and open-source semi-automatic segmentation of multi-center data to be able to perform large studies across different centers (111, 112). Ideally, it will be possible to make use of artificial intelligence algorithms to analyze large data sets efficiently, based on OCT measurements from different devices facilitating collaboration and use of bigger data.

3.3.5 Visual evoked potentials (VEP)

Our studies employed a classic VEP set-up according to the standards from the International Society for Clinical Electrophysiology of Vision (ISCEV) (113). This ensured that the NONMS eyes did not have suffered a subclinical ON. If we were to study different parts of the retina and their axonal out-put through the optic nerve, a multifocal VEP set-up would have been appropriate (114, 115).

There are currently devices under development to test for visual pathway anomalies as a screening in multiple sclerosis, showing promising results (116).

3.3.6 Eyetracking and pupillometry

Eye trackers follow the test subject's eye movements in terms of gaze shifts, such as saccades, and register fixations' durations and their locations. They are valuable tools with several applications such as in usability interface evaluation studies (117). Modern infrared eye trackers are possible to use with or without a chin-rest. We did not use a chin-rest in order to provide an environment as close to the SDMT-test situation as possible.

Saccade parameter tests are proposed as possible motor tests of fatigue (59), and underline our study hypothesis of motor deficits as confounding factors of cognitive tests. In MS, saccadic changes occur both in fatigued patients (59), and in the case of eye motor disturbances like INO (118). Cognitive test paradigms

demanding repeated saccades for test completion are in these cases prone to be biased. An additional test for our protocol could be a test of saccade latency without the need of initial deciphering of arrow direction, to test the saccadic motor reflex.

The eye pupils' own motor frequency is less than 9Hz so a sampling rate at 60Hz is adequate when studying pupillary oscillations. As we kept the luminance constant and the tasks were low-paced, we did not have to apply additional time-domain analysis to correct for rapid pupillary changes (119).

Repeated measures of up to 10-15 or 20 trials have been the standard in pupillometric studies of cognitive load. In paper II we refer to the work of Beatty, J. "Task-evoked pupillary responses, processing load, and the structure of processing resources" published in 1982 (Psychol Bull 91(2): 276-292), as well as the work of Kahneman, D. and J. Beatty "Pupil diameter and load on memory" published in Science in 1966 (154(3756): 1583-1585). These experiments contained relatively few repetitions and were performed without remote eye-trackers. To test if todays' eye-tracker systems are comparable to these standard experiments, Klinger and colleges replicated the old studies in a remote eye tracker system and presented it in 2008 at the symposium on Eye tracking research and applications in Savannah, Georgia with the title "Measuring the task-evoked pupillary response with a remote eye tracker". Comparing the relatively high frequency noise from the eye tracker with the low frequency noise of the pupil itself, they underline the fact that the pupil is a robust system with highly reliably obtainable measurements also with a remote eye tracker. Further underlining is found in the consensus article after the 32nd International Pupil Colloquium 2017 in Morges, Switzerland (22).

Presentation of all the stimuli were in the same order for all participants in paper II. Counterbalancing easy and hard problems could have let us further explore the analyses of fatigue. One could argue that what we actually measured was the fact that these participants became tired. Still, the difference in pupillary responses that we observed in some of the fatigued patients were evident even in

the very first, easier, tasks of the experiment. Thus, we do not believe we measured exhaustion during the experiment, but rather the patients' diminished ability to recruit cognitive resources (120).

3.3.7 Pupillary light reflex testing (PLR)

We collected our data in 2012-2013, while international pupillographic standards were published in 2019 (22). However, when looking into these criteria, post testing, reporting of our recordings are in line with the Minimum Information about a Neuroscience Investigation (MINI) adapted for pupillography. We also provided background demographic data on our study population as requested by these criteria.

The consensus paper states that twenty minutes of dark adaptation is advisable and the first stimulus response in each series should be discarded. We encourage incorporation of these two criteria in future replication studies. In our methods, the pre-test dark adaptation was five minutes for all the participants. Since we used a high intensity stimulus this was an adequate dark adaptation time considering the fact that with such a stimulus the pupillographic curves reach the plateau level much faster than with lower intensities, as used when studying rods, cones and intrinsically photosensitive ganglion cells separately (121).

Myriad factors influence pupil size and reactivity, and sleep disorders are prevalent in MS. To ascertain that participants did not suffer from sleep loss, our test battery could benefit from an additional pupillary study of sleepiness (122), in conjunction with the PLR testing.

3.3.8 Bedside orthostatic testing

The ANS test battery consisted of PROMs as well as bedside testing of BP and pulse in supine and upright position. The bedside tests were designed to discover orthostatism and POTS related to alterations in ANS regulation of cardiovascular function. We identified no orthostatism in Paper III, but postural tachycardia and POTS. Orthostatic bedside testing is not a fully sensitive way to apprehend early cardiovascular autonomic changes. The plasticity of the ANS often compensates

for early changes, with an upregulation of post-synaptic receptors leveling out the change. One of our goals was to employ an easy access and robust test battery, relevant for a clinical setting. A complete test battery regarding early cardiovascular change should also contain a valsalva maneuver test and continuous BP measurements (123), as mentioned in Paper III. In neuro-physiology, cardiovascular ANS dysfunction is tested with a head up tilt test combined with continuous heart rate and BP measurements and encompasses the entire autonomic regulation of the cardiovascular system. This test in combination with the abovementioned tests in addition to sudomotor reflex testing as well as thermoregulatory sweat tests, will distinguish where in the ANS the problem is located (124), if it is to be studied more extensively than with our bed-side battery.

I

3.4 Statistical methods

Our studies included all available participants during 2012 and 2013 as described above, and we performed no formal sample size calculations.

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We did however compare our sample size in Paper II with the sample in the original article of Hess and Polt from 1964. That experiment was based on four

tasks and had only five participants. Applying a calculated Cohen's d of -1.854, and a probability level of 0.05 from Hess and Polt's study, and our sample size of 41, we obtain an observed power of 0.999 in our study. This confirms that our sample size is reliable and prepare the ground for interpretation on group level.

The software packages from SPSS, versions 22 and 24, were used for all the statistical analyses. SPSS is a user-friendly software, originally designed for social sciences, and has a gradually learning curve. Limitations are for instance graphics manipulation, which would have been far better in a program like R (https://guides.nyu.edu/quant/statsoft) which is a free software, based on the S programming language. Rstudio has large applications, and is also used by commercial companies. R is a good choice for big data and machine learning like analysing medical imaging. So in future OCT multi-center studies this could be a valuable choice with advantaged compared to the SPSS package.

In Paper I-III, we applied independent samples t-test for continuous data to assess differences between groups. Our data were normally distributed, as this parametric test requires, and did not require logarithmic transformation. For categorical data the x^2-test was applied.

When analysing the PROMs we categorized the data based on previously described clinically relevant cut-offs. In general, categorizing continuous data may lower the statistical test power, but it is a good choice in order to label participants according to diagnostic criteria.

We applied Bonferroni correction for multiple correlations to adjust for Type 1 errors, in order not to reject the null-hypothesis based on a high number of false positives. On the other hand, this test is prone to Type 2 errors, not rejecting the null hypothesis although it should have been. We did not find any difference in pupil dilations between the groups in Paper II, there is however a change in the time spent on solving the mental calculations at task "9x15", indicating a difference between the groups, but this did not survive the Bonferroni correction. In case of Type 2 errors, further studies with a slightly different

design, balancing easy and difficult calculations, could give an answer to the question of what is the maximum possible cognitive mobilization in the two groups.

In Paper II, repeated measures on the same study object led us to use repeated measures analyses of variance (ANOVA) because of paired variables and not independent of each other. For each tested variable, there is a need of at least 10 participants (125). This is why our results concerning low cognitive score (LCS) should be replicated in a larger cohort since there were only 9 MS patients with LCS.

Linear regression assessed association between variables. This does not tell us anything about cause and effect, but shows time and space synchronicity in variables. Pearson correlation tested the extent of synchronicity. Figure 7 in Paper II and Figure 3 in Paper III captures in some sense the complexity when trying to model the difference between on one hand, cause and effect, and linearity on the other. The scatterplot in Figure 2 in Paper III underlines the spread in the data and a longitudinal follow-up would of course provide more information on the time-variation of disease duration in relation to retinal neurodegeneratioan.

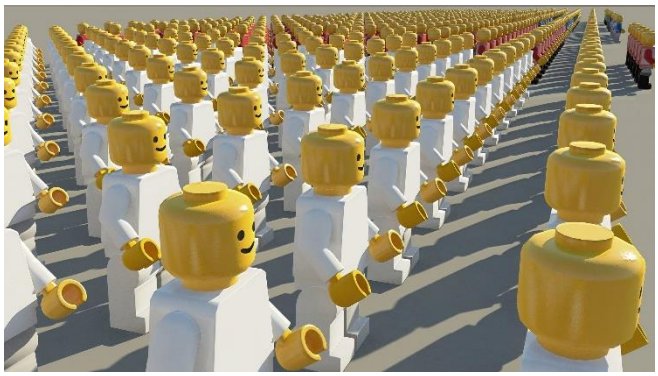
In a previous published article based on the same patient sample (63), we found no association between the use of disease-modifying therapies and levels of fatigue. Most of the MS patients used disease-modifying therapies. However, we did not analyze the correlation between fatigue and treatments in Paper II.

We reported all descriptive data as mean with standard deviation (SD). SD describes data variation not to be confounded with confidence interval (CI) describing the variation in the measured effect size with 95% reassurance. The CIs in our studies would have been narrower with a larger study cohort.

In our studies, we collected as much data as attainable on known possible confounders, and corrected for those in our analyses. We may have ignored factors influencing our data, and there are formulas handling such unknown

selection and confounding biases (126), we have however not applied such formulas in our studies. When conducting for instance epidemiologic studies, this is warranted.

To conclude our study designs deserve replication in lager cohorts.



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Chapter 4 Summary of results

This thesis focuses on neuro-ophthalmic signs of neurodegeneration in early MS while exploring symptoms of cognitive difficulties, fatigue and dysautonomia.

4.1 Paper I

Eye and hand motor interactions with the Symbol Digit Modalities Test in early multiple sclerosis

The existing research literature highlights the relationship between cognitive changes and neurodegeneration in MS. The Symbol Digit Modalities Test (SDMT) is widely used in cognitive MS assessment, and it is important to identify possible test confounders. Because of possible hand motor interactions, the oral version of the tests is recommended in MS (127, 128). On the other hand, oral motor slowing is also a known problem in MS so the oral test version is not ideal either. Saccadic initiation time (SI time) is reported to increase in MS-patients. To complete the SDMT the test person must be able to perform appropriate saccades and the time to perform a saccade affects the test result. SI time in relation to the SDMT in MS, is not tested before.

We compared patients with RRMS (N=44) with matched healthy controls (N=41) and found that SI time was longer in RRMS than controls ($p < 0.05$). Our study underlines difficulties and the test complexity when exploring early cognitive changes in MS. Eye, hand and oral motor changes must be considered antecedent to test conclusion.

4.2 Paper II

Fatigue and cognition: Pupillary responses to problem-solving in early multiple sclerosis patients

In line with the need of test improvement, we scouted for new paradigms. Pupillometry is an unexplored way to study cognitive load in relation to fatigue in

early MS. This test paradigm also explores possible CNS network disconnection hypotheses, encompassing regulatory neuropeptides, specifically noradrenergic activation via a brainstem nucleus called the locus coeruleus, an activation mirrored in the eye pupil.

The study design included assessment of neurologic impairment, MRI, VEP, depression and fatigue in MS patients (N=41). Processing speed, functional eye examinations and retinal imaging with OCT was comparatively tested in matched healthy controls (N=43) as well as pupillary eye-tracker responses during playback of multiplication tasks of increasing difficulty.

The experimental test results showed the same performance level in arithmetic calculations and overlapping curves of pupillary dilations during problem solving in both groups with all the tasks collapsed together, a mean of 0.55mm dilation in patients and 0.54mm dilation in controls.

A subgroup of patients (n=9) and controls (n=6) were classified with low cognitive score (LCS) and these participants presented significantly larger pupillary responses than then the rest of the participants. Comparing the LCS patients and controls, the LCS patients had significantly smaller pupillary responses than the LCS controls.

Dividing the patients into subgroups with (n=19) or without fatigue (n=21) we found no significant difference in pupillary dilations between the groups, but interpreted this as a tendency underlined by the different curve profile of the fatigued patients in comparison to the healthy controls.

We found no association between depression scores and pupillary responses.

Our study results on the eye pupils' dilation suggest there is a link between cognition and fatigue. The study raises the question whether the measured changes may be due to ANS changes.

4.3 Paper III

Neurodegenerative interplay of cardiovascular autonomic dysregulation and the retina in early multiple sclerosis

To explore the presence of ANS symptoms in a newly diagnosed MS-population and its' relation to neurodegeneration we therefore conducted this third study containing classical pupillometry measuring PLR and OCT evaluation of retinal neurodegeneration in MS patients (N=43) compared to healthy controls (N=45). A subgroup of MS patients (n=37) took part in further examinations with bedside orthostatic BP and heart rate measurements as well as PROMs.

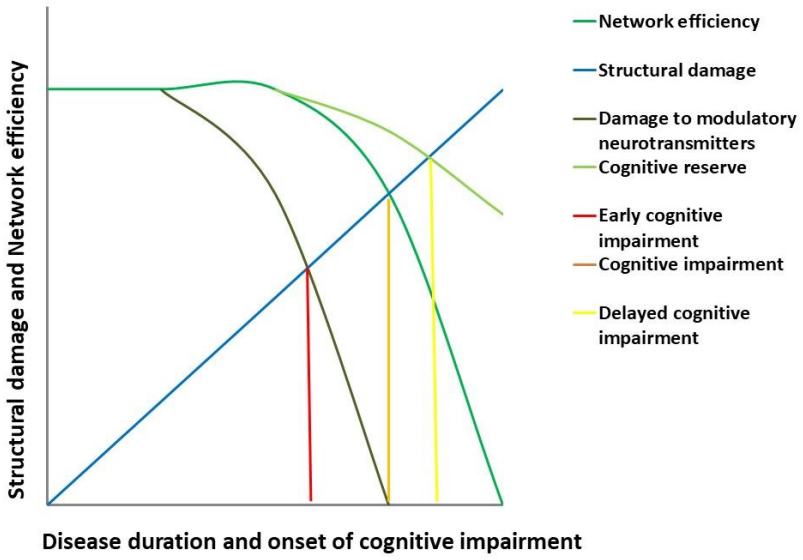
The MS patients presented with a significant burden of symptoms linked to the ANS, they did have premature asymptomatic thinning of the neuro-retina measured with OCT compared to the controls. We found no orthostatic hypo-or hypertension. Twenty-three percent of the patients had an abnormal pulse response during the orthostatic bedside testing and 3 patients (10%) fulfilled the POTS criteria. We found no difference in PLR measurements between patients and controls.

Future longitudinal studies are encouraged to explore if the present symptoms are to be considered as preclinical findings in relation to ongoing neuronal loss, and on the other hand if POTS is a transient symptom of neuro-inflammatory stress. Our study highlights the finding of a retinal functional reserve.

Chapter 5 Discussion

MS inflict hardship on everyday life. Disease duration, fatigue, motor and cognitive symptoms are factors stopping MS patients from keeping up with a professional career according to a study in Norway (129). In a long-term perspective, neurodegeneration is the reason MS patients experience considerable functional decline (4). Time-lime occurrence of structure and functional changes is essential to implement treatment algorithms.

Our research revealed both functional and neurodegenerative changes with prolonged saccadic initiation (SI)-time as shown in paper I, and thinner meanGCIPL layer as shown in paper III, as well as pronounced ANS burden of symptoms and a considerable amount of self-reported fatigue in a newly diagnosed MS cohort. Further, our studies support the cognitive reserve hypothesis where educational level and work status protect against cognitive difficulties as shown in Figure 7, Paper II.

