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II. Abbreviations

AASM	American academy of sleep medicine
ADL	Activities of daily living
αSYN	α-synucleinopathies
BDI	Beck's depression inventory
cMRF	Central mesencephalic reticular formation
CNS	Central nervous system
CTRL	Control
DAT	Dopamine transporter
DLB	Dementia with Lewy bodies
DLPFC	Dorsolateral prefrontal cortex
DMV	Dorsal motor nucleus of the vagus
EBN	Excitatory burst neurons
EEG	Electroencephalograms
EMG	Electromyograms
EOG	Electrooculograms
EW	Edinger Westphal nucleus
FDG	Fluorodeoxyglucose
FDGPET	18F-Fluorodeoxyglucose (18F-FDG)-positron emission tomography (PET)
FEF	Frontal eye field
FIX	Fixation
FV	Free viewing
GABA	Gamma-Amino-Butyric acid
GPE	External globus pallidus
IBN	Inhibitory burst neurons
INS	Spinal interneurons
ION	Inferior olivary nucleus
IPAST	Interleaved Pro/ Anti Saccade Task
iRBD	Isolated REM sleep behavior disorder

ITI	Inter-trial interval
LC	Locus coeruleus
MC	Motor cortex
MDS-UPDRS	The movement disorder society-unified Parkinson's disease rating scale
MIBG	Cardiac123I-Metaiodobenzylguanidine
MNS	Spinal motor neurons
MOCA	Montreal cognitive assessment
MRI	Magnetic resonance imaging
MSA	Multiple system atrophy
MSA-C	Multiple system atrophy cerebellar subtype
MSA-P	Multiple system atrophy parkinsonian subtype
NINDSSPSP	National institute of neurological disorders and stroke and the society for PSP
OPNs	Omnipause neurons
PD	Parkinson's disease
PDNMS	PD non-motor symptoms scale
PDRP	PD-related pattern
PET	Positron emission tomography
PMC	Premotor cortex
PPRF	Paramedian pontine reticular formation
PSG	Polysomnography
PSP	Progressive supranuclear palsy
PSTH	Peri-stimulus time histogram
RBDSQ	RBD screening questionnaire
REM	Rapid eye movement
riMLF	Rostral interstitial nucleus of the medial longitudinal fasciculus
SC	Superior colliculus
SD	Standard deviation
SLD	Sublaterodorsal nucleus
SN	Substantia nigra
SNc	Substantia nigra pars compacta
SNpr	Substantia nigra pars reticulata

SPECT	Single-photon emission computed tomography
SRT	Saccade reaction time
STN	Subthalamic nucleus
UMSARS	Unified multiple system atrophy rating scale
UPDRS	Unified Parkinson's disease rating scale
UPSIT	University Of Pennsylvania smell identification test-40
VMM	Ventromedial medulla

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I. Introduction

Neurodegenerative disorders occur when neurons lose their structure or function due to the process of neurodegeneration resulting in cell death (Oertel et al. 2012). Because the process of neuronal death is irreversible, these neurodegenerative diseases are incurable (Oertel et al. 2012). In order to prevent these disorders from occurring, biomedical studies have attempted to identify a variety of factors that affect the progression of neurodegenerative disorders, including age effects, genetics, environmental and systemic perturbations, diet, and pharmaceutical influences (Lees et al. 2009).

Accordingly, this dissertation investigates changes in the oculomotor function, the pupillomotor function, and the blink behavior 1) in two neurodegenerative disorders of the α-synucleinopathies (αSYN) type, i.e. in the relatively common disorder Parkinson's disease (PD) and in the rare disease Multiple-System Atrophy (MSA) in their manifest stage, 2) in isolated rapid eye movement (REM) sleep behavior disorder (iRBD)[1], which is accepted to be a specific prodromal stage of both PD and MSA and 3) – for comparison - in the manifest tauopathy Progressive Supranuclear Palsy (PSP) as a disease-control and 4) in healthy controls. To assess the changes in oculo- and pupillomotor function and in blink behavior, the Interleaved Pro/Anti Saccade Task (IPAST) and the newly developed method of Free Viewing (FV) are employed.

Until 2022, very few articles have discussed oculomotor and pupillomotor dysfunctions in manifest and prodromal αSYN (Hanuška et al. 2019; Perkins et al. 2021). In fact, the vast majority of the biomarkers for αSYN so far identified or proposed are related to motor and cognitive dysfunction and imaging of the central nervous system (CNS) (Miglis et al. 2021). We systematically examined saccades, pupil response, and blink behavior in manifest αSYN (PD and MSA) and their corresponding prodrome RBD as well as in PSP and healthy controls - with both methods (IPAST and FV) – as a novelty - in a comparative design. Wide ranges of eye movement parameters were quantified, and the patient groups were compared to control groups and each other.

[1] The dissertation has been simplified by using RBD rather than iRBD.

This study aimed to identify biomarkers in the oculomotor, pupillomotor, and blink domains among manifest and prodromal αSYN for possible use in future protection trials with disease-modifying compounds.

Ideally, a disease-modifying therapy should be administered in the prodromal phase of for example PD with the intention to delay or even prevent the manifestation of PD as defined by the presence of the cardinal motor symptom, akinesia combined with tremor at rest or rigidity (Miglis et al. 2021). With the inclusion of the RBD, this dissertation contains two further novelties: first) it provides the unique opportunity to systematically investigate RBD, the prodromal phase of PD and MSA in comparison to its manifest stages (PD and MSA) with the same design and second) presents for the first time, data in RBD versus PD and MSA with the technique of Free Viewing.

RBD has been identified as one of the most specific and common prodromal stages of PD and other αSYN such as dementia with Lewy bodies (DLB), and MSA (Iranzo et al. 2014). Up to 85% of patients with RBD progress to a neurodegenerative disorder of the type of αSYN within 10 to 20 years (Iranzo et al. 2014). However, the latency between RBD diagnosis and phenoconversion to αSYN is long, lasting many years to decades (Iranzo et al. 2014). Therefore, identifying patients with RBD who are likely to undergo phenoconversion using highly sensitive and specific prodromal biomarkers and progression markers is crucial.

In summary, the RBD population is an ideal candidate group to identify biomarkers for conversion and to benefit from disease-modifying therapies to delay or even prevent phenoconversion towards manifest αSYN groups.

The dissertation has five aims: 1) to confirm the limited data so far published in PD, MSA, and especially in RBD with IPAST (Hanuška et al. 2019; Perkins et al. 2021),

2) to generate data - for the first time ever - with the Free Viewing paradigm on changes in oculo- and pupillomotor function and blink behavior in PD, MSA, and RBD, 3) to perform the first comparative study in the three disorders PD, MSA and RBD under identical IPAST and identical FV conditions - with PSP and healthy subjects as control groups, 4) to identify - in combination with newly developed sophisticated algorithms for the analysis of IPAST and FV data - changes which are specific indicators (biomarkers) for PD versus MSA, 5) to investigate whether the changes identified in PD and/or MSA are already detectable (maybe to a smaller degree) in the prodromal stage RBD (for PD or MSA).

With this dissertation - to the best of our knowledge – the aims 2-5 (use of Free Viewing), are addressed for the first time ever in comparative research on PD, MSA, and RBD.

The ultimate aim of the study of this dissertation is to contribute biomarkers to the growing field of clinical trials with potential disease-modifying compounds not only in patients suffering from manifest PD and MSA (J. Levin et al. 2021; McFarthing et al. 2021) but in particular for future neuroprotective studies in subjects identified to still be in the prodromal stage of PD and MSA i.e. in RBD.

In the following section, we present the first paragraphs on the diseases investigated in the following sequence: the αSYN PD, MSA, and RBD, the tauopathy PSP followed by a section on sleep stages. The next section covers the anatomy and physiology of eye movement, pupil reaction, and blink response. It follows two sections on the two methods used: IPAST and Free Viewing, respectively.

I.1. Alpha-Synucleinopathies (αSYN)

Alpha-synucleinopathies (αSYN) are neurological disorders in which the protein alpha-synuclein forms pathological aggregates (Lees et al. 2009). These aggregates are toxic and damage certain classes of neurons (and glia cells) in the nervous system, which are particularly vulnerable to these aggregates. In this study, we have investigated patients suffering from the manifest αSYN PD or manifest αSYN MSA. Both diseases are characterized by the motor symptoms of akinesia and rigidity (Lees et al. 2009). PD also features about 70 % a tremor at rest, whereas the diagnosis of MSA requires the early presence of autonomic dysfunctions in the course of the disease. A further difference between PD and MSA is that MSA patients very often express symptoms of cerebellar dysfunction, whereas the cerebellum in PD is intact (Terao et al. 2019).

In the clinic especially in the early stages of the manifest disease, the clinical differential diagnosis is a challenge, as their clinical appearance can be very similar (Lees et al. 2009; Oertel et al. 2012; Terao et al. 2019). On the other hand, the situation for PD versus MSA patients is fundamentally different. For PD a highly effective symptomatic therapy is available (L-DOPA, dopamine agonists, and other pharmaca, in the late stage deep brain stimulation) whereas, for subjects suffering from MSA, no effective treatment is known (Oertel et al. 2012; Terao et al. 2019). In context with this dissertation, both PD and MSA have a decade-long prodromal phase. RBD is the most specific indicator for the prodromal stage of PD and MSA

(Iranzo et al. 2014; Miglis et al. 2021). Therefore, it has been difficult to distinguish between PD and MSA not only in the early manifest stage but also in the prodromal stage.

In this dissertation, we have selected oculomotor and pupillomotor tasks in order to search for a difference in oculo-pupillomotor functions in manifest PD and MSA and to see whether these differences - if found – would be already seen in patients suffering from RBD.

I.1.1. Parkinson's disease (PD)

PD is a neurological disorder with an etiology that up to 15 percent has a genetic course, whereas 85 percent are idiopathic, although in most cases the aggregation of the protein alpha-synuclein plays a role in its pathogenesis (Tran et al. 2020).

The clinical motor symptoms of PD are caused by the degeneration of dopamine-producing neurons in the substantia nigra pars compacta, which leads to a shortage of dopamine, a neurotransmitter that is essential for the control of movement (Lees et al. 2009). The symptoms of PD typically include tremors, rigidity, slow movement (bradykinesia), and difficulty with balance and coordination (Lees et al. 2009).

I.1.1.1. Epidemiology

According to reports, PD incidence and prevalence increase with age (Twelves et al. 2003). PD is a common age-related neurodegenerative disorder, affecting 1% of people aged 60–65, but rises to a prevalence of 5% by the age of 80, indicating an age-related tendency (Reeve et al. 2014). Parkinson's disease has an annual incidence of about 16 per 100,000 people (Twelves et al. 2003).

According to age-standardized prevalence rates, the male-to-female ratio was roughly 4 to 1 (Moisan et al. 2016). The prevalence of this chronic disease primarily affecting the elderly is predicted to double in the next 25 years (Dorsey and Bloem 2018). Patients' lives, as well as the entire healthcare system, are drastically affected by Parkinson's disease. For instance, the cost of managing and treating Parkinson's disease patients in Germany is 8.610 € per patient in a 6-month period (von Campenhausen et al. 2011). Therefore, the rising frequency of such diseases, along with growing aging populations, poses a serious challenge to any country's healthcare systems and society. There is an unmet need for novel disease-modifying neuroprotective therapeutic options to mitigate/improve or even prevent the course of the disease (Miglis et al. 2021).

I.1.1.2. Pathology- Braak staging

Besides the loss of dopaminergic neurons, the neuropathological hallmark of PD is the Lewy bodies that consist of aggregated alpha-synuclein (Lees et al. 2009). Braak et al. in 2003 attempted to classify the progression of pathological changes of Parkinson's disease, proposing that a pathogen inducing alpha-synuclein aggregation enters the central nervous system via the enteric nervous system of the gastrointestinal tract and the olfactory bulb. In stage I of Braak's staging hypothesis olfactory bulb and the dorsal motor nucleus of the vagus are affected. As the disease progresses to stage II, pathology moves up to the brainstem including the locus coeruleus (LC) in the pontine tegmentum (Braak et al. 2003). At the beginning of stage III, the substantia nigra (SN) is affected, and Lewy bodies are observed in the substantia nigra pars compacta (SNc) leading to the degeneration of dopaminergic neurons (Braak et al. 2003). Most dopaminergic cell destruction happens at stage IV, and the thalamus and amygdala start to degenerate. Stages V and VI are the beginning of neocortical involvement, and the disease is at its most severe condition (Braak et al. 2003).