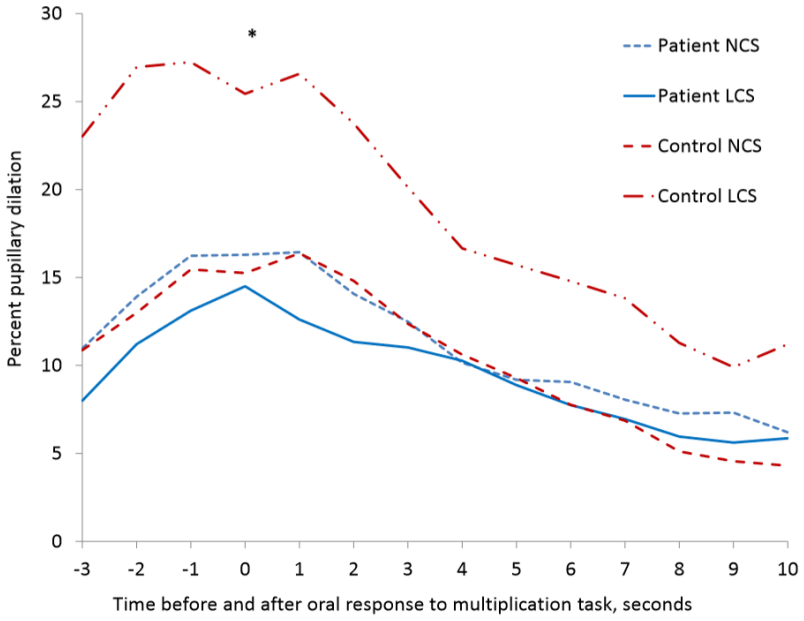


From paper II: Figure 7

A recent review article (77), points out that there is a difference in methodology between studies with high and low frequency of cognitive impairment. We present a newly diagnosed, cognitively well-preserved MS cohort. We had to recruit a local matched control group since the MS patients' test results

outperformed the established test norms adjusted for age and educational level. This underlines the patients well preserved cognitive capacities, a lack of CI so to speak, and not, insufficient test-sensitivity. Cognitive reserve may be supported (130), and our studies underline its' importance. A recent meta-analysis further strengthen this point (131).

The new application of eyetracking in conjunction with traditional infrared pupillometry underlines on one hand the *robustness* of the pupil. A set of symptoms without clinical findings of ANS dysfunction does not translate into altered PLR metrics. This robustness underlines the difference between ANS symptoms and ANS dysfunction. On the other hand our study display the pupil's *sensitivity* in LCS MS patients who present altered dilation curves upon testing of cognitive load (Figure 5 in Paper II) as a proxy of function of central neuropeptide levels.



From Paper II, Figure 5.

5.2 Efferent subclinical eye motor dysfunction

Traditionally, the eye motor function in MS has been tested bedside and the patients referred for supplementary eye examinations if they present with clinical findings or with subtle motor deficits and subjective symptoms like double

vision. Since the late seventies, there has been an increased interest in subclinical eye motor changes in order to establish a definite MS diagnosis (132-135). Structure and function studies have tried to demonstrate a correlation, but when it comes to saccades and subclinical findings in MS this is challenging (136). Since the new millennium, the focus on cognition and MS has brought a change in the research on subclinical saccadic alterations in MS. Because of the long-range nerve connections involved in saccades and the network theories behind eye motor control and cognition, focus is on the oculomotor system as a model system (137-141), to inspect intricate cognitive functions such as attention, working memory and decision-making. These studies do however not report on any eye related mesures nor on ON status. It is therefore difficult to draw any conclusions. The studies by Fielding in 2009 makes use of a black screen in a darkened room with red and green saccadic fixations and saccadic cues. MS patients may present with past ON with colour vision deficiencies, or a significant loss of foveal ganglion cells affecting their ability to adapt to the mesopic test condition. A test paradigm with black backdrop and coloured cues then represents not only a saccadic test, but rather a combined saccadic and cone adaptation test (http://www.leatest.fi/index.html?start=en/vistests/instruct/conetest/conetest).

Ill 4. Image by Stefan Keller at Pixabay

The idea of using the oculomotor system as a model system for attention is however intriguing, and a seminal study on saccades show that allocation of attention (142), does improve saccade parameters such as delay. This improvement of saccadic delay depends on a remapping process predicting the future eye movement. It is then timely to ask if this allocation again depends on a precise ability to predict this relocation, and if so could a change in oculomotor capacities affect this stability even with preserved cognitive efficiency?

To synthesize, subclinical eye motor deficits in MS pose challenges when conducting cognitive testing and our study underlines these challenges disclosing both eye and hand motor slowing even without cognitive changes in early MS.

A recent meta-analysis on motor rehabilitation in MS (143), establish that MS patients, despite the high frequency of cognitive impairment, are capable of learning new motor skills. Eye motor training may also improve saccadic latencies and peak velocities (144), making the detection of deficits important to discern at an early and subclinical stage to be able to install training regiments before deficits become symptomatic. This fact once again, underline the multilevel reserve capacities of the CNS and the physical training benefits in MS.

5.3 Afferent neurodegenerative visual pathway changes

OCT studies have revealed asymptomatic retinal thinning across different diseases like Alzheimer's, obesity and epilepsy (145-147). These changes translate into a neurodegenerative state in the CNS, and the eye serves as a sensitive and presymptomatic biomarker. In the case of Alzheimer's disease, early retinal changes correlates with the risk, in patients with mild cognitive impairment, of developing definite Alzheimer's. These patients do not have any visual problems.

In ophthalmology, OCT is valuable in the follow-up of patients with diabetes mellitus, age-related macular degeneration and glaucoma (148-150). It enables the clinician to measure structural changes both at a preclinical stage and in the setting where visual functional correlates lead to specific treatment choices.

In MS, the study of retinal OCT changes represent several viewpoints encompassing the status as potential biomarker of disease activity, ON sequela measuring tool predicting visual disability and also as a useful imaging measure to facilitate differential diagnostics between different antibody mediated demyelinating ONs and MS (151). There has recently been a joint effort to define clinically significant inter-eye OCT changes (57), to enable the use of OCT measures to differentiate between MSON eyes and NONMS eyes in the diagnostics of MS. This is important at an individual level, and for future recognition of the optic nerve as a lesion site in the diagnostic criteria. The same study established minimally clinical relevant OCT differences proposed implemented in future treatment studies in MS. It points out an important statement underlining that the ganglion cell measurements are more stable than the peripapillary retinal nerve fiber layer measurements, since the latter are more prone to fluctuations due to for instance edema.

The results in Paper III showed robust OCT intereye differences, and the difference between ON and MSNON is further underlined with a difference in latency to p100 on VEP. MSNON eyes had significantly thinner mean GCIPL compared to healthy controls, as shown in earlier studies in MS (152, 153). The novelty is however that our patients had a short disease duration and a low disability score and even at this stage the retinal thinning correlated with disease duration. This raises the question whether OCT may serve as prognostic marker in CIS pointing out who is at risk of developing MS.

A recent study on CIS (154), show that OCT and MRI together points out subclinical optic nerve lesions without other signs of optic pathway involvement supporting the inclusion of the optic nerve as a site of second involvement in MS diagnostics. This study underlines the OCT as tool to study the optic nerve involvement in CIS and not at as an early marker of CNS degeneration because of the high frequency of silent optic nerve lesions on MRI.

5.4 Higher order cerebral functions reflected in the pupil

MS affects information processing and contributes to working memory alterations. The underlying deficit is probably the speed with which new information is allocated and stored. In the general population, performance on cognitive tests concerning working memory, correlate negatively with age. Salthouse's theory (155), on altered processing speed explains this aging phenomenon, stating that two mechanisms operate in parallel and are responsible for the relationship between speed and cognition. The first is the limited time mechanism where the loss of speed leads to incompletion of cognitive tasks due to lack of time. The second states that the brain normally is capable of simultaneously processing several bits of information in order to obtain a complete scenery. With loss of speed, this scenery is not completed and there is less information available for higher order processing of a given problem. This framework fits for MS as well and when given more time to resolve given tasks the task accuracy improves (156). Increased cognitive load strains the processing speed, like a heavy burden on your back would do if you were out running. This duality was one of our objectives in Paper II.

We discovered no PLR abnormalities in Paper III and no brainstem lesion association with our pupillometric findings in Paper II. This facilitates the interpretation of the pupil as a proxy of function of higher order functions in our cohort.

The lack of PLR abnormalities underlines the difference between ANS symptomatology and ANS dysfunction, strengthening the hypothesis of stress related ANS symtomatology rather than loss of ANS function, with tachycarida translated into POTS in ten percent of the patients.

ANS symptomatology may have other explanations than ANS dysfunction or stress. Altered neural signalling is a known entity in MS and MS patients present with altered bodily signal sensing such as temperature, fatigue and chronic pain.

Orthostatic and ANS complaints could be due to changes in interoception (157). In our study, a possible explanation could be affection of autonomic-vagal interoceptive CNS pathways while the somatosensory pathways remain intact.

Chapter 6 Conclusions and future perspectives

It is important to establish sensitive biomarkers of neurodegeneration for experimental clinical trials (158), to develop medication to stop not only inflammation, but also to prevent loss of neuronal tissue. There are evidences of a separate ongoing neurodegenerative disease process in MS. As this thesis attests, it occurs even in early MS. Pinpointing neuronal loss at an early stage of the disease may prevent end-stage atrophy. Identifying such markers will bring the treatment algorithms closer to the goal of personalized medicine and no evidence of disease activity (NEDA) (159). For this to take place, study measures in large and well-defined research cohorts need translation into clinically relevant measures at an individual level.

Phenotypic time-line occurrence will provide a key to the MS pathology and ultimately the causes of this disease. This thesis contribute to the understanding of symptoms in early MS, providing new ways of measuring them utilizing a neuro-ophthalmologic and neuropsychological toolbox. Structure and function studies are important in MS and ON is put forward as a model system for remyelination research (160). Visual function and structural changes are accessible for direct examination and anatomical evaluation in contrast to other brain areas. This is a hot topic in MS research as a context for our studies showing the complex relationship between eye, brain and cognitive reserve.

Longitudinal studies are important to seize different aspects of disease development over time and to stratify patient risk profiles. Supported by a large review study on processing speed in MS (79), future studies in cognition should test for and report on visual functioning, covering both visual acuity and saccadic eye movements. A recent fMRI study supports the prominence of the eye and the visual networks as a key part in understanding cognitive challenges in MS (81). Fatigue, autonomic dysfunction and cognitive impairment are highly disabling symptoms in early MS. These features are often invisible to family,

friends and colleagues, and disproportional to motor and MRI findings as evaluated with traditional scoring systems as the EDSS.

A longitudinal neuro-ophthalmic study, with tests such as; circulatory retinal oxymetry (161); conjunctival circulatory tests (162); OCT and OCT angiography (163); pupillomtric tests of the PLR, fatigue, chronobiology (22), and cognitive load; autonomic testing supplied with peripheral circulatory tests; MRIs with restriction spectrum imaging (RSI) (164) and estimation of brain age (165), could provide further answers. A 10 years follow-up of this cohort could provide interesting long-term findings.

Identification of signs at an early stage with reliable tests, treatment of inflammation and neurodegeneration, and last but not least fortification of the natural reserves, are all key factors to improve our understanding of disease mechanisms.

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Errata

Articles I-III

I

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Eye and hand motor interactions with the Symbol Digit Modalities Test in early multiple sclerosis

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ABSTRACT

Purpose: Eye and hand motor dysfunction may be present early in the disease course of relapsing-remitting multiple sclerosis (RRMS), and can affect the results on visual and written cognitive tests. We aimed to test for differences in saccadic initiation time (SI time) between RRMS patients and healthy controls, and whether SI time and hand motor speed interacted with the written version of the Symbol Digit Modalities Test (wSDMT).

Methods: Patients with RRMS ($N=44$, age 35.1 ± 7.3 years), time since diagnosis <3 years and matched controls ($N=41$, age 33.2 ± 6.8 years) were examined with ophthalmological, neurological and neuropsychological tests, as well as structural MRI (white matter lesion load (WMLL) and brainstem lesions), visual evoked potentials (VEP) and eye-tracker examinations of saccades.

Results: SI time was longer in RRMS than controls ($p<0.05$). SI time was not related to the Paced Auditory Serial Addition Test (PASAT), WMLL or to the presence of brainstem lesions. 9 hole peg test (9HP) correlated significantly with WMLL ($r=0.58$, $p<0.01$). Both SI time and 9HP correlated negatively with the results of wSDMT ($r=-0.32$, $p<0.05$, $r=-0.47$, $p<0.01$), but none correlated with the results of PASAT.

Conclusions: RRMS patients have an increased SI time compared to controls. Cognitive tests results, exemplified by the wSDMT, may be confounded by eye and hand motor function.

1. Introduction

Multiple sclerosis (MS) is an inflammatory disease affecting the central nervous system showing both local and more widespread diffuse inflammation and neurodegeneration. Early features of relapsing-remitting multiple sclerosis (RRMS) are varied and may include eye motor disturbances (Reulen et al., 1983; Frohman et al., 2005; Graves and Balcer, 2010), fine motor control of the hand (Cutter et al., 1999) or cognitive dysfunction (Amato and Ponziani, 2001; Amato et al., 2010).

The Symbol Digit Modalities Test (SDMT) (Aron, 1982) is a widely used test of processing speed, recently suggested as sentinel test for cognitive impairment in multiple sclerosis (Van Schependom et al., 2014). It is part of several test batteries used in the assessment of cognitive impairment in MS patients (Benedict et al., 2002; Langdon et al., 2011) and is suggested for use in clinical trials (Benedict et al., 2012). Because of the wide use of the SDMT (Benedict et al., 2004; Drake et al., 2010; Langdon et al., 2011), it is important to identify possible input or output level problems related to the procedure of the test.

Saccadic initiation time (SI time), i.e. the time from a central

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visual cue appears to the onset of an appropriate saccade, may be increased in patients with MS (Reulen et al., 1983) and hence constitute an input problem when performing the SDMT. Complex tests of eye movements, like tests of anti-saccades, have been associated with cognitive dysfunction in MS (Fielding et al., 2009a, 2012), and MS patients appear to spend excessive time on saccadic tasks with distractor stimuli (Fielding et al., 2009b). Recently a test for eye motor speed has been suggested as a bedside assessment tool in MS, as the number of speeded saccades for 30 s was related to both visual and non-visual cognitive tests (Roberg et al., 2014). However, to our knowledge, SI time, relevant for an effective completion of the SDMT, has not been studied in MS patients.

Motor function could affect the response to cognitive tests, like the SDMT. The neuropsychological test batteries for MS patients, like the MACFIMS and BICAMS, where SDMT is included, have recommended the use of the oral version of the SDMT because of possible motor interactions with the written version (Benedict et al., 2002; Langdon et al., 2011). However, oral motor slowing has been found to affect the results of the oral SDMT (oSDMT) (Arnett et al., 2008), indicating that an oral response to the SDMT may not be ideal. The emergence of new disease modifying treatments requires clinicians to carefully monitor their patients' disease progression early in the disease course. In particular the increasing attention on cognitive dysfunction in MS warrants a need for quick and easy assessment of cognitive function in early MS patients. These patients may have a very low disability and minor motor dysfunction. The written version of the SDMT, (wSDMT) is easier to administer for the clinicians and probably would feel more discreet to complete for the patients, and it would therefore be an advantage to both parties if this version of the test could replace the oral version in some instances. It is, however, not known whether hand motor speed is associated with the test results on the wSDMT in such patients.

In this study we aimed at testing whether decrements in eye and hand motor control could confound the test score of the visual SDMT with written response (wSDMT) in early MS patients.

2. Materials and methods

2.1. Patients and controls

Relapsing-remitting MS (RRMS) patients diagnosed within the last three years with no drug abuse and no other neurologic or psychiatric disease, were investigated ($n=48$). Healthy controls ($n=47$) were included for the ophthalmological and eye-tracker analyses, but not tested neurologically. They were recruited from the hospital and university environment and had no medical conditions known to affect the visual pathways. They were matched on age and gender at a group level and, after exclusion (four patients and five controls because of technical problems with the eye tracker, one patient because of febrile acute illness and one control because of possible demyelinating disease), 44 patients and 41 controls were eligible for analyses (Table 1).

All participants gave written informed consent and the project was approved by the regional committee for medical and health research ethics (REK).

2.2. Clinical evaluation

All patients were tested by the same trained neurologist (GON) with the Expanded Disability Status Score (EDSS) and the 9 Hole Peg test (9HP) for hand motor speed. They also underwent a thorough neuropsychological assessment (Nygaard et al., 2014). Results of the auditory 3 s version of the Paced Auditory Serial Addition Test (PASAT) (Gronwall, 1977) performed by the patients, and wSDMT (Aron, 1982) performed by all participants, are reported. Ophthalmological examinations were performed by the same trained ophthalmologist (SRB).