I.1.1.3. Clinical symptoms and diagnosis

PD is clinically diagnosed according to the "UK PD Society Brain Bank Clinical Diagnostic Criteria" and clinically defined by the presence of the cardinal motor sign akinesia in combination either with rigidity or rest tremor and later on postural instability – and the absence of other neurological symptoms and signs (Gibb 1988; Calne et al. 1992; Lees et al. 2009; Eggert et al. 2012). The unilateral start of motor symptoms, the good response to levodopa, and the slow progression of disease support the diagnosis of PD (Eggert et al. 2012). In addition to motoric symptoms, other non-motoric symptoms may manifest themselves in PD patients, like constipation, orthostatic dysregulations, hyposmia, or psychiatric problems like apathy and depression (Eggert et al. 2012; Lees et al. 2009). Interestingly, these nonmotor symptoms precede the manifestation of motor symptoms for years (Eggert et al. 2012; Postuma et al. 2019).

PD patients always undergo a neurological examination to evaluate the core motor symptoms that are mandatory for diagnosis (Lees et al. 2009).

Unified Parkinson's Disease Rating Scale (UPDRS)

One of the clinical diagnostic tools used to quantify the intensity of PD symptoms is the Unified Parkinson's Disease Rating Scale (UPDRS) (Movement Disorder Society Task Force on Rating Scales for Parkinson's Disease 2003). UPDRS was created by neurologists as a screening tool for monitoring the responses to PD medications. Filling out the UPDRS questionnaire needs expertise and should be completed by an expert with experience in Parkinson's disease. Experts should be capable of rating the severity of symptoms and responding to the questions presented in each UPDRS segment after inspection of the patients. In total, UPDRS has six parts (Movement Disorder Society Task Force on Rating Scales for Parkinson's Disease 2003): 1) Mentation, behavior, and mood, 2) The activities of daily living (ADL), 3) Motor sections, 4) Complications of therapy (in the past week), 5) Modified Hoehn and Yahr Scale, and 6) Schwab and England ADL scale.

Since its first introduction, UPDRS has undergone several changes; The Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) is a revised UPDRS scale that addresses the limitations of the original UPDRS (Goetz et al. 2008). Additionally, UPDRS is used as a semi-quantitative assessment tool in a Parkinson's disease diagnostic method called the levodopa test, as described in the following section.

Levodopa test

Levodopa is an amino acid that is the precursor to dopamine and is commonly used by clinicians to replenish the dopamine levels in the brain of Parkinson's patients. Levodopa is usually taken as a pill (in combination with other medications) and unlike dopamine, it can get absorbed in the blood and travel toward the brain (Oertel et al. 2012). As soon as it enters the brain, it will be converted into dopamine by the enzyme, dopa decarboxylase. Therefore, restoring the dopamine level can improve motor function. Levodopa's effect on Parkinson's disease has therefore been used to differentiate it from other neurological disorders like MSA which is not responding to Levodopa (Oertel et al. 2012).

A neurologist conducts a levodopa test after the patient withdraws any other dopaminergic medication at least 8 hours after the last dose. The motor functions of the patients are evaluated before the test and again 60 to 90 minutes after levodopa administration (Oertel et al. 2012).

By using the UPDRS-III, patients' responses to the levodopa test are analyzed semi-quantitatively to assess their Parkinson's symptoms and if they have a significant improvement,

then it strongly supports the clinical diagnosis of PD (Oertel et al. 2012). In general, improvements over 30% are considered light, improvements over 50% are considered good, and improvements over 80% are considered very good (Oertel et al. 2012). Levodopa testing has a sensitivity of 75% and a specificity of 87% in patients with Parkinson's disease (Clarke and Davies 2000). Tests with levodopa assess only the brain's response to dopamimetic compounds and therefore have limited differential diagnostic significance and depend on the comparative disease (Oertel et al. 2012).

Imaging methods

Different parts of the brain are affected by PD and the most obvious loss is the degeneration of the dopaminergic neurons in the substantia nigra (Lees et al. 2009; Becker et al. 1995). Depending on the population studied, researchers find that about 60% to 90% of neurons in the substantia nigra are already destroyed before PD symptoms become clinically apparent (Becker et al. 1995). These neurons innervate the striatum with the dopaminergic nigrostriatal projection. The striatum is the largest nucleus in the basal ganglia, consisting of the putamen and caudate (Lanciego et al. 2012).

A feedback circuit connects the basal ganglia with the cortex through the thalamus, indicating that the circuit is tuned correctly (Lanciego et al. 2012). Information concerning movement planning is sent to the striatum from the cerebral cortex. Basal ganglia loop disruption would have a negative effect on the circuit's functionality, causing various problems (Lanciego et al. 2012). In Parkinson's disease, motor symptoms appear when approximately 60% of the nigrostriatal pathway volume has been damaged. In other words, akinesia, rigidity, and tremor symptoms arise when dopamine levels in the SNc and the basal ganglia, i.e., the putamen and caudate nucleus, are deficient by up to 80% (Scherman et al. 1989).

Dopamine transporter (DAT) single-photon emission computed tomography (SPECT)

Thus, when motor symptoms of PD become manifest and the clinical diagnosis of manifest PD can be made the pathological changes represent stage IV in the so-called Braak staging (Braak et al. 2003). To monitor the deterioration of dopaminergic neurons, brain imaging techniques are widely used as clinical diagnostic tools (Stiasny-Kolster et al. 2005; Meles et al. 2017; Iranzo et al. 2010). One of the imaging methods is called Dopamine transporter (DAT) single-photon emission computed tomography (SPECT) which can image dopamine transporter ligand binding in the brain. DAT-SPECT is now the most researched and widely

accessible SPECT imaging method for visualizing the integrity of the dopaminergic nigrostriatal innervation of the basal ganglia (Giza et al. 2012; Meles et al. 2017; Stiasny-Kolster et al. 2005; Iranzo et al. 2010).

As mentioned earlier, nigrostriatal dopaminergic neuron loss is one hallmark of PD. These neurons release dopamine as an essential neurotransmitter for the brain. Briefly, when dopamine is released in the synaptic cleft, the postsynaptic neuron uptakes a part of it, and some of it will be re-uptaken by the presynaptic neuron (Iranzo et al. 2011). DAT, which is a transmembrane protein, is responsible for the reuptake process. Imaging methods like DAT-SPECT use an agent (Ioflupane (123I)) to bind the DAT in the striatum to be able to capture and quantify the amount of presynaptic dopamine uptake site. Thus DAT SPECT visualizes the presence of transporters. Based on the literature, 50-70% of the DATs are reduced in Parkinson's disease when the disease starts to manifest (Scherman et al. 1989). In general, DAT SPECT is used to evaluate the presynaptic dopamine neuronal deficiency in the nigrostriatal pathway.

18F-Fluorodeoxyglucose (18F-FDG)-positron emission tomography (PET)

PD patients have displayed additional brain deficits rather than striatal dopaminergic loss. The brain activity pattern in PD patients should be investigated on a larger scale. Since the brain uses glucose for its activity, one helpful measure could be monitoring the glucose metabolism in the brain. A standard brain imaging method, 18F-Fluorodeoxyglucose (18F-FDG)-positron emission tomography (PET), visualizes the brain's glucose metabolisms (Janzen, Kogan, et al. 2022).

FDG-PET is a medical imaging technique that uses a radioactive tracer called 18F-FDG that allows for visualization and assesses the metabolic activity of tissues in the body (Meles et al. 2021; Janzen, Kogan, et al. 2022). It works by injecting a small amount of FDG, a radioactive glucose analog, into the patient's body. FDG is taken up by metabolically active tissues, such as cancer cells or regions of the brain that are more active than normal. The PET scanner then detects the radiation emitted by the tracer to show the distribution and intensity of metabolic activity in the body. 18F-FDG-PET has been used in movement disorders and Parkinson's disease to track brain metabolism or synaptic activity. Some studies have shown that 18F-FDG uptake has been reduced in PD patients (Meles et al. 2021; Janzen, Kogan, et al. 2022).

DAT-SPECT and 18F-FDG-PET are two commonly accessible radionuclide imaging modalities. DAT scan has long been established as a routine method in clinical routine. FDG-PET is especially valuable in the differential diagnosis of PD and atypical Parkinson's syndromes (Janzen, Kogan, et al. 2022; Meles et al. 2017).

Cardiac 123I-Metaiodobenzylguanidine (MIBG) scintigraphy

Cardiac 123I-Metaiodobenzylguanidine (MIBG) scintigraphy is another nuclear imaging method that visualizes the sympathetic innervation of the heart (Janzen, Vadasz, et al. 2022; Satoh et al. 1999). PD patients with autonomic dysfunction showed substantially reduced cardiac MIBG uptake (Braune et al. 1999).

I.1.2. Multiple system atrophy (MSA)

Multiple System Atrophy (MSA) is a rare neurodegenerative disorder that affects numerous parts of the body and brain and it is difficult to diagnose the MSA at the early stages as the symptoms are similar to those of PD (Wenning et al. 2004; Terao et al. 2019).

I.1.2.1. Epidemiology

The αSYN MSA is a relatively rare, fatal adult-onset neurodegenerative disease that affects 0.6-0.7/100,000 persons per year worldwide (Fanciulli and Wenning 2015). It is estimated that the prevalence of the disease under the age of 40 is about 1.9-4.9/100,000, while it may rise to 7.8/100,000 after that (Schrag et al. 1999).

I.1.2.2. Pathology

MSA is an alpha-synucleinopathy characterized by specific glioneuronal degeneration affecting the striatonigral, olivopontocerebellar, and autonomic nervous systems, as well as other regions of the central and peripheral nervous systems (Wenning et al. 2004; Fanciulli and Wenning 2015). The clinical and pathological subtypes of MSA are the MSA-Cerebellar type (MSA-C) and MSA-Parkinsonism type (MSA-P) (Gilman et al. 2008). Most MSA instances in the western world are MSA-P, while MSA-C is more common in Asian populations, most likely due to genes and environmental influences (Jellinger 2020).

In the various MSA subtypes, different parts of the brain are affected. MSA-P has a greater impact on the striatonigral system, whereas MSA-C has a greater impact on the olivopontocerebellar system (Wenning et al. 2004). MSA-P causes the putamen to shrink,

whereas MSA-C allows the striatum and substantia nigra to remain less affected (Wenning et al. 2004). MSA has also been linked to spinal cord cell loss in parasympathetic preganglionic nuclei (Wenning et al. 2004).

The following are the primary visual-related regions of the brain that are most often impacted in MSA: basal ganglia, cerebellum, inferior olivary nucleus (ION), LC, the motor cortex (MC), premotor cortex (PMC), pontine nuclei, spinal cord, substantia nigra, thalamus (Armstrong 2014).

I.1.2.3. Clinical symptoms and diagnosis

It is often difficult to diagnose MSA because it requires both clinical and laboratory evaluations (Oertel et al. 2012; Wenning et al. 2004; Braune et al. 1999). A comprehensive neurological examination and a complete history of medical conditions can help diagnose MSA. Imaging tests such as magnetic resonance imaging (MRI) or positron emission tomography (PET) can help with clinical diagnosis. Additionally, MIBG can be used to discriminate MSA patients from PD patients (Braune et al. 1999). However, only a post-mortem examination of brain tissue can provide a definitive diagnosis of MSA.

The symptoms of MSA can vary widely from person to person, but MSA usually presents with early and severe autonomic dysfunction, Parkinsonism, and/or cerebellar dysfunction (Wenning et al. 2004). Parkinsonian symptoms may include tremors, rigidity, bradykinesia (slowness of movement), and postural instability. Cerebellar symptoms may include ataxia (loss of coordination), dysarthria (slurred speech), and dysphagia (difficulty swallowing). Other symptoms may include sleep disorders (for example nearly all MSA patients present with RBD), breathing difficulties, and difficulty regulating body temperature (J. Levin et al. 2016).

Erectile dysfunction, urinary urgency/incontinence/nocturia, reduced blood pressure management, vocal cord paralysis (stridor), reduced sweating, and dusky hands are some of the consequences of autonomic dysfunction (J. Levin et al. 2016). Orthostatic hypotension is another indication of autonomic dysfunction, which frequently manifests as postural disorientation, fatigue/weakness, inability to concentrate, and blurred vision (Wenning et al. 2004; Armstrong 2014).

I.1.3. REM sleep behavior disorder (RBD)

As mentioned earlier, rapid eye movement (REM) sleep behavior disorder (RBD) has been identified as one of the most specific and common prodromal stages of αSYN, such as PD, DLB, and MSA (Iranzo et al. 2014). RBD is a parasomnia characterized by dream enactment of often vivid or aggressive dreams with aggressive movements such as kicking and punching and vocalizations during the dream phase of sleep (sleep phases have been explained in section I.5.Sleep) – the so-called REM sleep. (Miglis et al. 2021). These actions of patients can often cause injury to themselves and/or their bed partners.

I.1.3.1. Epidemiology

The exact prevalence of RBD in the general population is not well known, but it is estimated to be around 1% in people over 60 years of age, with men being more likely to be affected than women (Haba-Rubio et al. 2018). It was also found that a quarter of all PD patients retrospectively already suffered from dream sleep disorder before the manifestation of motor symptoms (Sixel-Döring et al. 2014).

RBD may be associated with several neurological disorders, including PD, DLB, and MSA. Usually, RBD occurs prior to the onset of these neurodegenerative diseases (Iranzo et al. 2014). Up to 85% of patients with isolated RBD develop PD or DLB and rarely MSA within 10 to 20 years (Iranzo et al. 2014).

I.1.3.2. Pathology

Patients with RBD manifest impairments in several neurological systems that are associated with PD; The sublaterodorsal nucleus (SLD) is located in the brainstem (Figure I-1) (Vetrivelan et al. 2009; Oertel et al. 2020). Interneurons in the spinal cord are activated by the SLD, resulting in the inhibition of spinal motor neurons (Vetrivelan et al. 2009). SLD has an indirect inhibitory influence on the spinal motor neurons via excitatory connections to the ventromedial medulla (VMM), which transmit inhibitory projections to the spinal motor neurons. The result is muscle atonia, which is characteristic of REM sleep (American Academy of Sleep Medicine 2014). In other words, inputs from REM atonia circuits inhibit motor neurons by activating glycinergic and Gamma-Amino-Butyric acid (GABAergic) premotor neurons. When this circuit is damaged (for example, by an SLD lesion), muscles may not be inhibited efficiently, allowing them to move during REM sleep. As a result, people may induce dream acting, which

is one characteristic of RBD. On the other side, LC might inhibit the initiation and maintenance of REM sleep; hence, LC must be suppressed before REM (Luppi et al. 2006).

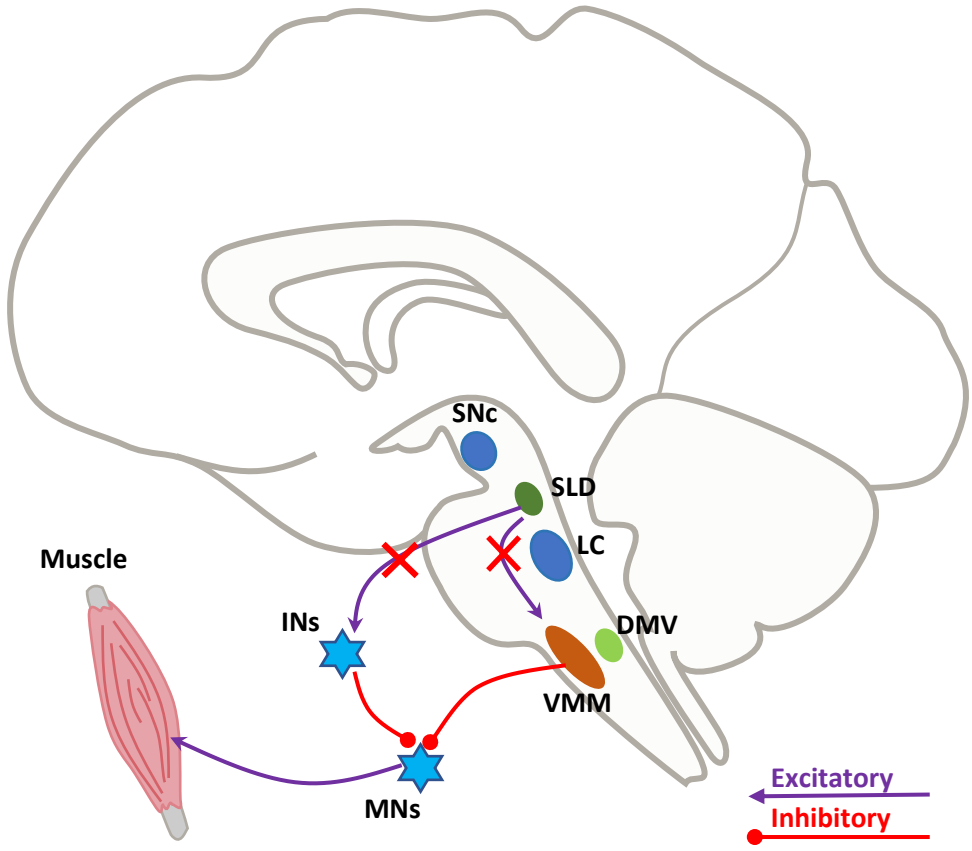

Figure I-1. Schematic brain circuits responsible for REM sleep. The red cross signs indicate the damaged pathways in RBD as a result of the lesion in SLD. SNc: substantia nigra pars compacta, SLD: sublaterodorsal nucleus, LC: locus coeruleus, DMV: dorsal motor nucleus of the vagus, VMM: ventromedial medulla, INs: spinal interneurons, MNs: spinal motor neurons (Adapted from (Oertel et al.*, 2020))*

RBD is also associated with neuronal degeneration in the brainstem. The coeruleus-subcoeruleus nuclear complex and LC comprise intracellular Lewy bodies similar to those found in premotor stages of PD (Iranzo et al. 2014). Braak reports that sleep-related centers are affected in stage II (Braak et al. 2003). According to Braak's hypothesis, PD patients have gone through constipation and olfactory dysfunction (Braak stage I) before experiencing a sleep disturbance (Braak stage II). PD is associated with αSYN pathology in the olfactory bulb, which contains a substantial amount of dopaminergic neurons. As RBD begins at Braak stage II, it implies that all RBDs should have olfactory bulb dysfunction, which is associated with Braak stage I (Braak et al. 2003). Therefore, RBD patients who have prodromal αSYN are expected to have olfactory dysfunction, which is a common symptom of PD (Hawkes et al. 1999; Janzen, Vadasz, et al. 2022). Compared to healthy controls, 35.7-97% of patients

diagnosed with RBD have been observed to have an olfactory impairment (Högl et al. 2018). Several studies have shown a significant likelihood that individuals with RBD who have olfactory impairment would develop manifest PD (Janzen, Vadasz, et al. 2022).

I.1.3.3. Clinical symptoms and diagnosis

Screening questionnaire

RBD can be screened by using some simple and widely used tools, such as clinical screening questionnaires. There are several screening questionnaires with varied degrees of sensitivity and specificity that can be used to identify patients that are suspected to have RBD (Stiasny-Kolster et al. 2007; Li et al. 2010; Postuma et al. 2012). In 2007, Stiasny-Kolster et al. introduced the RBD screening questionnaire (RBDSQ), which includes ten questions related to REM sleep behavior (Stiasny-Kolster et al. 2007). There is a total score of 13 on the RBDSQ, and any score greater than 5 indicates a potential RBD. Regarding sensitivity and specificity, RBDSQ has a coefficient of 0.96 and 0.92 when compared to patients with other sleep disorders (Stiasny-Kolster et al. 2007).

Imaging

The diagnosis of RBD usually involves a thorough medical history and physical examination, along with a sleep study (polysomnography) to monitor brain activity, eye movements, and muscle activity during sleep (Oertel et al. 2012). As a matter of fact, video polysomnography (PSG) is mandatory for the RBD diagnosis to detect the loss of muscle atonia during REM sleep (REM sleep without atonia) (Miglis et al. 2021).

Although RBD is primarily diagnosed with a clinical evaluation and PSG with video recording, imaging methods such as MRI and positron emission tomography (PET) can be useful for assessing the underlying causes or complications of the disorder (Meles et al. 2017; 2021).

For example, brain magnetic resonance imaging can be used to rule out other structural abnormalities or lesions in the brain that may cause RBD or mimic its symptoms (Meles et al. 2021). It can also detect specific patterns of brain atrophy or degeneration that are associated with neurodegenerative disorders that may cause RBD, such as PD, MSA, or DLB.

FDG-PET imaging can be used to measure the metabolic activity or the levels of specific neurotransmitters in the brain, which may help differentiate RBD from other disorders or

monitor the progression of the disease (Kogan et al. 2021). For example, DAT SPECT imaging can show a reduced uptake of dopamine in the striatum, a brain region involved in movement control and reward processing, in patients with RBD and Parkinson's disease.

I.2. Biomarkers for α-synucleinopathies (αSYN)

As mentioned earlier, people with RBD will be an appropriate cohort to study biomarkers. For a biomarker to be considered optimal, it must have a combination of the following characteristics: high sensitivity and specificity, reproducibility, affordability, and the ability to monitor the disease progress as well as the effect of treatment (Miglis et al. 2021). Some methods used in clinical practice to identify beneficial biomarkers will be outlined below.

I.2.1. Cognitive deficits

Cognitive deterioration is a common symptom in RBD patients (Högl et al. 2018). Those with cognitive deficits have a greater chance of phenoconversion (especially to DLB) than RBD patients with normal cognitive function (Postuma et al. 2019). In a follow-up study on RBD patients over six years, Marchand et al. showed that developing dementia was strongly associated with cognitive deficits (Génier Marchand et al. 2018). Therefore, cognitive impairment is linked to a higher risk of developing DLB in RBD (Miglis et al. 2021) and could be considered an early indicator for DLB.

One of the screening tests that assist in determining a person's risk of developing dementia is Montreal Cognitive Assessment (MoCA) test which has been introduced by a group at McGill University to identify mild cognitive dysfunction (Nasreddine et al. 2005). MoCA test takes 10-12 minutes to administer and consists of 30 questions that assess different aspects of cognitive function: Orientation, short-/long-term memory, executive function/visuospatial ability, language, verbal fluency, abstraction, attention, and working memory.

Using MoCA in clinical trials and research studies, when cognitive impairment is an exclusion criterion, is particularly advantageous since it is a readily accessible test that can be quickly completed.

I.2.2. Nigrostriatal dopaminergic neuron loss

The progress of functional and structural brain imaging holds promise for the early identification of prodromal Parkinson's disease. Accordingly, the reduction of dopamine transporter in the nigrostriatal area in individuals with RBD is common (Iranzo et al. 2013).

I.2.3. 18F-Fluorodeoxyglucose (18F-FDG)-positron emission tomography (PET)

Since RBD is a very critical prodromal stage for Parkinson's disease, 18F-FDG-PET has been used to probe the presence of abnormal brain glucose metabolism known as the "PD-related pattern" (PDRP) in subjects with RBD (Kogan et al. 2021). Increased PDRP expression has been associated with disease progression, making it a prodromal progression marker that could predict conversion to αSYN subtypes (PD/DLB) as well as monitor the course of the disease (Huang et al. 2007; Kogan et al. 2021; Meles et al. 2021).

I.2.4. Autonomic dysfunction

Autonomic dysfunction is present in both PD and MSA patients but in different manners; In PD these autonomic dysfunctions are postganglionic damage to sympathetic neurons while in MSA, the autonomic disturbances are due to damage to preganglionic neurons of the autonomic nervous system (Braune et al. 1999). Histopathological and in vivo studies have shown that there is no decrease in the postganglionic cardiac MIBG uptake in MSA, allowing differentiation of PD and MSA (Braune et al. 1999). MIBG scintigraphy has not been systematically used in RBD as a prodromal of PD and MSA.