All patients underwent detailed MRI within a week of the other examinations. White matter lesion load (WMLL) was estimated from FLAIR and MPRAGE sequences, using the Cascade software, previously applied to an overlapping MS sample (ki.se/en/nvs/cascade) (Damangir et al., 2012; Nygaard et al., 2014). A subset of the patients ($n=32$) were examined by a trained neuroradiologist (PS) and rated according to the presence of white matter lesions (WML) of the brainstem.

Patients' visual evoked potential latency to P100 (VEP P100) were obtained with dimmed light (~25 lx) and the screen placed 100 cm in front of the eyes with checkerboard patterns (check size 65′, 2 Hz with a 16″ cathode ray tube screen). Three hundred responses were averaged from the mid-occipital lobe (MO, defined to be 5 cm above inion) referenced Fz, (defined by the 10/20 system) with 1–100 Hz band-pass filter. Rejection level was set to $\pm 100\ \mu V$.

Saccades were acquired using an iView X Hi-Speed eye-tracking (SensoMotoric Instruments, Teltow, Germany). The participants were seated approximately 70 cm from the 18.5″ monitor, measuring a diagonal length of 47 cm, and the constant display resolution was set to 1680×1050 pixels. Binocular data were recorded at a sampling rate of 60 Hz. The eye-tracking system is accurate to less than 0.4°.

Participants first fixated on a central cross and made saccadic movements as fast as possible towards a star in the corner of the screen cued by a central appearing arrow instantly replacing the cross (Fig. 1a and b). The test's primary output was SI time, defined as the time from the appearance of the arrow until the onset of an appropriate saccade.

Target areas of interest (tAOI) were defined by circles surrounding the stars in the four corners of the screen (Fig. 1c). Time to tAOI (ttA) was calculated as the time from appearance of the central arrow to the participants' saccade entered the tAOI.

Each participant was given eight trials. The first trial was regarded as a test and discarded. Altogether, 286/308 (93%) of the trials of the patients and 273/287 (95%) of the trials of the controls had good quality and were included in the analyses.

2.3. Statistical analyses

SPSS version 22, Chicago, IL was used for statistical analyses. Independent samples t-test and χ^2-tests were used to test for differences between patients and controls and Pearson bivariate and partial correlations were used to test for associations between the same groups. Linear regression analyses were used to assess the association between motor function and cognitive tests. A significance level of 0.05 was applied for all analyses.

3. Results

3.1. Clinical features

The background and clinical characteristics are listed in Table 1. The groups were comparable concerning age, gender distribution, visual acuity and test results on the wSDMT. The controls' educational level was on average two years higher than that of the patients. VEP p100 was in normal range on group level. Almost half of the patients had undergone known or subclinical optic neuritis (ON) of either or both eyes prior to the examinations.

The patients had a significantly longer SI time than the controls while ttA was comparable (Table 2).

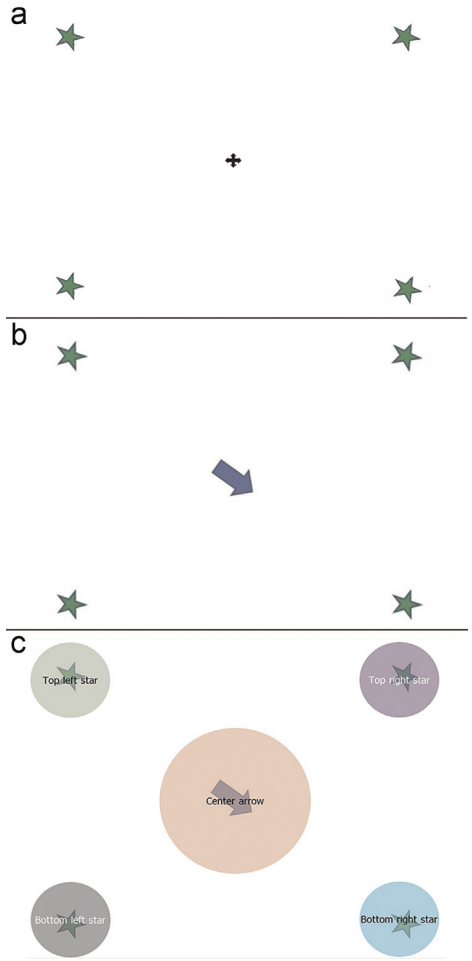

Fig. 1. Screenshots from the eye tracker. (A) fixation slide (corner stars=saccadic targets); (B) task (saccadic cue=pointing arrow); (C) AOIs.

Table 1
Background.

	Patients	Controls	Difference	
	N=44	*N*=41	95% CI	*p*-Value
Female, *n* (%)	32 (73)	30 (73)		1.000
Age, years ± SD	35.1 ± 7.3	33.2 ± 6.8	−1.2 to 4.9	0.225
Education, years ± SD	15.2 ± 2.1	16.9 ± 3.0	−2.9 to 0.6	0.003
Time since diagnosis, months ± SD	16.3 ± 11.2			
Disease duration, months ± SD	30.7 ± 28.3			
Disease modulatory treatment, n (%)	36 (82)			
EDSS, mean ± SD	1.8 ± 0.8			
EDSS, median (min–max)	1.5 (0–3.5)			
9HP, sec, mean ± SD[a]	20.8 ± 3.8			
T25FWT, sec, mean ± SD[a]	4.0 ± 0.6			
Visual acuity, left eye, LogMar ± SD	−0.04 ± 0.12	−0.08 ± 0.08	−0.01 to 0.08	0.097
Visual acuity, right eye, LogMar ± SD	−0.08 ± 0.09	−0.06 ± 0.11	−0.06 to 0.02	0.381
Previous optic neuritis				
Left eye, *n* (%)	9 (21)			
Right eye, *n* (%)	11 (25)			
Both eyes, *n* (%)	1 (2)			
White matter lesion load, mm^3, mean ± SD[a]	5.4 ± 3.6			
Presence of lesions in brainstem, *n* (%)	19/32 (59%)			
VEP, delay to p100, left eye, mean ± SD[b]	107.0 ± 9.0			
VEP, delay to p100, right eye, mean ± SD[b]	106.9 ± 7.1			
wSDMT, correct answers, mean ± SD[c]	53.9 ± 8.9	55.8 ± 8.5	−5.8 to 0.3	0.321
PASAT, correct answers, mean ± SD[a]	47.4 ± 9.3			

[a] *n*=42 s.
[b] For two patients VEP delay was not quantifiable on the left or both eyes.
[c] *N*=40 controls.

3.2. Association between motor function, disease characteristics and cognitive test results

The patients' SI time was associated with wSDMT, but not with PASAT, as shown in Fig. 2a and b. Furthermore, there were no associations between the patients' SI time and WMLL or VEP P100 (Table 3). ON patients did not present longer SI time than the other patients (t=1.559, p=0.126), and the patients with and without WML of the brain-stem had similar SI time (t=0.182, p=0.857). Furthermore, there was no significant association between SI time and age (r=0.232, p=0.129).

As illustrated in Fig. 2c and d, the patients' hand function (9HP) was associated with the wSDMT, but not with the PASAT. 9HP was also associated with WMLL, but not with VEP P100 latency (Table 3) or WML of the brain-stem (p=1.000). Hand function was, however, associated with age (r=0.481, $p<0.001$). The association between hand function, wSDMT and WMLL, was still significant after correcting for age (9HP versus wSDMT (partial): $rp=-0.349$, p=0.025, 9HP versus WMLL (partial): rp=0.576, $p<0.001$).

As expected from previous studies (Brochet et al., 2008; Drake et al., 2010) the results of the wSDMT and PASAT were associated (r=0.457, p=0.002). When controlling for 9HP (r=0.511, p=0.001), or SI time (r=0.455, p=0.003) this association was unchanged.

In the healthy controls, we found no association neither between SI time and wSDMT ($r=-0.189$, p=0.256), nor between wSDMT and age ($r=-0.227$, p=0.176).

Table 2
Test results of saccades in patients and controls.

	Patients	Controls	Difference	
	n=44	*n*=41	95% CI	*p*-Value
Saccadic initiation time, ms ± SD	351.7 ± 69.4	326.0 ± 45.0	0.3–51.2	0.047
Time to target AOI, ms ± SD	468.5 ± 84.6	446.0 ± 64.6	−10.2 to 55.1	0.175
Number of valid saccades per participant, total ± SD	6.5 ± 1.1	6.7 ± 0.8	−0.6 to 0.3	0.442

Univariate regression analysis showed 10% of the variance in wSDMT could be explained by the results on SI time, and every extra 10 ms of SI time was associated with a 0.41 point decrease in the wSDMT (Fig. 2a). Regression analysis revealed that 22% of the variance in wSDMT could be explained by the results on the 9HP, and every extra second spent on the 9HP was associated with a 1.1 point decrease in wSDMT (Fig. 2c).

Multivariate linear regression with 9HP and SI time as independent variables and wSDMT as dependent variable revealed that SI time did not add significantly (p=0.209) to the model when controlling for 9HP.

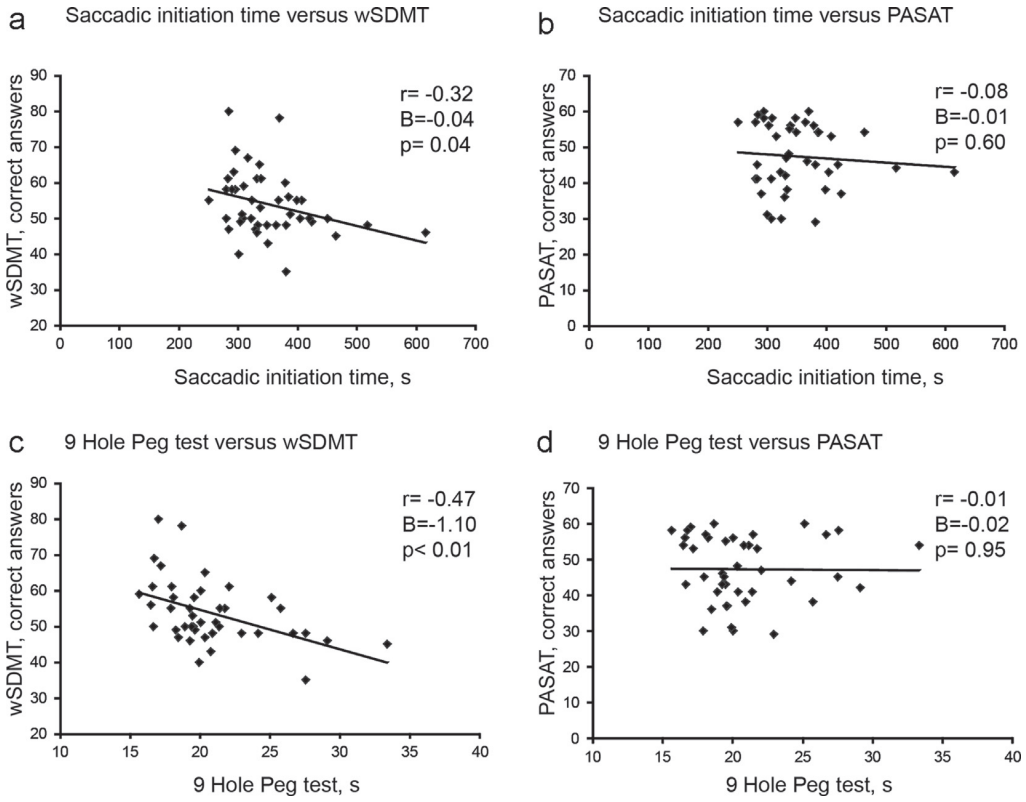

Fig. 2. Association between motor function and cognitive tests in RRMS patients. The scatter plots illustrate the association between (a) SI time and wSDMT, (b) SI time and PASAT, (c) 9HP and SDMT and (d) 9HP and PASAT, respectively. As illustrated, only wSDMT, and not PASAT, is associated with motor function in early RRMS patients.

Table 3
Correlations in RRMS patients.

	WMLL *r* (*p*-value)	VEP P100 *r* (*p*-Value)
SI time	0.038 (0.815)	−0.104 (0.519)
9HP	0.579 (< 0.001)	0.094 (0.560)

In the RRMS patients, SI time was not associated with WMLL or VEP P100, while 9HP was associated with WMLL, but not with VEP P100.

4. Discussion

This study shows that RRMS patients early in the disease course have a longer SI time than healthy controls. SI time was unrelated to other disease characteristics, WMLL and VEP. We also found that both hand function (9HP), and SI time were associated with the test results of the wSDMT, but not with the auditory and oral test of the same functional domains, PASAT.

In contrast to the study by Fielding et al. (2009b) who found prolonged saccadic delay in response to composite, but not to simple cues, we found a prolonged SI time in response to a simple cue. Our sample size is larger than previous ones, and our patients had a shorter disease duration and higher EDSS, possibly accounting for the different findings (Fielding et al., 2009b).

Saccades and cognition are thought to be linked, and recently a test for eye motor speed was suggested as a bedside assessment tool in MS (Roberg et al., 2014). These neuropsychological studies indicate that eye motor disorders may be a sensitive early marker of disseminated disease. Our study showed increased SI time in the absence of cognitive difficulties. We therefore hypothesize that SI time and cognitive decline are caused by separate anatomico-pathological alterations in early RRMS. These domains may not evolve in parallel and identifying the actual impairments may lead to improved care for the patients.

Slowing of hand function has been recognized as a sign in early MS (Cutter et al., 1999), and the patients in our study had a slower hand function than healthy controls in previous studies (Drake et al., 2010). We did not identify any association between 9HP and PASAT. In contrast, studies of the components of the MSFC have identified associations between 9HP and PASAT in large patient samples with long disease duration (Cutter et al., 1999; Drake et al., 2010). The absent association in our study may be explained either by short disease duration, cognitive intactness or by the limited patient sample. The significant association between 9HP and the wSDMT test score indicates that hand function is relevant in solving the wSDMT even in the absence of cognitive dysfunction.

We found associations between hand motor function and WMLL, but no association between SI time and WMLL or brainstem lesions. However, there is considerable evidence that both normal appearing white matter (Kutzelnigg et al., 2005; Bodini et al., 2009) and gray matter (De Stefano et al., 2003; Sailer et al., 2003; Ceccarelli et al., 2008) may be affected in early RRMS patients, and associated with specific symptoms, like cognition and fatigue (Sepulcre et al., 2009; Nygaard et al., 2014). Cerebellar pathology is also related to eye movements disorders, as recently shown in a study of antisaccades and cognition in early MS patients (Kolbe et al., 2014). Thus our study does not exclude associations between SI time and other disease related structural brain changes.

The oSDMT is the most commonly used test in the neuropsychological assessment of MS patients. From our results, showing that SI time affects the wSDMT results, we hypothesize that the results of the oSDMT would be affected by eye motor

decrements. This has, however, not been directly shown in this study, and should be tested separately in future studies. Our study is limited by the fact that the patients were not given both the written and the oral version of the SDMT. This would have contributed to clarify the different and possibly additional motor skills required for performance of the wSDMT. Moreover, the controls did not perform the PASAT, which would have been an additional strength to our analyses.

Low SDMT score is associated with early cognitive dysfunction (Deloire et al., 2006), disease development, imaging (Christodoulou et al., 2003; Benedict et al., 2004; Filippi et al., 2010) and vocational outcomes (Drake et al., 2010) in MS patients. The SDMT may be the best available cognitive test for MS patients (Langdon et al., 2011). The association between wSDMT test score, eye and hand motor function described in this study may in fact contribute to the value of the wSDMT in real life. This study therefore does not oppose the future use of this test, but highlights that impairments other than cognitive difficulties may confound the test results and these should be interpreted with caution in some patient groups.

In conclusion, slowing of eye movements, as well as hand motor function, is associated with the wSDMT test score. Clinicians should be aware that the wSDMT may measure not only cognition, but also eye movements and hand function in early RRMS patients.

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Conflict of interest

None.

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ORIGINAL RESEARCH

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Fatigue and cognition: Pupillary responses to problem-solving in early multiple sclerosis patients

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Abstract

Introduction: In early multiple sclerosis (MS) patients, cognitive changes and fatigue are frequent and troublesome symptoms, probably related to both structural and functional brain changes. Whether there is a common cause of these symptoms in MS is unknown. In theory, an altered regulation of central neuropeptides can lead to changes in regulation of autonomic function, cognitive difficulties, and fatigue. Direct measurements of central neuropeptides are difficult to perform, but measurements of the eye pupil can be used as a reliable proxy of function.