Regardless of the fact that autonomic dysfunction in RBD patients is a diagnostic marker (Miglis et al. 2021), additional research is needed to determine if autonomic impairment might predict the phenoconversion of RBD to αSYN subtypes due to intra-individual variability.

I.2.5. Eye movement biomarkers

Several studies have attempted to identify eye movement biomarkers for Parkinson's disease(Brooks et al. 2017; Hanuška et al. 2019; Perkins et al. 2021; Habibi et al. 2022). These studies have investigated eye movement abnormalities in RBD patients and have attempted to identify promising biomarkers. Further details of the abnormalities are provided in the data chapters of this dissertation.

There are several additional biomarkers, such as tissue biopsy, autonomic function, and genetic testing, but none of them meets the standards of precision and accuracy in predicting RBD phenoconversion to αSYN (Miglis et al. 2021; Doppler et al. 2017). In addition, despite a range of research methods, no readily available biomarker exists to date for separating the prodromal stages of PD, DLB, and MSA (Miglis et al. 2021).

The subsequent section addresses another neurodegenerative disease that is not included in the group of αSYN diseases (PD, MSA, and RBD).

I.3. Progressive supranuclear palsy (PSP)

Progressive supranuclear palsy (PSP) is a sporadic, progressive, neurodegenerative disease (Litvan et al. 1996). The vast majority of PSP patients suffer from Tauopathy. Several researchers have attempted to identify any environmental exposure as the reason for PSP. There are several theories concerning a fruit on the island of Guadeloupe known as Annona Muricata as a potential cause of PSP (Champy et al. 2004). Drinking well water, according to Litvan et al., might be another risk factor (Litvan et al. 2016).

I.3.1. Epidemiology

PSP is an adult-onset neurodegenerative illness that typically manifests after age 40 (Höglinger et al. 2017), and the prevalence is about 5-6 per 100,000 people (Schrag et al. 1999; Nath et al. 2001). However, Japan, Switzerland, and the United Kingdom have reported a greater frequency of PSP (Coyle-Gilchrist et al. 2016; Takigawa et al. 2016; Fleury et al. 2018). The average age of survival after the beginning of the illness is six years (Lubarsky and Juncos 2008). According to the studies available to date, the gender distribution cannot be definitively determined, but it does appear to be balanced in routine clinical practice. Nevertheless, men are diagnosed later than women (33.4 versus 24.1 months after clinical onset) and die earlier than women (37.0 versus 476) (Nath and Burn 2000).

I.3.2. Pathology

The atypical parkinsonian syndrome, PSP, is associated with Tau protein accumulation in the brain (Tauopathy) in contrast to the αSYN PD, MSA, DLB, and their prodromal stage RBD (Höglinger et al. 2017).

PSP is an atypical parkinsonian disorder that pathologically is differentiable from PD by symmetrical tissue loss in the frontal cortex and basal ganglia (Litvan et al. 1996; Höglinger et al. 2017; Armstrong et al. 2007).

Brain weight is reduced in PSP compared to normal, and brain abnormality often impacts the midbrain. Histological analysis has shown that the External globus pallidus (GPe), the subthalamic nucleus (STN), red nucleus, substantia nigra, periaqueductal grey matter, pontine tegmentum, and dentate cerebellar nucleus are nearly invariably involved (Steele et al. 1964; Armstrong et al. 2007). Additional brain areas affected by PSP include the reticular formation,

the oculomotor system, the vestibular system, the superior colliculus (SC), and certain cortical regions (Armstrong et al. 2007).

I.3.3. Clinical symptoms and diagnosis

PSP is associated with oculomotor dysfunction (slowed vertical saccades, vertical supranuclear gaze palsy) akinesia and rigor, postural difficulties (falling backward), and frontal lobe-related cognitive impairment (Oertel et al. 2012).

Similar to Parkinson's disease, early symptoms of PSP are imprecise, non-specific, and often misleading (Höglinger et al. 2017). Disturbed balance, clear falls (backward), and dizziness are some of the initial complaints of PSP patients. Akinetic rigidity, characterized by difficulty initiating movements and upright gait (hyperextension of the neck), is another early symptom. Slowdown and clumsiness are described, but to a lesser extent and more symmetrically (without lateral emphasis) than in Parkinson's disease (Oertel et al. 2012).

The clinical diagnosis of PSP requires the presence of vertical gaze palsy or slowing of the vertical saccades. It is important for physicians to measure the maximum amplitude of the upward/downward gaze movement, which is already decreasing with age or with other neurodegenerative diseases. The PSP also moves the head before moving the eyes when aiming to look at targets. Their eyes are wide open, they have an astonished look (amazed), and their blink rates are reduced (Oertel et al. 2012).

PSP patients respond poorly to adequate dopaminergic treatment, while PD patients respond well (Litvan et al. 1996). Another distinction is the existence of resting tremors in PD but the lack of tremors in PSP. Steele, Richardson, and Olszewski published the initial description of PSP in 1964 (Steele et al. 1964).

Additional diagnostic tools are imaging methods to prove the brain pathology n the PSP. The hummingbird sign is the most persistent indication of MRI in PSP, which refers to the appearance of the brainstem after pathology in sagittal view (Kato et al. 2003). Furthermore, severe midbrain atrophy in PSP can manifest in the axial plane as the appearance of the morning glory flower (Adachi et al. 2004).

I.4. Differential diagnosis

Both MSA and PSP patients are called atypical Parkinsonian diseases which refer to progressive and disabling neurodegenerative diseases exhibiting some motor symptoms associated with PD (J. Levin et al. 2016). As indicated above, atypical parkinsonian diseases

may look like Parkinson's disease, but many also suffer from additional symptoms not found in PD patients. Their symptoms usually progress faster, and they are not responding well to levodopa treatment (J. Levin et al. 2016; Höglinger et al. 2017).

MSA-P patients are often mistaken for PSP patients because both have some Parkinson's symptoms (J. Levin et al. 2016); The postural stability of people with MSA is impaired from the beginning, while recurrent falling backward is uncommon at the onset, unlike persons with PSP. Orthostatic hypotension is hardly noticeable in PSP, while neurogenic bladder emptying disorders occur as the disease progresses. Lastly, PSP patients have severe frontal executive dysfunction, unlike MSA-P patients. Having the so-called applause sign as evidence of frontal disinhibition speaks against MSA-P.

Table I-1 shows differential diagnosis comparisons between PD, MSA, and PSP. In contrast to Parkinson's disease, MSA usually lacks the classic resting tremor (J. Levin et al. 2016). Instead, it is characterized by tremors of the hands that are irregular and are interrupted by irregular movements (myoclonus). Accordingly, PSP patients do not tend to exhibit tremors, but they tend to fall backward, unlike patients with PD and MSA. The table below clearly outlines other differences. In the PSP, there is an inability to perform as many claps as the examiner, and this helps to distinguish it from PD and MSA (J. Levin et al. 2016).

Table I-1. Differential diagnosis. +: exist, (+): sometimes exist, -: not exist, ?: uncertain. PD: Parkinson's disease; MSA: Multiple system atrophy; PSP: Progressive supranuclear palsy.

	PD	MSA	PSP
Rest-tremor	+	(+)	-
Falls backward early	(+)	-	+
Autonomic dysfunction	-	+	-
L-dopa-response	+	(+)	(+)
Clapping /uninhibited	-	-	+
Hyposmia	+	-	?
RBD	+	+	-

Since RBD patients have a very important rule as the prodromal stage of αSYN disorders (PD and MSA), as explained earlier, and have severe sleep disturbance, a brief introduction to sleep is given in the next section.

I.5. Sleep

Sleep is a highly complex, dynamic, and very tightly controlled process, which can have significant effects on consciousness during wakefulness. In addition to assisting in physiological recovery processes, sleep plays an important role in memory formation and function, and nervous system development (Weeß and Landwehr 2009).

Sleep is not uniform and has different stages (Figure I-2). Non-REM1 (N1), Non-REM2 (N2), Non-REM3 (N3), and REM are among the four sleep stages according to the American Academy of Sleep Medicine (AASM 2014; American Academy of Sleep Medicine 2014) criteria based on polysomnography (PSG) which includes an electroencephalogram (EEG), electrooculogram (EOG), and electromyogram (EMG). As the body transitions from wakefulness to sleep, stage N1 is the first sleep stage characterized by theta activity in the brain and slow and rolling eye movements, and a decrease in muscle tone (Weeß and Landwehr 2009).

In N2, stable sleep is characterized by theta activity and the K-complex waveform, no eye movements, and diminished muscle tone. The deepest sleep stage, N3, is characterized by delta activity in the brain, no eye movements, and a decrease in muscle tone (Weeß and Landwehr 2009).

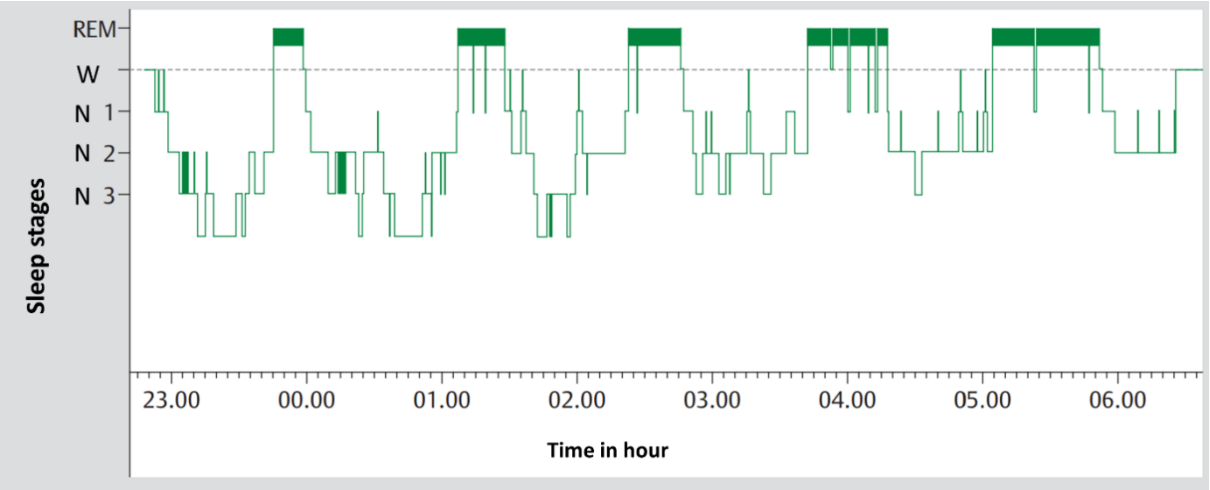

Figure I-2. Sleep stages of a normal young man. REM: rapid eye movement, W: wake stage, N1: non-REM stage 1, N2: non-REM stage 2, N3: non-REM stage 3 (adapted from Wess et al. (Weeß and Landwehr 2009)).

Phases N1, N2, and N3 are referred to as non-REM stages (Weeß and Landwehr 2009). In general, the sleep cycle is an oscillation between the REM phase and the non-REM phase of sleep. The REM (Rapid eye movement) stage is also known as dream sleep, paradoxical sleep, or active sleep. REM is the stage in which there is theta brain activity, and the eyes move very rapidly, but they do not send any visual information to the brain. This is when most of the dreams occur while muscles get temporarily paralyzed (atonia). This stage is also characterized by autonomic dysregulation, which manifests itself in faster and irregular pulse and respiratory rates as well as higher blood pressure (Diederich 2007).

PSG records overnight different functions like leg/arm movements, breathing irregularities, eye movement, cardiac rhythm, and brain activities via electroencephalography (American Academy of Sleep Medicine 2014). Using PSG, the different sleep stages can be determined, and potential abnormalities can be assessed. The recorded data are evaluated by a sleep medicine specialist.

As mentioned earlier, the REM phase of sleep is the phase that is disturbed in RBD patients. In RBD, the physiological atonia is abolished. Therefore, specialists can monitor patients' sleep stages in the sleep clinic to check whether sleep disturbances are present to confirm whether they have RBD or not (Oertel et al. 2012).