Methods: This study assesses pupil size during problem-solving in early MS patients versus controls. A difference in pupil size to a cognitive challenge could signal altered activity within the autonomic system because of early functional brain changes associated with cognitive load. We recruited MS patients (mean disease duration: 2.6 years, $N = 41$) and age-matched healthy controls ($N = 43$) without eye pathology. Neurological impairment, magnetic resonance imaging, visual evoked potentials, depression, and fatigue were assessed in all of the patients. In both groups, we assessed processing speed and retinal imaging. Pupil size was recorded with an eye-tracker during playback of multiplication tasks.

Results: Both groups performed well on the cognitive test. The groups showed similar pupillary responses with a mean of 0.55 mm dilation in patients and 0.54 mm dilation in controls for all the tasks collapsed together. However, controls ($N = 9$) with low cognitive scores (LCS) had an increased pupillary response to cognitive tasks, whereas LCS MS patients ($N = 6$) did not ($p < .05$). There was a tendency toward a smaller pupillary response in patients with fatigue.

Conclusions: This is the first study to investigate pupillary responses to cognitive tasks in MS patients. Our results suggest that MS-related changes in cognition and fatigue may be associated with changes in arousal and the autonomic regulation of

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task-related pupillary responses. This supports the theory of a link between cognition and fatigue in MS.

KEYWORDS
cognition, fatigue, multiple sclerosis, pupillometry

1 | INTRODUCTION

Multiple sclerosis (MS) patients frequently suffer from cognitive difficulties and fatigue, often present early in the disease course (Amato, Ponziani, & Pracucci, 1995; Krupp, Alvarez, LaRocca, & Scheinberg, 1988). The impact on everyday life may be severe (Nortvedt, Riise, Myhr, & Nyland, 1999). Neuropsychological testing, functional and structural brain imaging are now extensively used to investigate cognition (Amato et al., 2010; Bakshi, 2003). However, the cognitive symptoms are only partly explained by changes in the central nervous system (CNS) as demonstrated by magnetic resonance imaging (MRI) studies (Genova et al., 2013; Rocca, Parisi, et al., 2014; Rocca et al., 2015). Early brain changes in MS may affect central neurotransmitters and the autonomic nervous system, which could be effectively picked up with alternative methods that relate directly to autonomic function like pupillometry. Moreover, the possibility to measure this through the eye pupil is less expensive than MRI methods and with currently infrared eye-tracking systems is also elegantly noninvasive (Joshi, Li, Kalwani, & Gold, 2016). To the best of our knowledge, investigations of task-evoked pupillary responses have not been applied yet to this patient group despite the relationship between such a physiological measurement and cognitive effort. In this study, we therefore explored whether cognitive challenges and fatigue in early MS would be reflected in these patients' pupillary responses to problem-solving tasks.

Functional MRI (fMRI) studies have indicated that patients with MS or clinically isolated syndromes (CIS) show a different cerebral 'resting state' activation as well as task-related cerebral activation patterns compared to healthy controls (Audoin et al., 2003; Forn et al., 2006; Penner, Rausch, Kappos, Opwis, & Radü, 2003; Rocca, Parisi, et al., 2014; Roosendaal et al., 2010; Staffen et al., 2002). These altered cerebral activation patterns in MS patients may be signs of functional reorganization compensating for structural damage. Alternatively, such a reorganization may be dysfunctional and contribute to a less appropriate brain activation, cognitive difficulties, and fatigue.

fMRI studies of fatigue in MS are scarce, but some studies have found both altered resting state and different activation patterns in fatigued MS patients compared to healthy controls (DeLuca, Genova, Hillary, & Wylie, 2008; Engström, Flensner, Landtblom, Ek, & Karlsson, 2013; Genova et al., 2013). An intriguing possibility is that connectivity in partially-overlapping networks collapses over time and structural damage may lead to fatigue and cognitive difficulties (Hanken, Eling, & Hildebrandt, 2014). This hypothesis is supported by the observation that patterns of gray matter atrophy are similar in patients with fatigue and cognitive difficulties in MS (Calabrese et al., 2010; Pellicano et al., 2010), in contrast to the weak correlation between these symptoms and damages to normal-appearing white matter and white matter lesion load. A thalamo-striato-frontal disruption pattern has been suggested as the cause of MS-related fatigue (Pardini, Bonzano, Mancardi, & Roccatagliata, 2010). A similar disconnection syndrome has been proposed to explain MS-related cognitive difficulties (Louapre et al., 2014). Such a disconnection may not only be structural; a possible cause of the altered cerebral activation patterns in patients with cognitive difficulties and fatigue could be because of disturbances in regulatory neuropeptides. The role of noradrenergic activation of CNS is particularly interesting in relation to these symptoms as noradrenergic activation leads to wakefulness and is involved in the allocation of attention (Laeng, Sirois, & Gredeback, 2012).

Noradrenergic activation can be observed indirectly by measuring pupil size during task-related pupillary responses (Joshi et al., 2016; Szabadi, 2013). The pupil size in general depends on arousal, sleepiness and sleep-deprivation, demands on attention, and surrounding light conditions as well as visual acuity and refractional errors. It is governed by the autonomic nervous system, and alterations in the sympathetic or parasympathetic nervous system possibly affects the task-evoked pupillary response as well.

Pupillometry has been used in psychological research as a marker of intensity of mental activity and of changes in mental states. The task-evoked pupillary response provides a reliable and sensitive indicator of within-patient variations in processing load in memory, language, reasoning and perception tasks, and it is sensitive to between-group differences in intelligence (Beatty, 1982). At the neural level, this pupillary response is associated with the activation of locus coeruleus, a brainstem nucleus and hub of the noradrenergic system, that is involved in arousal and the allocation of attention (Alnæs, Sneve, Espeseth, Pieter, & Laeng, 2014; Murphy, O'Connell, O'Sullivan, Robertson, & Balsters, 2014). Both the parasympathetic inhibition and the sympathetic activation of pupillary dilator muscle are involved in regulating the pupillary response to cognitive tasks (Steinhauer, Siegle, Condray, & Pless, 2004). Studying the pupillary dilation may therefore be a suitable noninvasive technique to examine deficits of the catecholamines' and cholinergic system as well as the autonomic nervous system in general. More specifically, pupillary studies can give insight into deficits of cognition resulting from neurological disorders.

Optic neuritis (ON) is common in patients with MS and can possibly interfere with pupillary measurements. It constitutes the first symptom of the disease for 25% of MS patients and occurs in the course of the disease in about 70% (Toosy, Mason, & Miller, 2014). It is not known whether the disruption of the visual pathways caused by ON leads to an altered task-related pupillary response in MS patients.

Ophthalmologic assessment and evaluation of visual evoked potentials (VEP) should therefore prelude the application of this method in MS patients. Further, lesions of the brainstem could lead to a disruption of the tracts from LC and other brainstem nuclei and interfere with the regulation of the pupil size. MRIs of the brainstem should therefore be evaluated neuroradiologically in MS patients subject to this method. In this study, we combined a multidisciplinary approach with examinations of task-related pupillary responses. A history of optic neuritis or brainstem lesions could then be tested for interference with the pupillary responses in a group of early MS patients, that is, within a time from diagnosis of less than 4 years.

Our main hypothesis was that pupillary responses to problem-solving in early MS patients would be different from those of healthy controls. One possible prediction was that neurodegeneration in connectivity could reduce the neuromodulatory influence of the norepinephrine —Locus Coeruleus system, resulting in reduced pupillary dilations during cognitive challenges task (Granholm, Morris, Sarkin, Asarnow, & Jeste, 1997). In addition, we examined whether pupillary responses to problem-solving would be influenced by individual differences in cognition, depressive symptoms, and fatigue, and in the subgroups with and without ON and brainstem lesions. As we had no a priori knowledge of the direction of the possible group differences, we used an exploratory approach with two-tailed statistical testing.

2 | MATERIALS AND METHODS

2.1 | Patients and controls

Relapsing-remitting multiple sclerosis (RR MS) patients (n = 49) diagnosed according to the revised McDonald Criteria (Polman et al., 2011), initially enrolled in a study of cognition and neuroimaging (n = 76) (Nygaard et al., 2015), were asked to participate in this study. Healthy controls were recruited from the local community, the hospital and university environment by email or direct inquiry. Inclusion criteria for both groups were—age 18–50 years, fluency in Norwegian, no prior ophthalmological, neurological or psychiatric disease, no head injury, and no substance abuse. To avoid possible confounding by reduced visual acuity, refractive errors with a spherical equivalent of more than ±6 or lesions of the visual pathways were exclusion criteria. All participants underwent an ophthalmological examination. The MS patients were also examined with visual evoked potentials (VEP). We could therefore carefully select measurements from the healthy eyes of the participants. Eight patients were excluded [due to bilateral ON (n = 5), previous amotio retina (n = 1), other conditions that may have interfered with the measurements (n = 2)], leaving 41 patients eligible for analysis. Forty-seven healthy controls were considered for the experiment, of whom four were excluded [due to technical difficulties with the eye-tracker (n = 2) and other medical or neurological conditions (n = 2)]; thus, 43 controls were eligible for analyses. The patients were clinically stable with at least 3 months since an episode of ON in the contralateral eye and 6 weeks since any other relapse or corticoid treatment.

All participants gave written informed consent and the study was approved by the regional ethical committee of South Eastern Norway (REK).

2.2 | Neurological and neuropsychological examinations

The patients underwent a full neurological examination within 2 weeks of the ophthalmological and pupillary measurements. The Expanded Disability Status Score (EDSS) was used to assess neurological disability. Depressive symptoms were assessed with the self-report Beck Depression Inventory (Beck, Steer, & Brown, 1996) addressing both cognitive and somatic aspects of depression, and fatigue was reported with the self-report Fatigue Severity Scale (FSS) (Krupp, LaRocca, Muir-Nash, & Steinberg, 1989) to separate depression from fatigue. Patients with FSS >4 were considered to have fatigue and patients with BDI >12 were considered to have depression.

Both patients and controls underwent testing with the neuropsychological tests included in the test battery "Brief International Cognitive Assessment for Multiple Sclerosis" (BICAMS) (Langdon et al., 2011). Symbol Digit Modalities Test (SDMT) (Smith, 1982) was used to assess processing speed, the sum of the first five trials of the California Verbal Learning Test 2 (CVLT) (Delis, Kramer, Kaplan, & Ober, 2000) was used to test verbal memory and the sum of the first three trials of the Brief Visuospatial Memory Test-Revised (BVMT) (Benedict, 1997) was used to test visuospatial memory. The raw scores of the test results of the controls were used to create z-scores for the patients. We chose a cut-off of $z < -2$ for CVLT and BVMT and a cut-off for SDMT results of $z < -1.5$. Anyone who scored $z < -2$ on either BVMT or CVLT, or $z < -1.5$ on SDMT were classified as with a low cognitive score (LCS). The rest were classified as with a normal cognitive score (NCS).

2.3 | Ophthalmological and pupillometric examinations

All participants underwent an ophthalmological examination, including the swinging flashlight test, as well as visual acuity measured as the logarithm of the minimum angle of resolution (logMAR) and spherical assessment. Pupil data were acquired using the SMI (SensoMotoric Instruments, Teltow, Germany) R.E.D. eye-tracking device. All participants were seated approximately 70 cm from the monitor in the same room, lit with approximately 180 lux. The eye pupillary responses were registered with I-View Software (SMI). The pupil diameter of both eyes was measured at a sampling rate of 60 Hz. The RED can operate at a distance of 0.5–1.5 m. This device has two sources of infrared light from an infrared light-sensitive video camera, placed under the monitor frame. The RED keeps track of head position which allows measuring reliably the pupil diameters in mm, despite the presence of head movements. According to SMI specifications, the RED system can detect changes as small as 0.004 mm. Binocular data were recorded at a sampling rate of 60 Hz (i.e., every 16 ms). The constant

display resolution was set to 1680 × 1050 pixels. A 5-point calibration pattern was displayed to participants before running the eye-tracker sessions. A dispersion of <0.5 in both x- and z-space was considered a successful calibration; recalibration was initiated until a successful calibration was obtained. Measurements were randomly conducted throughout the day for both groups. Patients and controls were examined during the same period, but the examiners were not blinded regarding the status of the participants.

2.4 | Optic coherence tomography

Retinal imaging was performed by the same trained ophthalmologist (SADRB) with the spectral domain RS-3000 OCT Retina Scan (Nidek Inc., CA, USA). Peripapillary retinal nerve fiber layer thickness (RNFL) data were obtained with the Disc Circle protocol with a scan width of 3.45 mm and a scanning speed of 53,000 A-scans/sec, centered on the optic nerve head without crossing of the two inner scan circles. All scans included had a signal strength of 8/10 or better.

2.5 | Visual evoked potentials

Visual evoked potentials (VEP) delay to P100 were obtained with dimmed light (~25 lux) and the screen placed 100 cm from the eyes of the patients, with a Dantec Keypoint Focus system with checkerboard patterns (check size 65') presented at 2 Hz with a 16" cathode ray tube screen. Three hundred responses were averaged from the mid-occipital lobe (MO, defined to be 5 cm above inion) to the mid-frontal lobe (Fz, as defined by the 10/20 system) with 1 Hz – 100 Hz band-pass filter. Rejection level was set to ± 100 μV. The VEP results were evaluated by two experienced clinical neurophysiologists (KBN and LE), and VEP was regarded as pathological with a duration of more than 110 ms and/or with a greater delay of at least 6 ms compared to the contralateral eye.

2.6 | Magnetic resonance imaging

All patients underwent cerebral MRI examinations using the same 1.5 T Siemens Avanto scanner (Siemens Medical Solutions) with a 12-channel head coil. For clinical radiological evaluation, FLAIR, T2 and pre- and post-gadolinium T1 MP-RAGE sequences were used. Details concerning the sequences have been described earlier (Nygaard et al., 2015). For this study, we were interested in detecting the presence of brainstem lesions. An experienced neuroradiologist (PS), blinded to the pupillometry and clinical test results, evaluated the MRIs and rated the patients as with or without lesions of the brainstem.

2.7 | Experimental design

New infrared eye-tracker technology was employed to reproduce a classic pupillometric experiment of cognitive load on patients and controls. Playback of seven mathematical multiplication tasks of increasing difficulty, adapted from the classic study of Hess and Polt (Hess & Polt, 1964) were presented auditorily at the initiation time

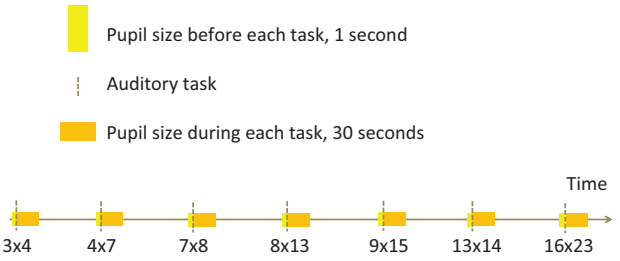

FIGURE 1 Experimental design. The participants were presented auditorily with mathematical tasks of increasing difficulty while they were told to fixate on a cross on a gray computer screen. The pupil size was measured continuously before and after the oral responses to the tasks

(Figure 1). Continuous pupil size recordings for each task started one second before the initiation time and lasted for 30 s after, irrespective of the participants' oral response time. The oral response time was recorded by a tap on the keyboard by a trained research assistant, coregistering whether the response was correct.

2.7.1 | Statistical analysis of baseline characteristics and neuropsychological test results

SPSS Statistics version 22.0 (SPSS, Chicago, IL, USA) was used for statistical analyses. Descriptive statistics of the differences between patients and controls were performed using independent samples *t* tests for continuous data and χ^{2-} tests for categorical data. Bonferroni-corrections for multiple comparisons were applied when appropriate. Pearsons correlations were used to test for association between the peak of the pupillary response and possible confounders.

2.7.2 | Statistical analyses of pupillary response to the experimental task

Initiation time and oral response of each task were coregistered with the dynamic measurements of the pupil size. The mean pupil size, during and after the participants' oral responses were extracted, using the open source programming language C++ (http://www.cprogramming.com/). The data were then transferred to SPSS for statistical analyses.