The remainder of the introduction is devoted to the principles of eye movement, eye anatomy, including the pupil, and a description of blink behavior.

I.6. Introduction to eye movement

Even though the eye can see anything in front of us, the best vision will be achieved in the fovea (see section I.9). The fovea is a tiny eye area with only 1 mm depth that can detect only a fraction of the visual field (Kandel et al. 2000). In order to explore an object, we should move the images of the objects to the fovea, which requires two components of the gaze system: Eye movement and head movement systems. The gaze and vestibulo-ocular systems are responsible for maintaining the stability of the picture of an item on the retina as the head or object moves. For more information regarding the vestibulo-ocular system, I refer the readers to the literature (H. Collewijn 1985; Kandel et al. 2000).

In 1902, Raymond Dodge identified several eye movement mechanisms that assist the eye in orienting itself so that the fovea may concentrate on the intended object (Dodge 1902). Some of these systems serve to maintain the head's positioning, while others facilitate the eye's movement toward the target. When it is necessary to move the eye from one position to another (target), saccadic eye movements are practical. Whenever there is a target that is moving and the eye must follow it, smooth pursuit eye movement is advantageous (L. Levin et al. 2011). Vestibulo-ocular eye movements refer to instances in which the eye shifts in the opposite direction of the head, necessitating a system to maintain the image's place on the retina.

Optokinetic movements consist of a slow, smooth pursuit phase and a rapid phase that assists the eyeball in moving in the same direction as the visual field (H. Collewijn 1985). Finally, when investigating a fixed object, the eye occasionally needs to remain motionless in orbit. Therefore, a fixation system should be present when observing a target to hold the eye still. In other words, other eye movements need to be suppressed. However, how does the eye move within the orbit, what signals does it receive, and which organs physically assist it in its movement? The following sections provide an overview of the eye muscles and the nervous system that transmits the movement signal. In addition, the various types of eye movement will be addressed.

I.7. Extraocular muscles

Understanding the anatomy of the eye, as well as the extraocular muscles, is necessary to understand the eye's movement. Eye movements are described as rotations around the eye's three axes of rotation - horizontal, vertical, and torsional (L. Levin et al. 2011).

In eye anatomy, different terms describe different eye movements, including abduction, adduction, elevation, depression, intorsion, and extorsion (L. Levin et al. 2011). The terms adduction and abduction are related to rotations of the eye away from the nose and rotations towards the nose, respectively. The term elevation refers to the rotation of the eye vertically upwards, and the term depression refers to the rotation of the eye vertically downwards. Intorsion brings the cornea's top closer to the nose, whereas extorsion brings it further from the nose. Different muscles attached to the eye utilize these certain movements shown in Figure I-3.

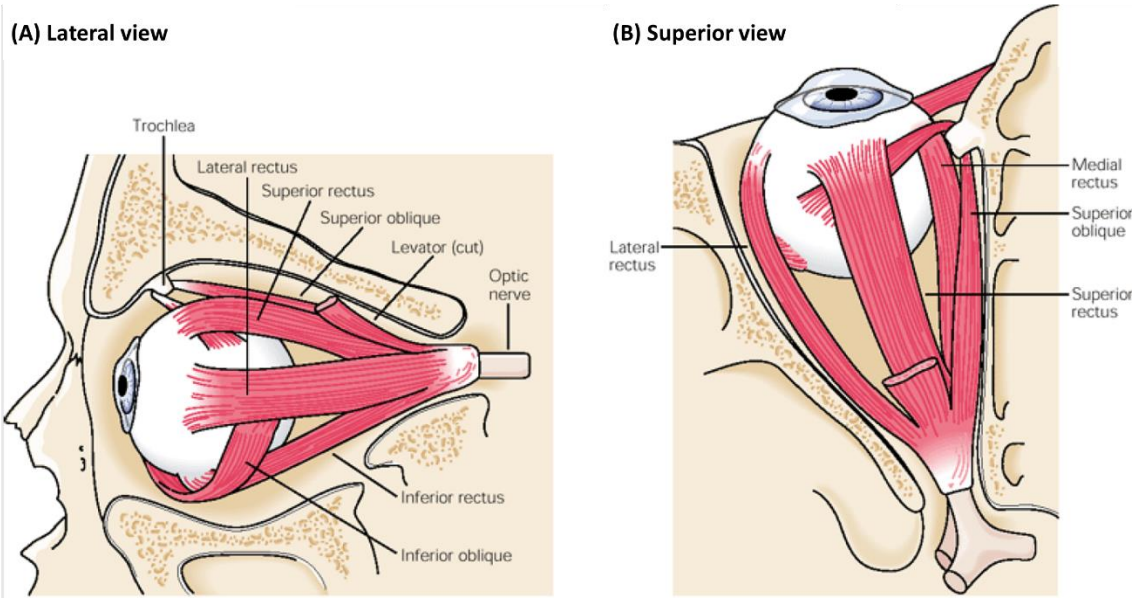

Figure I-3. Extraocular muscles. A. Left eye lateral view. B. Superior view of the left eye (adapted from Kandel (Kandel et al. 2000)).

Each eye is attached by six muscles: four rectus muscles (superior, inferior, medial, and lateral) and two oblique muscles (superior and inferior) (L. Levin et al. 2011; Kandel et al. 2000). Pupil tilting is caused by the oblique muscles pulling the rear of the eye toward their insertions in the occipital bone. As a result, the superior oblique elevates the eye, whereas the inferior oblique depresses it somewhat.

The medial rectus is responsible for adducting the eye, while the lateral rectus is responsible for abducting it (L. Levin et al. 2011; Kandel et al. 2000); The lateral and medial rectus coordinate a horizontal eye movement. Extraocular muscles in both eyes work together to move the eyes simultaneously in the same direction. Depending on the eye's direction, some muscles are contracted while others are relaxed (agonist and antagonist muscles). The agonist muscles

shift the eyes toward the desired direction, while antagonist muscles shift the eyes in the opposite direction. When both agonist and antagonist muscles are equally active, neither can win, and the eye remains motionless. For any type of eye movement to be generated, an appropriate eye movement command must be developed that allows sufficient increases in agonist muscles and decreases in antagonist muscles. The following sections explain how the antagonist and agonist muscles play a role in saccade generation. Understanding how the brain controls these muscles may help monitor any damage to the brain through eye movements.

I.8. Saccades

Saccades are the rapid eye movements that help us to adjust the direction of attention within the visual field. A sequence of fixations interconnected by saccades enables us to bring visually intriguing things to the center of our visual field, where eyesight sharpness is greater (Fovea) (L. Levin et al. 2011; Kandel et al. 2000).

In a saccade, an eye movement is made by moving the eye as quickly as possible. When the eye makes a saccade, its velocity increases and then decreases smoothly. Saccades occur at speeds up to 900° per second (Kandel et al. 2000). The velocity of the saccadic eye is only determined by the distance between the target and the fovea. The duration and direction of saccades can be altered intentionally, but their velocity is beyond our control (Kandel et al. 2000). The only factors that can slow saccades are fatigue, drugs, or pathological conditions (Dodge 1917; Bahill and Stark 1975).

I.8.1. Eye position and velocity coded by extraocular motor neurons

The motor impulses provided to the extraocular muscles must be understood in order to determine how the brain creates eye movements. Extraocular muscles are innervated by extraocular motor neurons (L. Levin et al. 2011). A direct correlation exists between the velocity and position of the eye and the discharge frequency of extraocular motor neurons (Kandel et al. 2000).

The underlying controller signal generating and directing the saccades has two components: pulse and step (Leigh and Zee 2015). The pulse is a burst of action potentials in the extraocular muscle motor neurons that generates the force to drive the eyes from one location to another. Neurons that contribute to the pulse component are known as excitatory burst neurons (EBN) located in the brainstem reticular formation (Scudder et al. 2002). As the pulse contracts the agonist muscle, burst neurons inhibition in the medullary reticular formation relaxes the

antagonist muscle. Afterward, the pulse gradually changes to a new tonic activity called “step”. The step response is the change in the tonic discharge of motoneurons that is required to maintain constant force with the muscles to keep the eyes in a new position after the saccade.

Consequently, a saccade signal generated by an ocular motor neuron displays pulse-step characteristics (Figure I-4). Saccade amplitude is determined by the step's height, while the pulse's height determines its speed (Scudder et al. 2002). Saccade duration is determined by the pulse duration. Different neural pathways are involved in determining the pulse and step components of the motor signal. Whenever the interaction between these components is altered, referred to as pulse-step mismatch (Bahill et al. 1975b), the behavior of saccades is changed. When no saccade is needed, the Omnipause neurons (OPNs) in the Pons disinhibit EBNs (Scudder et al. 2002).

In summary, two distinct stages comprise the pulse: antagonist and agonist. Movement of the eyes requires the burst discharge to be sent to the agonist muscle to be contracted. At the same time, the activity of motoneurons for the antagonist muscle is decreased so that they relax. For high-velocity saccades, inhibition of the antagonist is just as critical as the excitation of the agonist (Bahill and Troost 1979).

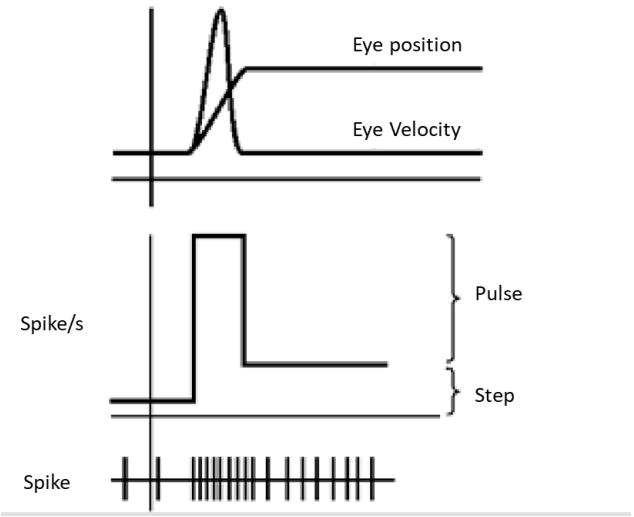

Figure I-4. Signals from motor neurons indicate eye position and velocity. The bottom plot shows the neural activity (spike), and the above plot shows the eye position and velocity (adapted from Kandel (Kandel et al. 2000)).

I.8.2. Main sequence

The link between duration and magnitude, as well as between peak velocity and amplitude, in the human saccade is referred to as the “main sequence” (Bahill et al. 1975a) that could be

applied to distinguish the saccades from other unknown types of eye movements. Indeed, saccade duration and peak velocity are correlated. On the other hand, as the saccade's amplitude rises, its duration and peak velocity increase (Bahill et al. 1975a).

I.8.3. Saccade circuits in the brain

How are the desired location and velocity of the eye determined? The higher centers that regulate gaze define only an intended shift in eye position. Interneurons in the brain stem reticular formation subsequently convert this signal into the appropriate velocity and location instructions for the motor neurons (Büttner-Ennever and Büttner 1988). Paramedian pontine reticular formation (PPRF) and rostral medulla coordinate the horizontal component of the eye movement, with impulses traveling to the horizontal recti muscles (Büttner-Ennever and Büttner 1988). The Rostral interstitial nucleus of the medial longitudinal fasciculus (riMLF) organizes the vertical component. The step and pulse components of the motor signal are handled by separate neurons in each of these circuits.

Omnipause neurons control both burst neurons (inhibit them) in the pontine and mesencephalic nucleus that contribute to the production of oblique saccades with horizontal and vertical components (all directions) (Büttner-Ennever and Büttner 1988). Therefore, patients with brain stem lesions manifest different eye movement deficits (Armstrong 2014; 2011).

I.8.4. The cerebral cortex controls saccades

Although the pontine and mesencephalic burst circuits control motor impulses necessary for the generation of saccades, the question is which part of the brain sends the command to initiate a saccade. Whenever a saccade is required, this command is executed. Consequently, higher brain areas related to more cognitive activities, such as the cerebral cortex, play a crucial role in this process (Büttner-Ennever and Büttner 1988). Normally, the cortex exerts control over the saccadic system through the superior colliculus (SC). In other words, the subject sends a signal to the frontal eye field (FEF) to tell where the target is, and when a saccade is needed, the FEF sends a signal directly to the premotor nerves or through a relay in the SC (Büttner-Ennever and Büttner 1988).