The pupillary dilation was calculated as percent difference in pupil size from each pre-task baseline: [(mean pupil size of each second during the task/task-dependent baseline pupil size) −1] × 100. The results were analyzed using repeated measure analyses of variance (ANOVA), with group (e.g., patients or controls) as independent variables, and pupillary dilation during the task as dependent variables. To control for possible confounders, we performed relevant analyses with analyses of covariance (ANCOVA), with age, gender, and brainstem lesions as cofactors. For the ANOVA and ANCOVA analyses, we tested for differences in the dependent variables throughout the experiment (main effect), for differences in the dependent variable between patients and controls (between-group effect), and for an interaction between the two (interaction effect).

To assure that any group differences were not caused by the lack of a pupillary response of participants not responding or by error-related negativity (i.e., increased cognitive processing after making a mistake), we performed relevant analyses in subgroups of participants with trials with only correct answers.

A significance level of $p < .05$ was applied to all analyses.

3 | RESULTS

3.1 | Clinical characteristics of patients and controls

The demographic characteristics of patients and controls are summarized in Table 1. The groups were matched on age ($t = 1.12$, $p = .222$) and gender ($\chi^2 = 0.0214$, $p = .884$). The controls had on average an education level 2 years higher than the patients ($t = -3.31$, $p = .001$).

TABLE 1 Baseline characteristics

	Patients	Controls
	n = 41	*n* = 43
Gender, female, *n* (%)	28 (68)	30 (70)
Age, years, mean (*SD*)	35 (7.4)	33 (6.7)
Education, years, mean (*SD*)	15 (2.1)	17 (3.0)[a]
Disease duration, years, mean (*SD*)	2.6 (2.1)	–
Time since diagnosis, years, mean (*SD*)	1.6 (0.9)	–
Neurological disability, EDSS, mean (*SD*)	1.9 (0.8)	–
Depressive symptoms, BDI, mean (*SD*)[b]	7 (5.9)	–
Fatigue, FSS, mean (*SD*)[b]	4 (1.7)	–
Brain stem lesions on MRI, *n* (%)[c]	22 (69)	–
Disease modifying treatment		
None, *n* (%)	7 (17)	–
First line, *n* (%)	30 (73)	–
Second line, *n* (%)	4 (10)	–

[a]Difference between patients and controls, $p = .001$.
[b]Data available on 40 patients.
[c]Data available on 32 patients.

3.2 | Neurological and neuropsychological test results

All patients had a mean disease duration of less than 4 years and a low disability level with a mean EDSS of 1.9 and a median EDSS of 1.5. MRI revealed that 69% of the patients had brainstem lesions, and the presence of brainstem lesions were thus included as covariate in analyses of the experimental task results.

The neuropsychological test results of the patients have previously been reported, comparing their results to published norms (Nygaard et al., 2015). The controls performed well on SDMT (mean 56.1, *SD* 8.1, median 56) and BVMT (mean 27.4, *SD* 4.9, median 29). They had very high scores on CVLT (mean 70.5, *SD* 6.3, median 72); most of the controls were able to list all 16 words of the word list on the second trial. The patients performed on average worse than the healthy controls on the CVLT, and similarly on the BVMT and SDMT. Similar proportions of patients and controls failed at least one neuropsychological test (LCS: 9/39 patients and 6/41 controls, $\chi^2 = 0.463$, $p = .496$). Two patients failed two of the neuropsychological tests, whereas none failed three tests. Z-scores of the neuropsychological tests were generated from the raw scores of the controls. Both CVLT and BVMT results of the controls showed a right hand skewness (CVLT skewness: −0.9, BVMT skewness: −0.642), while SDMT results of the controls were normally distributed.

As previously reported (Nygaard et al., 2015), the patients had a mean FSS of 4 (*SD* 1.7), and 48% had a mean FSS >4 and were thus classified as with fatigue. The mean BDI was 7 (*SD* 5.9) and 25% were classified as with depressive symptoms with BDI >12.

3.2.1 | Ophthalmological test results of patients and controls

The patients and controls had similar visual acuity and spherical equivalents on both the tested and the contralateral (non-tested) eye. The patients had a thinner RNFL than the controls in the tested eye, and even thinner RNFL on the contralateral (non-tested) eye, where 51% of the patients had a history of ON. No relative afferent pupillary defect was observed on the tested eyes (Table 2).

For the pupillometric analyses, we used the results from the left eye of the participants, except from the participants with a history of left eye ON, prolonged VEP latency (eight patients) or other pathology (one patient and one control with left side amblyopia). In these cases, the results from the right eye were used.

3.3 | Task results of the experiment

The patients and controls performed comparably well on the arithmetic calculations [patients mean 4.5 (*SD* 1.2) correct responses, controls mean 4.4 (*SD* 1.6) correct responses]. All the participants gave a correct reply to the easiest task, while only a few participants gave correct replies to the most demanding tasks. The patients and controls had similar response times for the tasks, except for the task 9 × 15, where the patients spent slightly, but not significantly longer time ($t = 2.42$, Bonferroni-corrected $p = .126$) (Figure 2).

3.4 | Pupillary responses to problem-solving

Initiation time was defined as the time when a new auditory task was given, and oral response was defined as the time when the research assistant registered the response from the participant (Figures 1 and 3).

The *maximum pupil size* of patients and controls was observed in the time interval from 2 s before the oral response to 1 second after the response, as illustrated in Figure 1. We therefore defined the *task-related pupillary dilation* as the difference between the maximum pupil size in this time interval and the pupil size at rest before each task. This measure of pupillary dilation, in millimeters, was used in the further data analyses.

TABLE 2 Results of the eye examinations

	Patients	Controls
	n = 41	*n* = 43
History of optic neuritis on any eye, *n* (%)	21 (51)[a]	0 (0)
Left eye tested with pupillometry, *n* (%)	32 (78)[a]	42 (98)
Visual acuity of tested eye, Log MAR, mean (*SD*)	−0.05 (0.10)	−0.08 (0.07)
Visual acuity of other (non-tested) eye, Log MAR, mean (*SD*)	−0.05 (0.11)	−0.06 (0.11)
Spherical equivalent of tested eye, mean (*SD*)	−0.67 (1.74)	−0.58 (1.35)
Spherical equivalent of other (non-tested) eye, mean (*SD*)	−0.73 ± 1.40	−0.61 (1.83)
Retinal nerve fiber layer thickness of tested eye, mean (*SD*)	98.9 ± 10.2[a]	104.6 (11.3)
Retinal nerve fiber layer thickness of other (non-tested) eye, mean (*SD*)	94.8 ± 13.9[a]	104.6 (11.5)
VEP of tested eye[b], Latency to p100, mean (*SD*)	104.7 (5.0)	–
VEP of other (non-tested) eye[b], Latency to p100, mean (*SD*)	108.7 (7.0)	–

Approximately, half of the patients had a history of optic neuritis. In the patients with a history of optic neuritis on one eye, data from the other eye were used in the analyses. Visual evoked potentials (VEPs) were only tested in the patients and were longer in the non-tested than in the tested eyes, probably because of a history of optic neuritis in a large proportion of the non-tested eyes.

[a]Difference between patients and controls, $p < .05$.

[b]Test results of 40 patients. For one patient neurophysiological visual evoked potential test results were inconclusive.

Patients and controls had similar curves of pupillary dilation during problem-solving, as illustrated in Figure 3.

The term *task accumulated pupil (TAP) size* was used to describe the pupil size at baseline before each new experimental task.

The patients and controls had similar pupil size at baseline, and both patients and controls had increasing TAP size as the experiment advanced (ANOVA: interaction effect: Wilks lambda = 0.92, $F_{6,76} = 0.14$, $p = .328$, main effect: Wilks lambda 0.512, $F_{6,76}$, $p < .001$, partial eta squared = 0.488, between-groups effect: $F_{1,81} = 0.14$, $p = .709$).

We collapsed the pupillary dilation for all tasks and performed an ANCOVA with age, gender and baseline pupil size as covariates, group (patient or control) as a fixed factor and pupillary dilation as dependent variable (Table 3). There was no difference in pupillary dilation in response to the mathematical tasks between the groups ($F = 1.31$, partial eta square = 0.063, $p = .272$). We further tested for differences in pupillary dilation between the groups for each of the different mathematical task in order to see whether the groups would behave differently with increasing task difficulty. There were no group differences in pupil dilation with increasing task difficulty (data not shown).

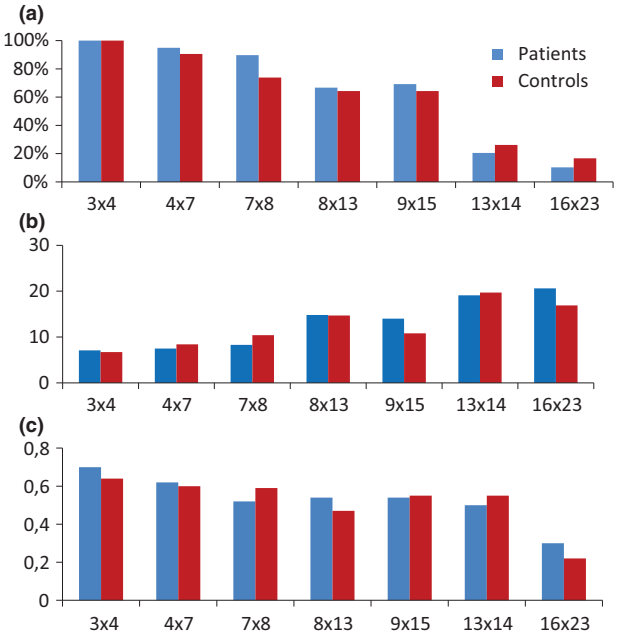

FIGURE 2 Experimental test results. (a) The percent of participants with correct answers, (b) the time spent to answer (seconds), and (c) pupillary dilation (millimeter) for each mathematical task is illustrated. Patients and controls had similar proportions of correct answers, spent similar time to complete the mathematical tasks and had similar pupillary dilations for each task

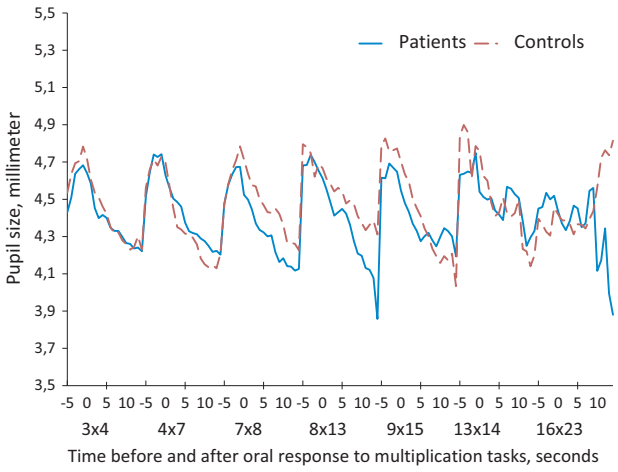

FIGURE 3 Pupil size of patients and controls during mathematical tasks of increasing difficulty. The patients and controls had similar curves of pupillary responses to mathematical tasks. Both groups showed a pupillary dilation with a maximum in the time interval from 1 s before and to 1 s after the oral response to the mathematical tasks

3.5 | Pupillary responses of different subgroups

Both patients and controls generally performed well on the neuropsychological tests. Regression analyses showed that the scores for processing speed (SDMT), were not associated with the pupillary

TABLE 3 Pupillary dilation during response to mathematical tasks

	Patients $n = 41$	Controls $n = 42$	ANCOVA		
	Pupillary dilation, mm (*SD*)	Pupillary dilation, mm (*SD*)	*F*	Partial eta square	*p*-value
Pupillary dilation, all tasks	0.55 (0.26)	0.54 (0.29)	1.31	0.063	.272

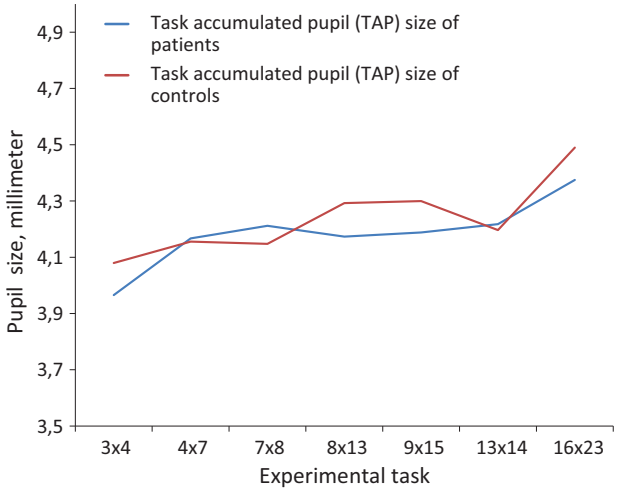

FIGURE 4 Task accumulated pupil (TAP) size of patients and controls before each new task. Both patients and controls had increasing TAP size as the experiment advanced

dilations of the participants in general ($r = -.002$, $p = .985$), nor in the MS patient group alone ($r = -.075$, $p = .690$). Further, we found no correlation between verbal memory (CVLT) and pupillary dilation, neither in the whole group ($r = 0.060$, $p = .599$) nor in the patients ($r = -.007$, $p = .966$). There was also no correlation between performance on the visuospatial tests and pupillary dilations in all participants ($r = 0.171$, $p = .131$), nor in the patients ($r = 0.261$, $p = .108$). Controlling for age, gender, and baseline pupil size did not alter these results (data not shown).

However, in a subgroup of both patients ($n = 9$) and controls ($n = 6$) classified as LCS (low cognitive score) the pupillary response was significantly larger than both NCS controls and patients (Figure 4). The LCS patients had significantly smaller pupillary responses than the LCS controls ($F_{2,25} = 8.10$, $p = .009$, Wilks lambda = 0.131, partial eta squared: 0.245). There was no significant difference in pupillary response between LCS and NCS patients ($F_{13,18} = 0.375$, $p = .961$, Wilks lambda = 0.787) (Figure 5).

Patients were divided into subgroups with no fatigue (FSS≤4, $n = 21$) and with fatigue (FSS >4, $n = 19$). There was a tendency toward smaller pupillary responses in patients with fatigue compared to the non-fatigue group ($F_{2,30} = 2.60$, $p = .118$, Wilks lambda = 0.150). When examining the curves of pupillary dilation of healthy controls and patients with and without fatigue, we noted that some of the patients with fatigue seemed to have a different profile of the pupillary response to problem-solving compared to healthy controls and non-fatigued patients. An example is given for the simple task for a typical healthy patient and a fatigued patient (Figure 6).

As illustrated, the fatigued patient has a smaller pupillary response, and the response lasted for slightly longer than the normal pupillary response.

The patients were further divided into groups of no depression (BDI≤12, $n = 30$) and depression (BDI >12, $n = 10$). We found no significant association between depression scores and pupillary responses ($r = -.251$, $p = .119$).

3.6 | The effect of possible confounders on the pupillary responses

Approximately, half of the patients had a history of optic neuritis. This could interfere with the pupillary response, and we therefore tested whether pupillary dilations were different between patients with and without a history of optic neuritis on the non-tested eye. To test this, we performed an ANCOVA with pupillary dilations for all the mathematical tasks as the dependent variable and a history of optic neuritis as the fixed factor. We found no differences between the groups ($F = 1.056$, partial eta squared = 0.026, $p = .310$). We further controlled for age, gender, and baseline pupil size with similar results (data not shown). The patients' pupil size at baseline was unrelated to RNFL ($r = -.019$, $p = .908$) and VEP ($r = 0.171$, $p = .292$). Moreover, the peak of pupillary dilation was unrelated to RNFL ($r = -.09$, $p = .593$) and VEP ($r = -.257$, $p = .109$).

Sixty-nine percent of the patients had visible brainstem lesions on MRI. There was no difference in the pupillary response of the patients with and without brainstem lesions ($t = 0.668$, $p = .509$).

4 | DISCUSSION

In this study, we investigated the pupillary response to problem-solving in early MS patients and healthy controls. Both patients and controls showed similar pupillary responses to cognitive tasks at a group level. However, while LCS controls had an increased pupillary response to cognitive tasks, LCS MS patients did not show such an increased response. We further identified a nonsignificant altered pupillary response in a subgroup of fatigued patients. Thus, we conclude that the early MS patients as a group had similar task-evoked pupillary responses as their healthy controls. This indicates that for most of the patients task-related allocation of attention is well-functioning, facilitating efficient cognitive processing. This is in accordance with the recent fMRI results of cognitive processing of early MS patients,

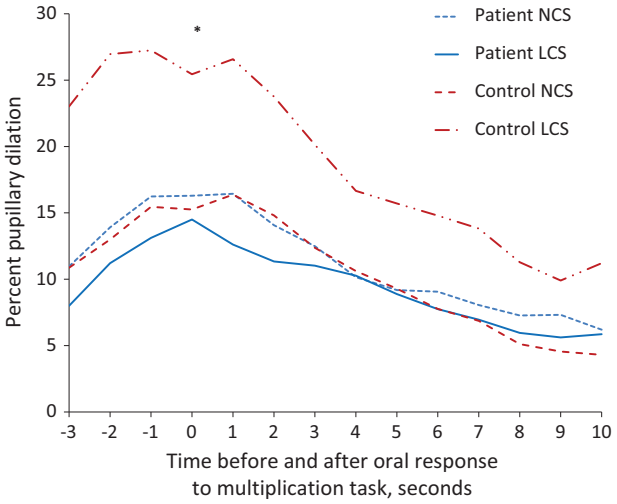

FIGURE 5 Pupillary responses of different subgroups. The mean of the correct responses of the three easiest tasks is shown for different subgroups of patients and controls. In (a) the LCS controls have a significantly larger pupillary response than the NCS controls. In (b) there are no significant differences between the patients with or without fatigue and in (c) there are no significant differences between patients with or without depressive symptoms. LCS: low cognitive score, NCS: normal cognitive score, *Significant difference in response between patients and controls with LCS, $p < .05$

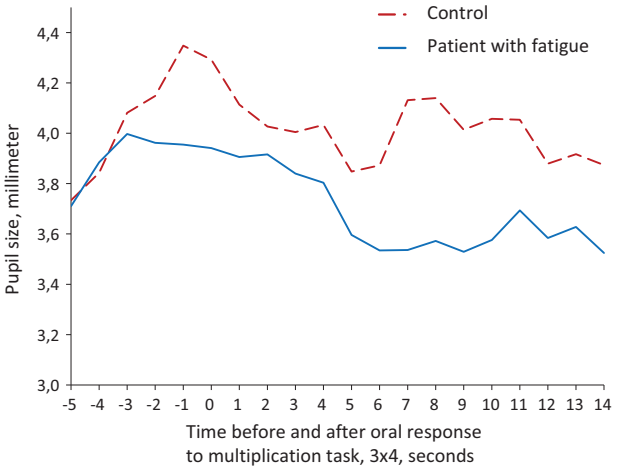

FIGURE 6 Patients with and without fatigue

where normal activation patterns were found in the cognitively preserved patients (Rocca, Valsasina, et al., 2014).

Our patient group succeeded as well as the controls on both the neuropsychological tests and the mathematical tasks, indicating that they had no obvious cognitive difficulties in the domains of processing speed, short-term memory, and problem-solving. We have previously reported that the patients mostly were in part- or full-time work (Nygaard et al., 2015), and their high level of education also underscore their status as a well-functioning group. The "cognitive reserve" hypothesis in MS has suggested that cognitive decline can be delayed in patients with high education or intellectual leisure habits, even in

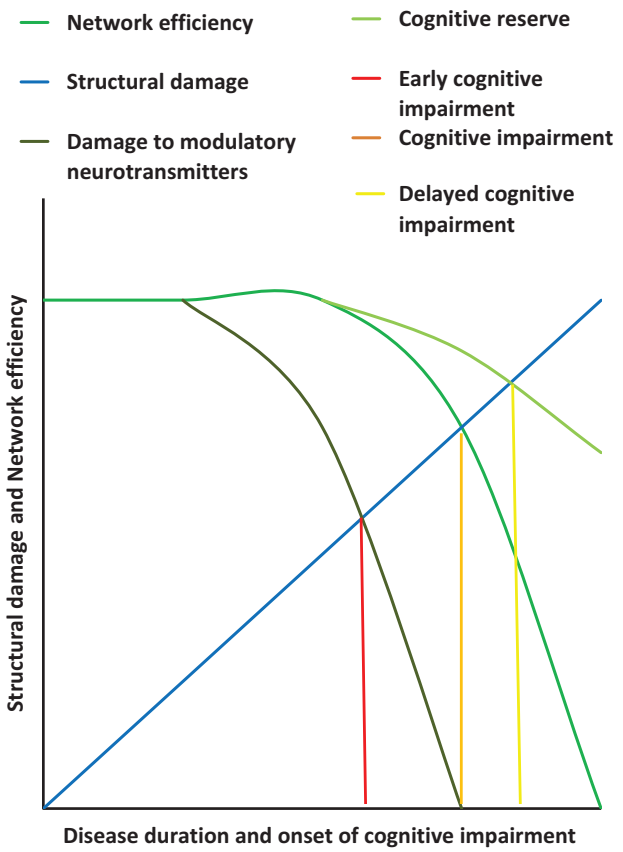

FIGURE 7 Factors contributing to the onset of cognitive impairment in MS patients. Probably both structural damage and network collapse contributes to the onset of cognitive impairment in MS patients. Damage to the regulation of strategic modulatory neurotransmitters may expedite cognitive impairment, while cognitive reserve may delay such a collapse

the presence of structural brain changes (Sumowski et al., 2013). In line with this, we have recently published results on cognition and gray matter changes from the same cohort showing that the patients performed well on all domains of cognition tested, in spite of widespread gray matter thinning (Nygaard et al., 2015).

We identified different pupillary responses between LCS controls and LCS patients. As expected, the LCS controls had larger task-related pupillary responses to the easy mathematical task, which indicated that they experienced a higher processing load than the NCS controls. The LCS patients lacked this extra recruitment. On the contrary, the LCS patients had a tendency toward a smaller task-related pupillary response than the NCS patients. We propose that this reduced response to cognitive tasks may index a neural deficit that can contribute to a worse cognitive performance.

Although the patients and controls had similar task-related pupillary responses on a group level, we observed that some of the fatigued patients had different response curves. These patients appeared to have a reduced and more prolonged pupillary response to problem-solving than healthy controls. We propose that this reduced response to the cognitive tasks may index a neural deficit, likely affecting

connectivity of brain areas to the norepinephrine-Locus Coeruleus system that will in turn be expressed in the pupil and contribute to a worse cognitive performance.

Interestingly, studies of patients with Alzheimer's or Parkinson's disease have shown an association between a slower pupillary light reflex and cognitive impairment, indicating that a cholinergic deficiency (affecting the parasympathetic branch of oculomotor control on the pupillary muscles) may be related to both the cognitive and the autonomic alterations in these patient groups (Fotiou et al., 2009). Thus, a central autonomic dysregulation in neurological diseases may be associated with cognitive dysfunction.

The smaller pupillary response in some fatigued patients observed in this study suggest central tiredness as the causal link between fatigue and pupillary responses. Lowenstein and Loewenfeld proposed a model of disintegration of central control due to post-task fatigue expressed as a change in the shape of the pupillary light reflex curve (Lowenstein & Loewenfeld, 1951), and it is also known that sleep deprivation changes the pupillary light reflex curves (Wilhelm, Wilhelm, Lüdtke, Streicher, & Adler, 1998). As fatigue and sleep disorders are tightly linked in MS patients (Veauthier et al., 2011), the altered pupillary response in our subgroup of fatigued MS patients may be related to a central tiredness caused by changes in Locus Coeruleus projections to sleep-promotion nuclei (Szabadi, 2013). Both patients and controls were randomly examined throughout the working hours; therefore, the effect of sleepiness in both groups should be evenly distributed. The baseline measures before the first cognitive task shows no difference between the groups and suggests that we have corrected for different levels of wakefulness. In normal sleepiness, we would have expected to find a reduced pupillary diameter at baseline if one of the groups was significantly more sleepy than the other (Wilhelm et al., 1998). Our results may therefore touch upon the true nature of fatigue as a condition different from sleepiness. This could in part explain why testing of autonomic instability by pupillary unrest (PUI) measurements done by Egg et al. did not show an association with MS-related fatigue, since PUI is a test of arousal and sleepiness (Egg, Högl, Glatzl, Beer, & Berger, 2002). Studies of the association between fatigue, cognition, and autonomic dysfunction in MS have shown conflicting results and warrants further research (Flachenecker, 2007; Flachenecker et al., 2003; Keselbrener et al., 2000).

While fMRI identifies altered recruitment of neural networks, the pupillometry results presented here could indicate a central autonomic dysregulation caused by reduced activity in the cognitive alert system of MS patients. Both an ongoing inflammatory process in MS patients and a more disseminated disease could lead to a decreased autonomic response, expressed as reduced pupillary dilation in response to cognitive tasks (Wilhelm & Wilhelm, 2003). This is in line with other studies of the pupillary light reflex in early MS patients, that have identified both sympathetic and parasympathetic disturbances without associations to neurological disability (Pozzessere et al., 1997; Surakka et al., 2008).

Reduction in CNS's noradrenergic levels and damage to Locus Coeruleus neurons have been described both in the experimental autoimmune encephalitis (murine MS model) and in autopsies of chronic MS patients, (Polak, Kalinin, & Feinstein, 2011). The same group has found that Locus Coeruleus damage increases the symptom severity in experimental autoimmune encephalitis, and that increasing noradrenergic and noradrenergic precursor levels can reverse this effect (Simonini et al., 2010). Treatment of experimental autoimmune encephalitis mice with a vincamine derivate vindeburnol, which temporarily depletes CNS noradrenergic and promotes an upregulation of Locus Coeruleus's noradrenergic levels and metabolism, leads to a reduction in experimental autoimmune encephalitis symptoms (Polak et al., 2012). A randomized controlled trial of treatment to normalize noradrenergic levels of the CNS (lofepramine, phenylalanine, and B12) in 69 MS patients in different stages of the disease lead to a reduction in MS symptoms in the treated patients (Wade, Young, Chaudhuri, & Davidson, 2002) and, in a small subgroup of patients, to a reduction in hypointense T1 lesions and a slower atrophy rate on MRI (Puri et al., 2001). Thus, there are indicators, though limited in number and based on small and mixed patient samples, that Locus Coeruleus's noradrenergic levels are reduced and that a reversal of this reduction may improve symptoms in both animal models and MS patients. This Locus Coeruleus -noradrenergic pathology may explain the small task-related pupillary response of some patients in this study.

A recent opinion paper has proposed that cognitive impairment in MS occurs as a result of network collapse (Schoonheim, Meijer, & Geurts, 2015). We propose that such network collapse could be expedited by a damage to the regulation of modulatory neurotransmitters. Cognitive reserve, on the other hand, may delay such a collapse. We therefore expand Schoonheim and colleagues' model of cognitive impairment as illustrated in Figure 7.

Our study has several limitations. First, these are the baseline results of a cohort study. We do not have longitudinal data and cannot draw conclusions concerning causality. Surprisingly, the patient group was cognitively well-functioning and we therefore could not study a cognitively impaired patient group. Studies of patients with a longer disease duration and a larger proportion of patients with low cognitive scores should be performed to assess whether a reduced Locus Coeruleus's activation may be linked to cognitive impairment, depressive symptoms, and fatigue in MS patients. Further, MS pathology may cause both focal and global CNS damage. We have as far as possible corrected for confounding factors by performing careful ophthalmological examinations, age and gender matching of patients and controls, similar timing of the examinations, and identical test situations, but we cannot distinguish between a local Locus Coeruleus's dysfunction and other CNS changes as a cause. Medications such as antidepressants could have interfered with autonomic function. Our participants were asked for a full list of currently used medications and only one patient was on antidepressive treatment (amitriptylin hydrochlorid). In this study, we did not test for clinical symptoms of dysautonomia which would have added further insight into these complex possible neurological associations. This warrants further studies including test for differences in patients with low versus normal cognitive score and fatigued versus non-fatigued differ in terms of autonomic function.

The experiment consisted of mathematical tasks of increasing difficulty and some of the participants may have been particularly

stressed by the nature of the tasks, possibly leading us to measure a stress response instead of just cognitive load. With our study design, we cannot distinguish between the autonomic response caused by stress as in fear of not mastering the task, and by alertness caused by the mental load applied to perform the actual task. A normal stress response depends on a state of alertness. Studies of the association between dysfunction of alertness and fatigue have found that these two phenomena are closely correlated in MS patients (Weinges-Evers et al., 2010). Indeed deficits in alertness networks have been found in MS patients and could explain some aspects of cognitive dysfunction in these patients (Urbanek et al., 2010). Thus, a possible difference in autonomic response between patients and controls could be caused by dysfunctional alertness networks leading to a different stress response in the patients. Another limitation is the fact that the healthy controls were not tested for fatigue and depression. This would have contributed to clarify the impact on cognitive performance in healthy individuals and it would have been an additional strength to our analyses. We did not interview our participants about sleep problems and the distinction between fatigue and sleepiness/alertness is difficult to draw only based on FSS and baseline pupil size, this must be taken into account when drawing conclusion about these results. Due to the design of the experiment, it is difficult to evaluate the effect of the hard tasks, as the error rate was very high. Future studies should therefore attempt at a design in which the effect of the increasing task difficulty would be easier to monitor.

Our relatively limited number of participants in the subgroup analyses constitutes another limitation of our study and warrant further replication in a larger sample size to prove the usefulness of pupillary responses as a reliable proxy to test levels of central neuropeptides.

In conclusion, this may be the first study to investigate pupillary responses to cognitive tasks in MS patients. The pupillary responses were similar comparing patients and controls. We propose that a reduced response to cognitive tasks may index a neural deficit that can contribute to a worse cognitive performance. Further, some fatigued patients did not show the same activation as the healthy controls, indicating that the Locus Coeruleus's response in MS patients with symptoms of fatigue may be altered.

Pupillometry research in psychiatric patients has had a sharp increase in recent years, mainly due to the reduced costs and the easy administration compared to MRI. Accordingly, the knowledge about pupillometry in patients with psychiatric diseases has accumulated (Graur & Siegle, 2013). With basis in our evidence that task-related pupillary responses are preserved in patients with a history of ON or brainstem lesions and preserved vision, we argue that this method could be useful in future studies of cognition, fatigue, and eventually psychiatric symptoms in MS patients longitudinally from early on in the disease course and preferably in combination with autonomic testing and fMRI.

Finally, we propose pupillometry as a valid method for unveiling task-related changes of attention that may link cognition and fatigue in multiple sclerosis patients.

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CONFLICT OF INTEREST

None declared.

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III

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Neurodegenerative Interplay of Cardiovascular Autonomic Dysregulation and the Retina in Early Multiple Sclerosis

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Introduction: Autonomic nervous system (ANS) symptoms are prevalent in multiple sclerosis (MS) as is neurodegeneration. Our aim was to explore the occurrence of ANS symptoms and retinal neurodegeneration in a newly diagnosed MS population with tools available in a clinical setting.

Methods: Forty-three MS patients and 44 healthy controls took part in the study. We employed a bedside cardiovascular ANS test battery together with classical pupillometry, optical coherence tomography (OCT) evaluation of retinal neurodegeneration in eyes without previous optic neuritis (MSNON) and patients' self-report forms on fatigue, orthostatic and ANS symptoms.

Results: Half of the patients presented with ANS symptoms and a high level of fatigue. There was a significant difference in ganglion cell layer thickness (mean GCIPL) evaluated by OCT in MSNON compared to healthy control eyes. We found a negative linearity of mean GCIPL on group level with increasing disease duration. Three patients fulfilled the criteria of postural orthostatic tachycardia syndrome (POTS).

Conclusion: Our results demonstrate retinal neurodegeneration in MSNON, a high frequency of fatigue and a high prevalence of ANS symptoms in newly diagnosed patients. Whether neurodegeneration precedes ANS dysfunction or vice versa is still open to debate, but as unveiled by the presence of POTS in this MS population, differences in stress-response regulation add to the understanding of variation in onset-time of ANS dysfunction in early MS.

Keywords: multiple sclerosis, neurodegeneration, autonomic nervous system, optical coherence tomography, pupillometry, fatigue, postural orthostatic tachycardia

INTRODUCTION

Multiple sclerosis (MS) is a chronic disease characterized by inflammation and neurodegeneration in the central nervous system (CNS). The disease course is highly variable and the cause is unknown (1). MS patients often present symptoms of autonomic dysfunction (2, 3). Autonomic dysfunction and neurodegeneration seem to be linked in MS (2). Correlations between autonomic symptoms and specific parts of the CNS exist (4, 5), but studying autonomic dysfunction in MS is complicated because of the plasticity of this system and its' widespread activation. This complexity may in part be the reason why there is no common standardized autonomic test battery for MS.