Visual and motor information is integrated by the SC in the midbrain into oculomotor signals to the brain stem (Dorris et al. 1997). SC consists of the following functional regions: the superficial layers and the intermediate and deep layers. Studies performed on monkeys have demonstrated that the superficial layers respond to visual stimulation (White et al. 2017). Meanwhile, the intermediate layer receives visual data from the prestriate, middle temporal, and parietal cortex, as well as motor information from the FEF. It has been found that lesions of a small part of the SC have a negative effect on the latency, accuracy, and velocity of saccades (May 2003).

There is a representation of the fovea in the area of the SC named the "fixational zone". During active visual fixation, the intermediate layers in this region get discharged strongly. In order to suppress a saccade and facilitate visual fixation, the basal ganglia inhibition on the SC should be removed. Then, SC will continue exciting the Omnipause neurons, whose activity prevents the saccade generation. These circuits and how they work have already been covered in more detail elsewhere (Munoz et al. 2000).

I.8.5. Saccadic subtypes

After different clinical experiments and studies on different diseases, several saccadic subtypes have been demonstrated. This led to introducing and classifying saccades into different subtypes (Figure I-5). Based on the various analyses of different saccadic eye movement recordings, terms like hypometric saccades, slow saccades, dysmetric saccades, and other types have been used (Bahill and Troost 1979). Each saccadic subtype occurs based on responsible neurological signals. Important saccadic subtypes will be described below however in this dissertation based on the designed experiments we only measure normometric saccades.

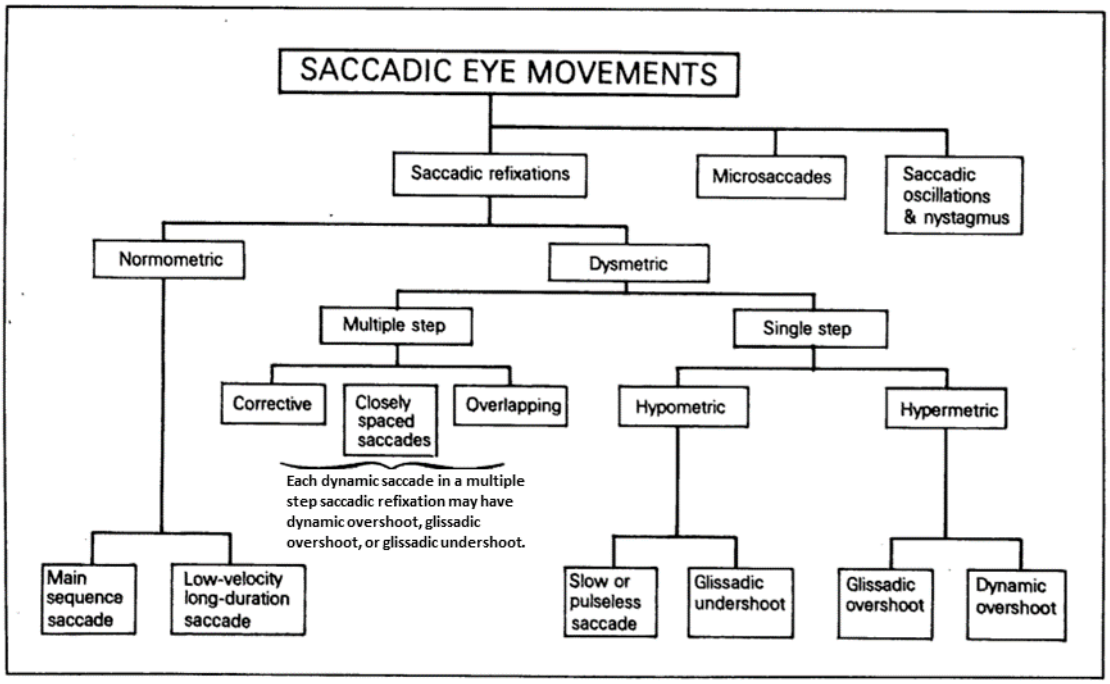

Figure I-5. Subtypes of saccadic eye movements (taken from (Bahill and Troost 1979))

I.8.5.1. Normometric saccades

A saccade is referred to as a normometric saccade when the pulse and step are matched to produce the appropriate refixation of the eye, thereby positioning the eye precisely in one step to the desired location (Bahill and Troost 1979). Normometric saccades exhibit the main sequence relationship. According to the amplitude of the saccades, we can classify them into macro-saccades and micro-saccades. Nevertheless, macro-saccades and micro-saccades cannot be determined exclusively from their amplitudes.

Macro-saccades

The eye has a different vision resolution depending on where the stimulus is located on the sensory surface. Therefore, the human eye tries to orient itself with minimal steps so that the visual target is located on the fovea. These eye steps are in a sequence of the fixation and saccade when scanning a scene (Rolfs 2009). Acute vision is significantly dependent on the ability of the eye to align itself with the appropriate sequence of the saccades. When the length of the saccade is ≥2 degrees (different definitions among literature), researchers usually label it as a macro-saccade (Rolfs 2009).

Micro-saccades

Micro-saccades are small rapid eye movements that happen during fixations and shift the eye position a couple of times per second. Dodge first coined the term micro-saccade in 1907, and it was widely adopted over the years (Dodge 1907). However, various other terms are used in the literature, like jerks, fixational saccade, minisaccade, and a few other terms (Bahill et al. 1975b). Although detecting the micro-saccades needs more consideration, most researchers, especially those doing free-viewing tasks with no visual targets, usually define them based on their amplitude (Han Collewijn and Kowler 2008; Habibi et al. 2022). Therefore in this dissertation, micro-saccades have been defined as saccades with an amplitude of fewer than two degrees.

I.8.5.2. Dysmetric saccades

Dysmetric saccades are caused when the controller signal's step and pulse are not properly matched (Bahill and Troost 1979). This implies that the eye cannot place itself at the destination point during a single saccade and may either overshoot or undershoot the target. The way eyes deal with saccadic displacement is of great interest because it could result from different pathologies. As a matter of fact, eyes try to locate themselves in the correct position by either a single step or multiple steps, which are called dysmetric single step and dysmetric multiple steps, respectively.

Dysmetric single step

During saccades, when the eyes are unable to reach the target, a minor eye movement known as a glissadic eye movement (slow, drifting eye movements (Bahill et al. 1975b)) is used to redirect the eye toward the intended location. Depending on whether the eye falls before or after the target, single-step dysmetric saccades are classified as either hypometric or hypermetric, respectively.

Hypometric

Hypometric saccades are a subgroup of dysmetric single-step saccades and are produced when the eyes undershoot the desired location and can result if the pulse component of the saccadic control signal is too small (an error). In this case, a glissadic eye movement aids in moving the eyes toward the target (glissadic undershoot). Sometimes there is no pulse in the

control signal, which is termed "slow" or "pulseless" saccades, and has been reported in individuals with PSP (Bahill and Troost 1979; Troost and Daroff 1977).

Hypermetric

Hypermetric saccades are another type of dysmetric single-step saccades that occur when the eyes overshoot the intended target. This can occur as a result of either a dynamic or a glissadic overshoot. Dynamic overshoot is a type of saccade behavior with a large return of 10-100 deg/s, while glissadic overshoot is a slow drifting eye movement with a return velocity of 2-20 deg/s (Bahill et al. 1975c). Dynamic overshoot occurs when neuronal control signals are reversed nonrandomly, and glissadic overshoot happens when the relationship between the pulse and step components is damaged (Bahill and Troost 1979).

Dysmetric multiple-step

As mentioned above, multiple-step saccades often occur when the initial saccade is dysmetric. There are different dysmetric multiple-step saccades defined below:

Corrective saccades

Corrective saccades often occur after a large saccade and serve to get the eyes to the target after an undershoot or overshoot. In a typical corrective saccade, the brain should evaluate visual data and deliver visual feedback to the eye to readjust it in the correct position. Time is required for this process; thus, the corrective saccade must be triggered after roughly 150 milliseconds (Bahill and Troost 1979). Otherwise, it is unlikely to be a corrective saccade because a visual feedback signal requires a long intersaccadic delay. Studies on the monkey visual system have shown that visual response latency on awake monkeys' primary visual cortex (V1) and superior colliculus is up to 50 ms, which needs time to select the target and generate a motor response leading to about 150 ms (White et al. 2017; Schmolesky et al. 1998).

For all the saccades described above, a specific experiment design is required. When watching a movie, the subject can choose any target on the screen without telling the examiner. There are no predefined targets on the screen for such tasks. It is therefore impossible to measure the difference between the desired target location and where the eyes dropped (gained amplitude). For this dissertation, we measure only normometric saccades. Various saccadic subtypes have been explained to the reader for their information.

I.9. Human eye anatomy

In Figure I-6, A, the key components of the eye that aid in human vision are shown. The white portion of the eye is called the sclera, whereas the colored portion of the eye is called the iris, which is covered by the cornea.

The eye is intended to minimize optical distortion by focusing the visual picture onto the retina (L. Levin et al. 2011). A hole in the iris called the pupil controls the luminance variability of the light entering the eye to keep the image quality and contrast; hence, visual acuity will be optimized. The cornea and lens focus light that enters the pupil, which is subsequently transmitted to photoreceptors, rods, and cones at the retina's rear. The majority of the retina contains retinal neurons ahead of the photoreceptors, however, there is a spot in which light could be projected directly onto photoreceptors. This region is referred to as the fovea (Kandel et al. 2000). Additionally, the optic nerve fibers leave the retina via a region known as the optic disc.

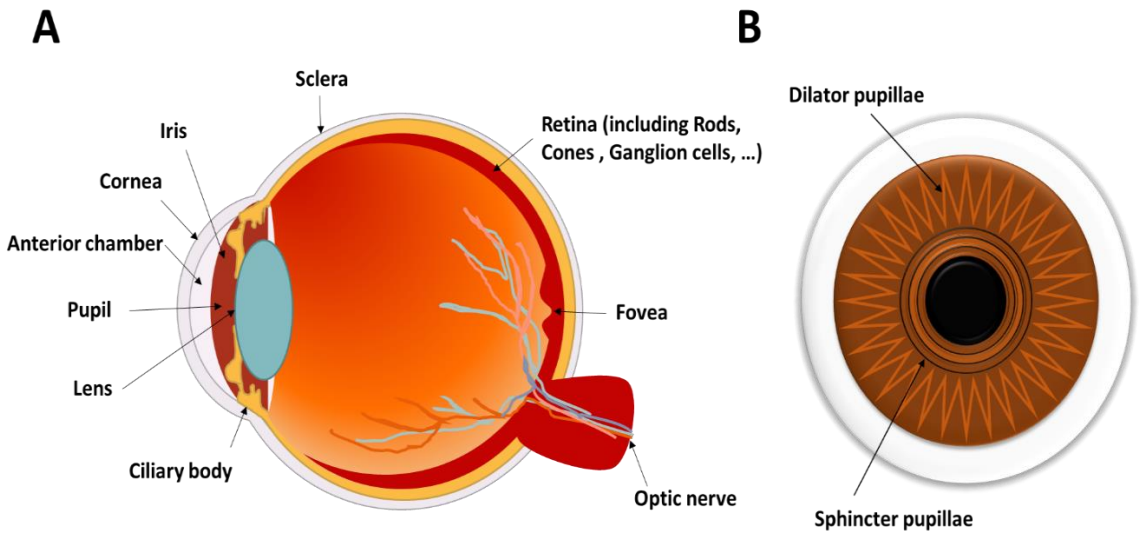

Figure I-6. Anatomy of the human eye. (A) The eye's major components and (B) a schematic enlargement of the pupil (Modified from (L. Levin et al. 2011)).

The iris influences the size of the pupil in a significant way. The iris is composed of two distinct groups of smooth muscles, as illustrated in Figure I-6 B. Muscles forming the sphincter pupillae constrict the pupil at the pupillary margin in a circular pattern. Dilation of the pupil occurs when dilator pupillae, a radially oriented muscle, is constricted. As illumination becomes dim, the radial dilator pupillae muscle constricts and pulls the pupil open while the

sphincter pupillae relaxes (ten Doesschate Jurriaan and Alpern Mathew 1965). On the other hand, a bright illumination will cause the circular sphincter pupillae muscle to constrict. Consequently, the pupil becomes smaller to control the amount of light that enters the retina (ten Doesschate Jurriaan and Alpern Mathew 1965).

The iris sphincter (which is regulated by the parasympathetic nervous system) has a more potent and active influence over pupil size than the iris dilator system (which is controlled by the sympathetic nervous system), even though pupil size relies on the balance between a parasympathetic and sympathetic nervous system (Giza et al. 2011).

I.9.1. Pupil

The resolution and preciseness of the image on the retina are fine-tuned by the lens and pupil size (L. Levin et al. 2011). Light entering the eye is the primary source of input that controls the sympathetic and parasympathetic innervation of the iris muscles. Multiple studies have investigated the relationship between pupil size and different aspects of cognition like certainty level, perception, learning, and decision-making (Wang and Munoz 2015). A comprehensive eye examination should include a pupillary test. The proper diagnosis of a visual pathway and autonomic nervous system can be achieved through careful observation, as well as a comprehensive and detailed case history.

Recent advances have allowed quantitative pupillometry to be developed as an autonomic testing tool (Bremner 2009). Standardization and consensus of testing protocols are required to further develop pupillometry as a noninvasive routine test for autonomic dysfunction. Muppidi et al. established a standardized pupil light reflex technique to evaluate the parasympathetic and sympathetic contributions to the pupil light reflex separately (Muppidi et al. 2013). They set up an experiment starting with one minute of darkness adaptation, followed by light stimulation of the eye while recording the pupil light reflex. They concluded that the initial pupil contraction after a light stimulation indicates parasympathetic innervation, the 75% recovery pupil diameter indicates mixed sympathetic and parasympathetic innervation, and the pupil diameter at 5 seconds indicates pupil sympathetic innervation (Figure I-7). As a result, they presented distinct parameters showing the autonomic nervous system's characteristics, which varied dramatically across different patient groups.

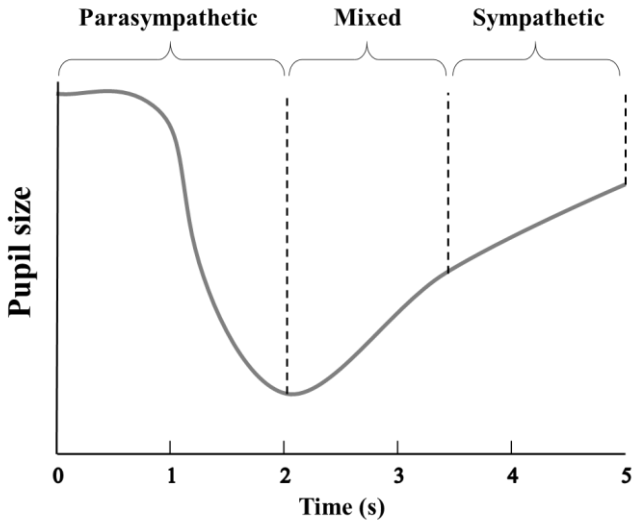

Figure I-7. Pupil light reflex. A schematic representation of the pupil light reflex and its associated components that are regulated by the sympathetic or parasympathetic nervous system. Mixed refers to innervations that are both sympathetic and parasympathetic (Adapted from (Muppidi et al. 2013).

I.10. Blink rate

Twelve spontaneous eye blinks occur per minute, interfering with the visible eye field (L. Levin et al. 2011). The blinking aids the secretion and dispersion of tears throughout the ocular surface, thereby preventing eye dryness. The regular tear cycle is disrupted by delayed blinking (L. Levin et al. 2011). Consequently, a person may have dry eye or secondary reflexive tears. It has been shown that blink is influenced by various characteristics such as age, emotion, and performance in mental activity (Sahlin et al. 1998). Studying blinks in great depth necessitates well-isolated tasks and consideration of other environmental variables, as well.

I.11. Main dissertation objectives

The assessment of the oculomotor system is critical for the differential diagnosis of neurodegenerative movement disorders such as αSYN – PD, DLB, MSA, and their prodrome RBD, as well as the Tauopathy PSP. It might be difficult to distinguish between PSP and αSYN in the early stages of the disease, especially when unusual features are present. Different saccades, pupil behavior, and blink rate can be measured using video-based eye tracking to determine the integrity of cortical and subcortical neural circuits, which can help to facilitate clinical diagnosis and improve oculomotor assessment and accuracy. With the development of potentially neuroprotective therapeutics for αSYN and Tauopathy, changes in saccade and pupil behavior components during prodromal stages of αSYN and early stages of Tauopathy are of great interest and may eventually serve as prodromal biomarkers.

The goal of this dissertation is to use video-based eye-tracking to identify novel biomarkers of disease in PD, RBD, MSA, and PSP. RBD is prodromal for αSYN, and abnormalities in RBD that are indicative of conversion to PD or MSA will be investigated here. Another goal is to identify differences between PSP and αSYN. Therefore, all patient groups were compared with each other and with the healthy control (CTRL) groups. Accordingly, this dissertation comprises two distinct research projects, each with a distinct focus:

Interleaved Pro/Anti Saccade Task (IPAST): The second chapter describes participants' behavior in the IPAST task: Previous research has demonstrated that patients with PD exhibit systemic abnormalities in oculomotor and pupillometric parameters (Perkins et al. 2021; Hanuška et al. 2019), which have been attributed to basal ganglia damage in the brain. Thus, the research objective is (1) whether saccadic abnormality, pupillary reaction, and blinks during the IPAST are different in RBD patients than in healthy controls, and (2) to what extent these target parameters already exhibit abnormalities in RBD patients comparable to PD, MSA, and PSP. (3) What are the distinctions between the αSYN and Tauopathy PSP?

Free viewing (FV): The third chapter describes the behavior of participants in the free viewing (FV) task that has been published (Habibi et al. 2022): We use a straightforward FV paradigm in which patients are presented with a series of brief video clips on a computer screen and are then free to view them in any way they like. This technique does not require extensive preparatory instructions for the participant to perform the task. In FV, we specifically address the following questions: 1) which saccade or pupil parameters are altered in patients with the manifest αSYN PD and MSA or the Tauopathy PSP? 2) are the abnormal pupil and saccade responses observed in PD or MSA also detectable in the prodromal αSYN stage RBD? 3) using these parameters, can we differentiate between patients with αSYN and PSP?

II. Task 1: Interleaved Pro/Anti Saccade Task (IPAST)

All of us interact with the environment with various voluntary and involuntary behaviors. Our behavior is under flexible and controlled action. For example, we know how to swing a ball with a bat and how to take a cup, among others. In some cases, there are complex ways to accomplish a task that makes it challenging to describe how they work.

The movement problems and the inability to inhibit automatic behavioral responses and initiate voluntary responses are characteristic of patients with PD (Wang et al. 2016). Neurodegeneration of the substantia nigra and its damaged striatal pathway in PD causes a slowing down of movement execution and impairment of cognitive control and movement initiation (Becker et al. 1995).

Research on saccadic eye movements could provide insight into motor impairment and response suppression in PD. Additionally, saccadic brain circuits have been extensively studied (Munoz et al. 2000; Leigh and Zee 2015). The SC mediates saccades that happen when visual stimuli appear suddenly (Hanes and Wurtz 2001). There are also saccades that occur when there is no visual stimulus, and these saccades depend on upper brain circuits like the frontal cortex and basal ganglia, providing critical input to SC (Hanes and Wurtz 2001). In order to reveal how PD initiates and executes voluntary motor responses, an appropriate task needs to be designed.

One of the tasks that capture and differentiate these types of saccades from each other is called Interleaved Pro/Anti-Saccade Task (IPAST). In 1978, Hallett established the IPAST, which has since become one of the most commonly used endogenous saccade paradigms (Hallett 1978; Everling et al. 1997; Munoz and Everling 2004; Hanuška et al. 2019). Pro-saccades commonly occur when participants make a rapid saccade to a peripheral target with an abrupt onset. Pro-saccades are usually fast and without error. The pro-saccade seeks to elicit the oculomotor system's stimulus-driven feature. On the other hand, there are anti-saccades that require suppressing the automatic saccade toward the stimulus and instead making a saccade away from the stimulus. Anti-saccades are

voluntary saccades that are triggered in the absence of a visual target and are associated with a higher error rate.

Thus an anti-saccade is defined as a saccade that is directed away from a peripheral stimulus to the opposite direction. To correctly perform the anti-saccade task, participants need to inhibit the automatic response towards the stimulus, invert the stimulus vector to produce the correct motor vector, and execute a voluntary saccade away from the stimulus.

IPAST can also be used to quantify pupil size which is controlled by a balance between sympathetic and parasympathetic pathways described in section I.9.1 (Loewenfeld 1993). Pupil size changes are also related to the LC function, which is damaged in PD (Braak et al. 2003; Wang et al. 2016; Joshi et al. 2016). In addition to saccade and pupillary responses, IPAST allows for examining the blink rates. The IPAST is, therefore, a well-designed task that can monitor a wide range of behaviors.

Several studies have investigated IPAST in PD patients, showing that PD suffers from saccadic impairments such as increased direction error and prolonged saccadic reaction time (Perkins et al. 2021; Chan et al. 2005). In the introduction, it was described that the objective of this study was to identify biomarkers in the prodromal stage of αSYNs diseases. Because RBD as the prodromal stage of PD and MSA is suitable to discover biomarkers by comparing the abnormalities between these groups (Miglis et al. 2021). Also, PSP, as another atypical parkinsonian disorder, is often misdiagnosed from PD and MSA in the earlier stages (Litvan et al. 1996). Therefore, we additionally investigated PSP in order to find out whether IPAST can facilitate early detection of this chronic disorder.

RBD has shown some similarities with PD; for example, a recent study has reported an increased direction error for RBD (Hanuška et al. 2019). A recent publication in our lab found that both RBD and PD exhibited significantly lower blink rates, lowered pupillary constriction, and dilation responses than CTRL (Perkins et al. 2021). Although there are pupil, saccade, and blink changes reported in PD, no study has compared the RBD to PD, MSA, and PSP. Accordingly, we made the following questions and hypotheses in IPAST:

Research question 1: Do RBD patients differ in their oculomotor parameters from healthy CTRL, PD, and MSA? Abnormalities in the specific parameters should show up gradually between the groups according to the slow disease progression. According to the preliminary studies (Terao et al. 2016; Perkins et al. 2021; Hanuška et al. 2019; Rottach et al. 1996) a significant difference between PD and MSA patients with CTRL subjects can be shown, whereas in RBD, as a prodromal stage, rather smaller differences in oculomotor parameters can be shown compared to healthy CTRL groups (CTRL < RBD < PD & MSA).

Research question 2: Do PSP patients differ in their oculomotor parameters from healthy CTRLs? Moreover, do PSP patients differ in their oculomotor parameters from αSYN groups (RBD, PD, and MSA)? In PSP patients, oculomotor dysfunction is one of the most prominent symptoms. Several studies have been conducted to investigate the abnormalities that occur in these patients (Troost and Daroff 1977; Armstrong 2011; Chen et al. 2010; Habibi et al. 2022). On the basis of preliminary studies, we expected to find significant impairments in oculo-pupillomotor deficits in PSP patients. We also compared PSP groups with αSYN groups in order to gain an understanding of similarities and differences in order to aid in the early identification of these diseases.

The hypothesis of comparing αSYN groups to CTRL:

1. PD patients show longer latencies of saccades than healthy controls.
2. MSA patients show longer latencies of saccades than healthy controls.
3. PD patients show higher error rates in the execution of anti-saccades than healthy control subjects.
4. MSA patients show higher error rates in the execution of anti-saccades than healthy control subjects.
5. RBD patients show higher error rates in the execution of anti-saccades than healthy control subjects.
6. PD patients show smaller saccade amplitude than healthy controls.
7. MSA patients show smaller saccade amplitude than healthy controls.
8. PD patients show a lower blink rate than healthy control subjects.
9. MSA patients show a lower blink rate than healthy control subjects.