The visual system offers a possibility to observe both the autonomic nervous system (ANS) and ongoing neurodegeneration utilizing pupillometry and optical coherence tomography (OCT). Classic study design, measuring the pupillary light reflex (PLR) with defined indices for parasympathetic and sympathetic function (6–9), has yielded changes in ANS function in MS patients (10–12). A link between PLR values and retinal architecture in MS patients has also been showed in advanced stages of the disease (13). OCT performs optical slicing of the retinal layers evaluating both the ganglion nerve cell layer and their peripapillar axons before leaving the eye and continuing as the optic nerve (14). OCT studies in MS have shown neurodegeneration in eyes with and without prior optic neuritis (MSNON) (15, 16). In order to examine the presence of ongoing neurodegeneration in early MS, we chose to examine MSNON.

MS may affect the ANS not only through neurodegeneration, but also by modulating the sympathetic nervous system peripherally through catecholamine release from inflammatory lesions, or by inflammatory induced expression of catecholamine receptors (17). Symptoms of orthostatic intolerance are frequent in MS (18–20) and may be explained as a result of inflammatory stress such as in orthostatic tachycardia syndrome (POTS) (21).

A recent ANS meta-analysis in MS (22) included 16 studies with over 600 patients tested with three or more cardiovascular autonomic tests showed a high rate of cardiovascular dysfunction among patients with MS.

Employing for the first time a clinical bed-side cardiovascular ANS test battery together with classical pupillometry and OCT evaluation of retinal neurodegeneration, our aim was to study early signs of ANS dysfunction and its' relation to neurodegeneration in a newly diagnosed MS population.

MATERIALS AND METHODS

Participants

Newly diagnosed relapsing remitting MS (RRMS) patients ($N = 49$), according to the revised McDonald Criteria (2010) (23) and healthy controls ($N = 46$), enrolled in an ongoing longitudinal prospective study on cognition and neuroimaging (24) were invited to participate in this study of autonomic pupillary function in relation to retinal architecture. Forty-three RRMS patients and 45 healthy controls were eligible for analyses. A subset of the included MS patients ($N = 37$) were examined with a set of self-report forms as well as bed-side orthostatic blood pressure (BP) and heart rate tests ($N = 31$). A flow chart of the included participants is presented in **Figure 1**.

Measurements were randomly conducted throughout the day for both groups. Patients and controls were examined during the same period, but the examiners were not "blinded" regarding the status of the participants.

All participants gave written informed consent and the study was approved by the regional ethical committee of South Eastern Norway (REK 2011/1846 A).

Neurological and Neuropsychological Examinations

The patients had a complete neurological examination and cerebral magnetic resonance imaging (MRI) performed within 2 weeks of the ophthalmological and pupillary measurements. All patients were clinically stable between the examinations. Grading of neurological disability was assessed using The Expanded Disability Status Score (EDSS) (25). Fatigue was assessed with the Fatigue Severity Scale (FSS) (26). We applied a cut off at FSS > 4 classifying the patients as fatigued.

Magnetic Resonance Imaging (MRI)

The cerebral MRI scans were performed on the same 1.5 Tesla scanner (Avanto, Siemens Medical, Erlangen, Germany) equipped with a 12-channel head coil. The following sequences were acquired: (1) sagittal 3D FLAIR, (2) pre-contrast sagittal 3D T1 MPRAGE, and (3) post-contrast sagittal 3D T1 MPRAGE started approximately 7 min after the contrast agent injection at a dose 0.2 ml/kg (Dotarem, Laboratoire Guerbet, Paris, France). All scans were evaluated by one neuroradiologist (PS) (blinded to clinical symptoms and findings in the patients) for the presence of brain stem lesions. The location of the lesions was registered (pons or/and medulla oblongata).

Ophthalmological and Pupillometric Examinations

The patients underwent an ophthalmological examination, including the swinging flashlight test. For all the participants we measured best corrected visual acuity (BCVA) expressed as the logarithm of the minimum angle of resolution (logMAR), in both eyes. Spherical equivalent was calculated and noted in diopters.

The PLR was tested with the Compact Integrated Pupillograph (CIP) version 13.00 from AMTech (Dossenheim, Germany) on both eyes in the patients and randomly on one eye in the healthy controls as far as in 16 who underwent examination of both eyes to balance the number of eyes in the three eye groups as described in the flow chart (**Figure 1**).

Dark adaptation for 5 min preceded the tests which were conducted with a fixated gaze, but without accommodative cues to avoid confounding pupillary constriction. Measurements were undertaken in darkness with chin and forehead rest in fitted position. The tests were conducted by the first author. When the trigger button was pushed by the examiner a clear yellow visible LED (585 nm) omitted an optical stimulus for 200 ms with an intensity of 784 cd/m^2 while 2 infrared (880 nm) bluish gray ones illuminated the tested eye and the acquisition of the horizontal

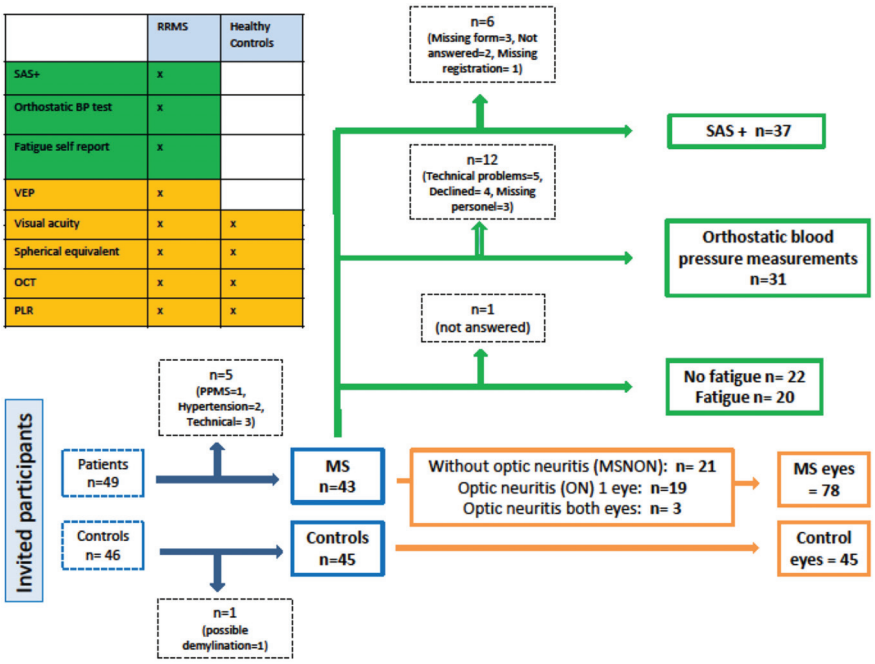

FIGURE 1 | Study flow chart. SAS+, Survey of Autonomic Symptoms and orthostatic symptom scores from the Autonomic Symptom Profile; BP, Blood pressure; VEP, Visual evoked potential; OCT, Optical coherence tomography; PLR, Pupillary light reflex; RRMS, Relapsing remitting multiple sclerosis; PPMS, Primary progressive multiple sclerosis; MS, Multiple sclerosis.

pupil diameter was measured with a sampling rate of 250 Hz during 4 s (2 s longer than the default setting from AMTech). The data was directly transferred to a computer with LoOK! software (AmTech) installed for storage and further analyses. Continuous measurement of the pupillary diameter permitted repeated measures of PLR with attended baseline diameter between each measurement. In this study 4 valid measurements were stored and used for further analyses for each included eye.

Various variables describing the function of the autonomic nerves in the eye were calculated according to previous publications (7, 8). LoOK! automatically calculates mean values for the initial diameter (the dark adapted diameter, hereby called the baseline) before each stimulus, the latency from the stimulus is given to the first sign of contraction, the constriction velocity and the amplitude of the contraction. The amplitude and the constriction velocity are dependent on the parasympathetic branch of the PRL. Whereas the 75% re-dilation time and velocity, extracted using a separate software application from AMTech made for this study, is mostly dependent on the sympathetic branch of the PLR.

Optical Coherence Tomography (OCT)

Optohistologic slicing of the retina was carried out with the spectral domain RS-3000 OCT Retina Scan (Nidek Inc., CA, USA) with a scanning speed of 53,000 A-scans/s and 4 µm digital resolution. The examinations were performed by the first author (SRB). Peripapillary retinal nerve fiber layer thickness (pRNFL) data were obtained with the Disc Circle protocol with a scan width of 3.45 mm centered on the optic nerve head without crossing of the two inner scan circles. The ganglion cell layer (GCL+IPL) thickness measurements were automatically generated from the 9 × 9 mm macula map scan glaucoma segmentation in the included software. All scans included had a signal strength of 8/10 or better.

Visual Evoked Potentials (VEP)

Visual evoked potentials (VEPs) delay to P100 were obtained as described previously (27) and were evaluated by two experienced clinical neurophysiologists (KN and LE). According to the laboratory-specific reference values, VEP was regarded as pathological with a P100 latency of more than 110 ms and/or with a difference in delay of at least 6 ms between the eyes.

Autonomic Self-Reports and Bed Side Orthostatic Testing

The patients conducted the self-report Survey of Autonomic Symptoms (SAS) questionnaire (28) consisting of 11 items in women and 12 in men. SAS aims to discover mild autonomic neuropathic symptoms. It is brief and easy to score, but has not previously been tested in early MS patients. We also used questions from the Autonomic Symptom Profile (ASP) (29) covering orthostatic symptoms with timeline. The SAS questionnaire and the ASP questions have been properly translated (and back translated) to Norwegian for another study (30).

As described in the original article of Zilliox et al. the patients were grouped according to SAS-score > 3, while the total impact score (TIS) cut off was > 7. A 4 points' cut-off as described by Suarez et al. at, was used to define orthostatic intolerance. The orthostatic sub-score provided by ASP both graded the complaints and their duration.

Bedside orthostatic testing was performed by the first author the same day as the ophthalmological examinations. The examination room was normally lit, with regulated heating and air conditioning and without any surrounding disturbances. BP and heart rate measurements were conducted automatically at preset intervals with a Dinamap procare V100 (GE Healthcare). Baseline was established with the participant comfortably in supine position for 10 min before asked to stand up for 5 min to calculate orthostatic mean values after 2, 3, 4, and 5 min of standing. The participants were then asked to lay down again for another 2 min for repeated measurements.

Statistical Analyses

SPSS version 24, Chicago, IL was used for statistical analyses. Independent samples *t*-test were used to test for differences between patients and controls. Linear regression analyses were used to assess the association between signs of retinal neurodegeneration and disease duration. A significance level of 0.05 was applied for all analyses. Levene's test was used to assess homogeneity of the population variances. A Pearson correlation coefficient was produced to assess the relationship between variables.

RESULTS

Ophthalmic and PLR Group Characteristics

Newly diagnosed patients with low EDSS and healthy controls were matched on age and gender as summarized in **Table 1**. Time since diagnosis is of shorter duration than disease duration and is due to the fact that patients often have had symptoms in concordance with MS for some time when the diagnosis is made. Half of the patient group presented with a history of optic neuritis, in all patients more than 3 months prior to the examinations. Brain stem lesions on MRI were visible in 56%.

The ophthalmic characteristics of all the examined eyes are summarized in **Table 2**. Eyes with prior optic neuritis, confirmed with the presence of prolonged VEP latency to P100, differed from the two other groups concerning retinal architecture and latency of the PLR.

For further analyses we therefore used one asymptomatic eye from the patients and one eye from the healthy controls to study the link between neurodegeneration and dysautonomia.

Table 3 presents a comparison of MSNON eyes compared to the healthy controls, one eye from each participant. It shows no difference in BCVA or in spherical equivalent between patients and controls. There was a difference in the ganglion cell layer thickness in the MSNON eyes compared with the healthy controls. There was no difference in the peripapillary nerve fiber layer (pRNFL) or in the PLR latency. Patients and healthy controls were not different concerning PLR measures of parasympathetic and sympathetic function.

TABLE 1 | Group characteristics of patients and controls.

	Patients ($n = 43$)	Controls ($n = 45$)	*P* value
Age, years, mean (SD, range)	34.23 (6.96, 21–49)	33.20 (6.91, 21–46)	0.487
Gender, female, *n* (%)	31 (72)	31 (69)	0.884
Neurological disability, EDSS, mean (SD, range)	1.88 (0.87, 0.0–4.0)	n.a.	
Time since diagnosis, years, mean (SD, range)	1.47 (1.0, 0.08–3.58)	n.a.	
Disease duration, years, mean (SD, range)	2.02 (2.53, 0.33–10.67)	n.a.	
Optic neuritis, *n* (%)	22 (51)	n.a.	
Presence of brain stem lesions on MRI, *n* (%)	24 (56)	n.a.	

EDSS, The Expanded Disability Status Score; SD, Standard deviation; MRI, Magnetic resonance imaging.

TABLE 2 | Characteristics of eyes with (ON eyes) and without previous optic neuritis (MSNON) and eyes of healthy controls.

	ON eyes ($n = 23$)	MSNON eyes ($n = 55$)	Control ($n = 45$)
Spherical equivalent, in diopters, mean (SD)	−0.91 (1.17)	−0.69 (1.57)	−0.79 (1.66)
Best corrected visual acuity in logMar, mean (SD)	−0.02 (0.10)	−0.07 (0.08)	−0.09 (0.01)
pRNFL in μm, mean (SD)	92.90 (12.86)	101.17 (10.94)	104.33 (12.18)
tRNFL in μm, mean (SD)	60.35 (17.37)	68.13 (13.78)	68.16 (11.53)
Ganglion cell layer + inner plexiform layer, superior half, μm (SD)	91.80 (13.49)	101.85 (8.68)	105.96 (8.20)
Ganglion cell layer + inner plexiform layer, inferior half in μm, mean (SD)	93.25 (11.39)	102.72 (7.91)	107.22 (8.12)
PLR baseline in mm, mean (SD)	6.42 (0.82)	6.50 (1.01)	6.60 (0.96)
Pupillary light reflex latency in ms, mean (SD)	251.50 (16.21)	240.28 (13.83)	239.69 (15.68)
Latency p100 in ms, mean (SD)	118.59 (10.56)	104.00 (4.33)	–

pRNFL, Peri-papillar retinal nerve fiber layer; tRNFL, Temporal section of peri-papillar retinal nerve fiber layer; PLR, Pupillary light reflex.

Linear regression showed lower mean GCIPL with longer disease duration as presented in **Figure 2**.

Autonomic Bed Side Orthostatic Testing

Results of the bed side orthostatic testing are presented in **Table 4**.

Seven patients (23%) presented test results compatible with postural orthostatic tachycardia syndrome (POTS) with sustained heart rate increase of ≥30 beats/min within the first 5 min of standing and without signs of orthostatic hypotension. Three of those reported having had symptoms for a longer period than 3 months fulfilling the diagnostic criteria for POTS (31). The patients with POTS had a mean age = 30.3 years (range 29–33); mean EDSS = 1.3 (range 1.0–1.5), and a mean FSS = 2.7 (range 1.3–4.7) representing 10% of the test group.

Self–Reports

ANS, orthostatic and fatigue symptoms are presented in **Table 5**. Upon subgrouping, according to standardized cut-off values (28) our patient group showed that 43% reported symptoms of autonomic dysfunction (SAS $\geq$ 3) and 32% had pronounced symptoms (TIS $\geq$ 7).

The patients presented a mean score above 4 on the orthostatic questionnaire, revealing a significant level of symptoms. We also discovered that almost half of the patients (45%) reported a high level of fatigue with FSS $\geq$ 4 based on the fatigue questionnaire.

Correlation analysis between FSS and SAS showed a Pearson coefficient of 0.44 ($r = 0.044$, $n = 42$, $p = 0.004$). There were no significant differences in the mean FSS scores between patients with or without ANS symptoms.

TABLE 3 | Eye characteristics of MS patients without previous optic neuritis (MSNON) and the healthy controls.

	MSNON ($n = 38$)	Control ($n = 45$)	p-value
Spherical equivalent in diopters, mean (SD)	−0.82 (1.58)	−0.79 (1.66)	0.94
Best corrected visual acuity in LogMar, mean (SD)	−0.07 (0.09)	−0.08 (0.09)	0.65
pRNFL in μm, mean (SD)	100.24 (10.72)	104.33 (12.18)	0.11
tRNFL in μm, mean (SD)	67.26 (13.32)	68.16 (11.53)	0.74
Mean ganglion cell layer + inner plexiform layer in μm, mean (SD)	102.30 (7.87)	106.59 (8.02)	0.02
PLR baseline in mm, mean (SD)	6.54 (0.97)	6.60 (0.96)	0.78
PLR latency in ms, mean (SD)	240.87 (13.46)	239.69 (15.68)	0.71
Constriction velocity in mm/s, mean (SD)	5.7 (0.69)	5.50 (0.77)	0.40
Time to minimum diameter, s, mean (SD)	0.84 (0.06)	0.83 (0.08)	0.74
75% redilatation velocity in mm/s, mean (SD)*	2.80 (1.55)	3.15 (1.87)	0.82
75% redilatation time in mm, mean (SD)*	2.64 (0.51)	2.68 (0.62)	0.48

**MSNON, n = 32, and controls, n = 41. Pupillary light reflex = PLR. pRNFL, Peri-papillar retinal nerve fiber layer; tRNFL, Temporal section of peri-papillar retinal nerve fiber layer; PLR, Pupillary light reflex.*

Test Battery

Of the 31 patients who undertook all the tests in our study no one presented with findings on the PLR test. However, 74% reported significant findings on the self-report questionnaire SAS+, and 23% had signs of orthostatic dysfunction on bed-side testing. As shown in **Table 6** 16% were positive on these latter two tests.

DISCUSSION

We found a significant difference in mean GCIPL in MSNON eyes compared to matched control eyes. Mean GCIPL showed as expected a negative linearity with increasing disease duration. There was no difference in mean pRNFL between MSNON and healthy controls. If there is a primary retinal neurodegeneration with a functional correlate to pick-up on early it is in the macula where our visual acuity is situated and the ganglioncell density is the highest that one would be able to detect it first.

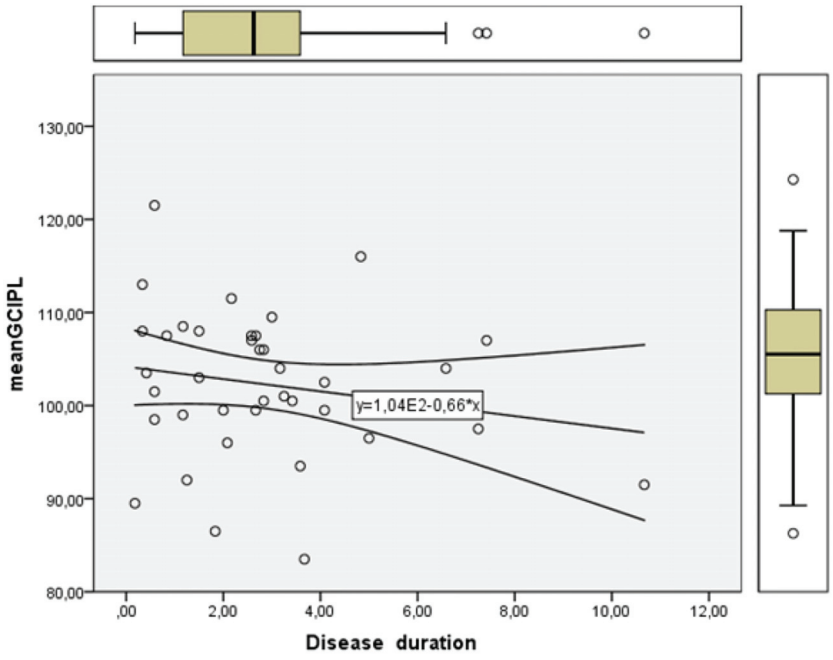

FIGURE 2 | Linearity plot of retinal ganglion cell layer thickness in μm (mean GCIPL) measured with optical coherence tomography showing a gradual reduction with increasing disease duration in years.

TABLE 4 | Orthostatic bed side tests.

	Baseline		Standing			
			Mean of 2 and 3 min standing		Mean of 4 and 5 min standing	
	Mean BP (SD), mmHg	Mean HR (SD), per min	Mean BP (SD), mmHg	Mean HR, (SD) per min	Mean BP (SD), mmHg	Mean HR (SD), per min
All included patients ($n = 31$)	109 (8)/64 (6)	58 (8)	112 (11)/69 (8)	76 (12)	113 (9)/69 (8)	76 (13)
HR increase ≥30 beats/min ($n = 7$)	110 (8)/64 (5)	57 (11)	112 (9)/70 (7)	88 (10)	113 (6)/70 (8)	90 (11)

BP, Blood pressure; HR, Heart rate.

Secondary degeneration of the axons from these dead neurons would also occur and possibly be measured as a loss of pRNFL on OCT, especially in the temporal region where the axons from the papillomacular bundle lies and the axons are the thinnest and most vulnerable. An ongoing loss of retinal ganglion cells without any previous clinical signs of inflammatory relapses or significant loss of retinal axons depicted with the peripapillary ring scan on OCT is described in MS (15), and confirmed by the present study. Nonetheless our results underline the premature emergence of retinal neurodegeneration. Longitudinal studies of patients diagnosed with clinically isolated syndrome may confirm if this neuronal loss is an onset feature of MS.

Half of the patients presented with ANS symptoms and a high level of fatigue in line with previous reports (2, 32). We found a medium level of correlation between the SAS score and the FSS score and no significant difference between patients with or without ANS symptoms. Self-reports are assessments of self-perceived health status often designed for population studies. The choice of tests is important to ensure its' relevance and proven efficacy regarding what we are searching for and in our case there are few tests that cover ANS symptoms in early MS and publications are scares. One might object to the use of self-reports on the basis of subjectivity since patients might over rate their symptomatic burden and they may also find the tests tiresome and become biased toward their inner feeling of distress produced by the fact that we ask them to complete the questionnaire.

Even though we did not find classic orthostatic hypo-or hypertension, 23% of the MS patients presented a high HR response upon bed side testing. Three patients (10%) fulfilled the criteria (33) of POTS in line with the few prior MS studies on this field (34, 35). Our patients with POTS tended to be young patients with a low EDSS score, only one had fatigue. The diagnostic recognition of this entity is important in order not only to treat the symptoms and to help patients resume their regular daily activities and work life, but it is also in line with the hypothesis of an altered stress response in MS (36) occurring early in the disease course. As described in a recent review "the onset of POTS is typically precipitated by immunological stressors" (21), this underlines the fact that POTS patients often present with positive autoimmune tests and immune system dysfunction. Cardiovascular autoantibodies with a causative role have been proposed in POTS, but another hypothesis is the presence of abnormally increased sympathetic activity alongside findings of peripheral autonomic neuropathy. Studies of cardiovascular risk in MS support this latter theory with upregulation of the sympathetic stress response early in the disease course (37). However, the present findings do not indicate that peripheral neuropathy can explain the POTS findings among our patients. A schematic illustration of different profiles of neurodegeneration, ANS symptoms and stress-response in early MS is proposed in **Figure 3**. As this theoretical scheme underlines, we are faced with changes in highly adaptive and complex systems when studying the central nervous and the immune system. The finely tuned communication between these systems in healthy subjects, is disrupted and probably a highly pathogenic factor in MS due to an excess of inflammatory processes that may be further amplified by coexisting psychological distress (33).

TABLE 5 | Patients self-reported autonomic symptoms and fatigue.

		MS, *n* (%)	Mean score (SD)
Survey of Autonomic Symptoms (=SAS)		37	2.2 (1.4)
	SAS score of <3 points	21 (57)	1.1 (0.7)
	SAS score of 3 or more points	16 (43)	3.6 (0.5)
Total impact score (=TIS)		37	5.3 (4.1)
	TIS score of <7 points	25 (67)	2.9 (1.9)
	TIS score of 7 or more points	12 (32)	10.3 (2.6)
Orthostatic subscore		36	4.3 (2.5)
Fatigue Self Report (FSS)		41	3.8 (1.9)
	FSS score of <4 points	23 (55)	2.6 (0.9)
	FSS score of 4 or more points	18 (45)	5.3 (0.9)

The sympatho-adrenergic branch of the ANS is particularly important in the cross-talk between the immune system and the CNS. Both adrenalin and noradrenaline are synthesized from dopamine which has known immune effects (38). Hypothetically for some MS patients an initial overshooting sympathetic upregulation caused by inflammatory stress may be followed by a negative feed-back loop that counteracts the innate immunosuppressive effect of adrenaline and noradrenaline. Hence the immune cell driven neurodegeneration in the CNS

TABLE 6 | The total of patients with findings either on the autonomic symptom questionnaire, the orthostatic bed-side test, or testing of the pupillary light reflex.

	SAS+, *n* (%)	**Bed side test with HR ≥ 30 beats/min, *n* (%)**	**PLR, *n* (%)**	**SAS+ AND Bed side test with HR ≥ 30 beats/min, *n* (%)**
Patients participating in all the tests $n = 31$	23 (74)	7 (23)	0 (0)	5 (16%)

SAS+, Survey of Autonomic Symptoms together with orthostatic symptom scores from the Autonomic Symptom Profile; PLR, Pupillary light reflex.

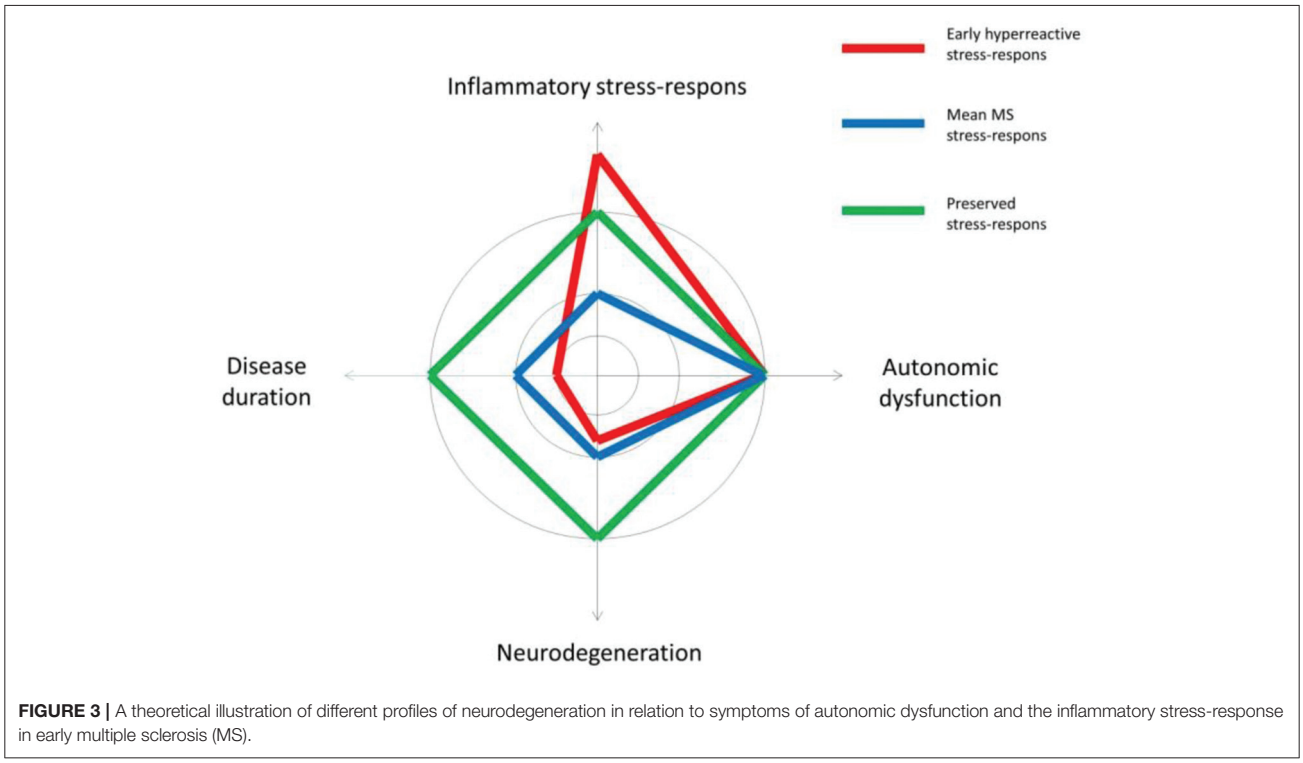

FIGURE 3 | A theoretical illustration of different profiles of neurodegeneration in relation to symptoms of autonomic dysfunction and the inflammatory stress-response in early multiple sclerosis (MS).

in MS is in these cases free to expand itself. Dopamine on the other hand is synthesized from phenylalanine through several steps and metabolomics have showed diminished levels of phenylalanine in the cerebrospinal fluid of MS patients (39), this could both counteract the innate immunosuppressive effect and alter the ANS output. Further studies focusing on the connection between ANS dysfunction, dopamine and phenylalanine levels could give insight into what causes the cardiovascular and neurodegenerative interplay in MS.

This study employed bedside tests with equipment available in most hospital settings together with a classical research tool, the pupilometer, in order to search for a link between early signs of neurodegeneration and ANS dysregulation in MS. The bedside tests are designed to discover orthostatism and POTS related to alterations in ANS regulation of cardiovascular function. Our test battery would have been more sensitive and complete regarding early cardiovascular ANS changes if supplemented with a Valsalva maneuver test and continuous BP measurements (40). Previous PLR studies (4, 10, 11, 41) have mostly studied patients with advanced stages of MS and a possibly much higher degree of retinal ganglion cell and axonal loss. However, this is the first time OCT is used in conjunction with an ANS test battery consisting of other test than PLR measurements.

There was no difference in the retinal nerve fiber layer depicted with the peripapillar ring scan on OCT or in the PLR latency between MSNON and healthy controls. Changes in PLR measures could then have been interpreted as alterations in ANS branches of the PLR since the optic nerve had no signs of disease affection. We did not find any changes in the PLR comparing patients and controls in this study, underlining the robustness of the eye pupil as a proxy of function and usability in other settings as for instance as our group has published earlier, in cognitive testing in MS (27).

Additionally the melanopsin-expressing retinal ganglion cells influencing the PLR are more resistant to injury like optic neuritis than other ganglion cells (42) and this adds to the fact that small structural retinal changes may not translate into PLR changes.

Our study is limited by the small subgroup numbers. Further testing of the concept of altered stress response in a larger group of patients is warranted. A study design

where both patients and controls are tested with all the tests is advisable in order to expand the normal reference standards. Health related quality of life scales would also add value to our self-reports repertoire. Another future aim is to establish robust structure –function correlates of retinal changes and PLR measurements, in other words at what level PLR results reflect structural retinal neurodegeneration and not ANS changes. Longitudinal cohorts may give the answer. PLR testing in the blue light spectrum of 400–500 nanometer (43) may disentangle at what level a possible loss of melanopsin containing ganglion cells affects ANS regulation of the pupil through input from the retina. Another interesting field in CNS and ANS cross-talk is the concept of interoception, the sensing of bodily signals, which is shown to be altered in MS (44) and should be considered in future MS ANS study design.

The autonomic test battery in our study is robust. We tested for ANS dysfunction and neurodegeneration employing the same input organ, the eye. Nevertheless, future studies may consider using a more extensive battery of ANS tests. We note that autonomic testing is a difficult domain and believe that establishment of screening tests batteries in the clinic will provide answers to the questions our patients are struggling with every day.

In conclusion, our study unveiled retinal neurodegeneration in asymptomatic eyes in newly diagnosed MS patients with a high frequency of fatigue and a high prevalence of ANS symptoms. Several of the patients had symptoms and signs consistent with POTS. The possible interplay between presymptomatic neurodegeneration, fatigue, and symptoms of autonomic dysfunction needs further investigation.

ETHICS STATEMENT

All participants gave written informed consent and the study was approved by the regional ethical committee of South Eastern Norway (REK 2011/1846 A).

AUTHOR CONTRIBUTIONS

SdRB wrote the manuscript, conducted the ophthalmological, pupillometric, optical coherence tomographic, and bed-side autonomic examinations as well as the statistical analyses. GN planned the study, conducted the neurological examinations, administered, and gathered all the self-report data and provided data for **Table 1**. KN contributed with study planning, analyzes of the VEPs and valuable input on how to interpret the autonomic findings and structuring of the manuscript. LE analyzed the VEPs. PS performed the MRI analyzes. MW-H conducted the neurological examinations. HH, LD, EK, and BL contributed with research planning and supervising the study. EC was the main supervisor of this study and gave valuable input throughout the whole process including statistical analyzes as well as structuring and writing of the manuscript. All authors reviewed and approved the final manuscript.

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