10. RBD patients show a lower blink rate than healthy Control subjects.
11. PD patients show smaller pupil dilation size than healthy control subjects.
12. MSA patients show smaller pupil dilation size than healthy control subjects.
13. RBD patients show smaller pupil dilation size than healthy Control subjects.

Comparisons between the αSYN groups:

14. RBD patients differ from PD and MSA Patients in their latencies of anti-saccades.
15. RBD patients differ in their rate of error in the execution of anti-saccades from PD and MSA patients.
16. RBD patients differ in their saccade amplitude from PD and MSA patients.
17. RBD patients differ in their blink rate from PD and MSA patients.
18. RBD patients differ in their pupil dilation size from PD and MSA patients.

Tauopathy group PSP versus CTRL:

19. PSP patients show longer latencies of saccades than healthy controls.
20. PSP patients show higher error rates in the execution of anti-saccades of saccades than healthy controls.
21. PSP patients show smaller saccade amplitude than healthy controls.
22. PSP patients show a lower blink rate than healthy controls.
23. PSP patients show smaller pupil dilation size than healthy control subjects.

Tauopathy group PSP versus αSYN groups:

24. PSP patients differ from RBD and PD Patients in their latencies of anti-saccades.
25. PSP patients differ in their rate of error in the execution of anti-saccades from RBD and PD patients.
26. PSP patients differ in their saccade amplitude from RBD and PD patients.
27. PSP patients differ in their blink rate from RBD and PD patients.
28. PSP patients differ in their pupil dilation size from RBD and PD patients.

Regarding the Tauopathy group, it should be mentioned that the PSP cohort has been included in this dissertation as a confirmatory and exploratory cohort. We expected to

find confirmatory oculo- and pupillomotor deficits in PSP as reported in the literature (Armstrong 2011) but also sought to find more alterations.

II.1. Materials and methods

II.1.1. Participants

This study consisted of five different groups of participants. We recruited patients with PD, MSA, RBD, and PSP at University Clinic Marburg, department of neurology. Healthy controls (CTRL) were recruited as part of a major research study at Queen's University in Kingston, Canada (Yep et al. 2022). Human research ethics committees of the Faculty of Medicine, University of Marburg (Protocol ID: 147/16) and the Faculty of Health Sciences, Queen's University (Protocol IDs: PHYS-007-97; CNS-005-10) approved the protocol. The Declaration of Helsinki was followed to obtain voluntary informed consent from each participant (included in the appendix).

Patients were coming from different places in Germany. All patients were recruited from the outpatient clinic and the ward at the department of neurology in Marburg. RBD patients and some PD patients were seen in the outpatient department by Prof. Wolfgang Oertel and Dr. Annette Janzen. In addition, some RBD patients were recruited via the hospital's recruitment system. At the beginning of the PhD project, I was responsible for finding suitable patients for my research project, talking to the neurologists, and then obtaining consent from the patients to participate in my study. Only individuals whose condition had been verified by video-assisted polysomnography and by the neurologists (often following numerous tests, DAT-SPECT, and biopsies-see below) were enrolled.

However, later in the data collection phase, a recruitment nurse was in charge of assigning patients to each research group and obtaining consent forms. Therefore, PD patients, MSA patients, and PSP patients were recruited when they visited the outpatient clinic or were hospitalized. Most of the patients came from a place near Marburg, but there were also patients from all over Germany. Patients invited to our study were reimbursed for petrol or cab costs. For patients who had a long journey (mostly RBD patients), hotel costs were also covered by the study resources.

A variety of clinical tests were performed on all patients, including UPDRS III, the MoCA (Nasreddine et al. 2005), RBDSQ (Stiasny-Kolster et al. 2007), Beck’s Depression

Inventory-II (BDI-II) (Beck et al. 1996), and PD Non-Motor Scale (PDNMS) (Storch et al. 2010).

As mentioned earlier, the MoCA questionnaire is used to monitor the risk of developing dementia in RBD patients and is a cognitive assessment tool. Also, the RBDSQ was used to determine the presence of RBD symptoms in participants. Additionally, we collected the BDI-II scores that measure characteristics attitudes, and symptoms of depression using 21 self-report items. The other questionnaire we collected was PDNMS which is used to assess a wide range of non-motor symptoms associated with Parkinson's disease (PD).

In all cases, I gathered questionnaires myself, except for UPDRS, which was assessed with the patient's corresponding doctor. Since questionnaires like MoCA cannot be completed twice within a short time period, if the physicians had previously completed them, I could not do them again. Instead, I could only benefit from them. Patients who had already been hospitalized for a few days had their questionnaires collected on the same day or before the experiment, depending on the situation. Patients who came to the outpatient clinic for an appointment or were only invited to our experiment were asked to fill out the questionnaires on the same day. Approximately 40 minutes were required to complete all the questionnaires.

II.1.1.1. Exclusion criteria

We did not recruit the following patients: 1) patients with a secondary RBD or parkinsonian syndrome (e.g., drug-induced, subcortical arteriosclerotic encephalopathy); 2) patients who were taking medications that may alter pupillary responses (e.g., anticholinergics, benzodiazepines, beta receptor blockers, pilocarpine, or other drugs if indicated in the manufacturer’s information); and 3) patients with glaucoma, pronounced strabismus, or uncorrected refractive error > ± 5 diopters.

An extensive medical and drug history was obtained from all RBD patients by corresponding doctors, along with a complete neurological examination. This procedure was repeated twice a year to avoid including subjects suffering from secondary RBD in the study. This was part of the routine clinical diagnosis conducted by a neurologist,

independent of my project. In addition, we excluded RBD patients with cognitive impairment (MoCA < 25), and this would presumably minimize the number of patients likely to convert to DLB (Miglis et al. 2021). A MoCA score of less than 25 was considered an exclusion criterion only for RBD patients but not for other patients.

Each cohort had the maximum possible number of participants measured. The size of each group was determined mainly by the disease's epidemiology and the number of patients who had attended Marburg's University Hospital and could therefore be recruited.

Following data collection, all data was processed through a fully automated pipeline, where various objective filters and criteria were applied regardless of the participant's characteristics. For example, a good calibration, a reasonable number of saccades during the task, a small number of blinks (or loss of data as a result of eyelid closures or head movements), or specific filters for certain parameters (acceptable reaction time for the pupil when searching for pupil constriction, explained in SectionII.8.1). Patients with poor data quality were excluded from these analyses.

II.1.1.2. Participant's characteristics

CTRL. One hundred thirty-two healthy age-matched CTRL participated in the study (86 female: 62.30 ± 9.87, 46 male: 62.95 ± 9.92) that were collected in Canada. The CTRL participants were subject to a similar test structure in that we tried to have a common room, experiment, and rules for implementation at each center. There were no clinical questionnaires provided for CTRL subjects. Table II-1 has provided clinical and demographic data. There was not a one-to-one matching of the control cohort to the patient cohorts. Instead, the approach aimed to utilize the extensive control group and encompass the entire age range of various patients.

Table II-1. IPAST participants' clinical data. CTRL: Control group; RBD: REM sleep behavior disorder; PD: Parkinson's disease; MSA: Multiple system atrophy; PSP: Progressive supranuclear palsy; MoCA: Montreal cognitive assessment; UPDRS: Unified Parkinson's disease rating scale; BDI: Beck's depression inventory; PDNMS: PD non-motor symptoms scale; RBDSQ: RBD screening questionnaire.

Group	participants	Age at time of measurement (years)	MoCA score	UPDRS III Score	BDI-II	PDNMS	RBDSQ
CTRL	132 (86F, 46M)	All: 62.67 ± 9.90 F: 62.30 ± 9.87 M: 62.95 ± 9.92	-	-	-	-	-
RBD	39 (3F, 36M)	All: 64.66 ± 5.73 F: 72.33 ± 1.15 M: 64.02 ± 5.57	28.18 ± 1.75	1.63 ± 1.45	8.01 ± 7.52	8.55 ± 4.47	10.45 ± 1.87
PD	37 (8F, 29M)	All: 66.05 ± 8.28 F: 67.12 ± 5.71 M: 67.75 ± 9.06	27.51 ± 3.45	14.02 ± 10.22	10.91 ± 10.43	7.60 ± 5.10	5.95 ± 3.94
MSA	14 (6F, 8M)	All: 63.71± 7.36 F: 62.33 ± 8.73 M: 64.75 ± 7.14	27 ± 2.97	29 ± 10.12	13 ± 5.16	11 ± 4.43	5 ± 3.08
PSP	8 (2F, 6M)	All: 67.62 ± 6.98 F: 66.50 ± 0.70 M: 68 ± 8.78	27 ± 1.2	24 ± 8.07	12 ± 5.17	9 ± 3.96	2 ± 1.08

RBD. Thirty-nine patients (3 female: 72.33 ± 1.15, 36 male: 64.02 ± 5.57) with video polysomnography-confirmed RBD (Darien IL, AASM, 2014) were included in this study. Mean UPDRS-III, MoCA, and BDI-II scores in RBD were 1.63, 28.18, and 8.01, respectively. There are 39 highly phenotyped RBD patients who constitute a substantial and sufficient number of participants (see power calculations in the appendix: Tables of statistics)

PD. In this study, PD patients were diagnosed in accordance with the United Kingdom Brain Bank Criteria. Thirty-seven PD (8 female: 67.12 ± 5.71, 29 male: 67.75 ± 9.06) were included in the study: 7 patients had de novo PD, 12 had been treated with dopaminergic medication (on-state), 14 patients had not been administered medicines for at least 12 hours (defined as off-state), and 4 had an unknown medication status. Accordingly, all four groups were pooled into one PD group due to the relatively slight variance between on and off states in saccadic behavior (Cameron et al. 2012). Mean UPDRS-III, MoCA, and BDI-II scores in PD were 14.02, 27.51, and 10.91, respectively.

The average disease duration for PD patients at the measurement date was 2.66 ± 3.38 years. As a result, PD patients were recruited relatively early after being diagnosed.

In terms of the number of participants, 37 patients with PD are considered to be a sufficient and substantial number of participants (see power calculations in the appendix: Tables of statistics)

MSA. Fourteen MSA patients (6 female: 62.33 ± 8.73, 8 male: 64.75 ± 7.14) were diagnosed according to the second consensus statement on the diagnosis of MSA (Gilman et al. 2008). Mean UPDRS-III, MoCA, and BDI-II scores in MSA were 29, 27, and 13, respectively. Although the number of 14 appears to be low, for a monocentric trial this is acceptable.

PSP. Eight PSP patients (2 female: 66.50 ± 0.70, 6 male: 68 ± 8.78) were diagnosed according to the National Institute of Neurological Disorders and Stroke and the Society for PSP (NINDS-SPSP) and Höglinger et al. (Höglinger et al. 2017) criteria. Mean UPDRS-III, MoCA, and BDI-II scores in PSP were 24, 27, and 12, respectively. Originally a group of 13 PSP patients was investigated, but 5 had to be excluded from the data analysis due to bad calibration and bad data quality (i.e. if a patient moves his head).

II.1.2. Task description

The pro/anti-saccade task consisted of two different trials (Figure II-1): Pro and anti-saccade trials. Each trial started with a black screen (0.1 cd/m2) on an empty page that lasted 1000 ms (ITI: inter-trial interval). Then a fixation point (0.5° diameter, ~44 cd/m2) appeared in the middle of the screen. Subjects were supposed to look at the fixation point as long as it was there. The fixation point was displayed for 1000 ms, and its color was either red or green. The green color fixation point revealed the pro-saccade trial, and the red color revealed the anti-saccade trial. Fixation was followed by a 200 ms gap, and then a peripheral white stimulus (0.5° diameter, ~62 cd/m2) was displayed on either 10° to the left or right of the fixation point.

There were written instructions for the patients on how to perform the pro/anti-saccade task. These instructions were provided to each participant prior to the task (are included in the appendix). In pro-saccade trials, participants were told to look toward the stimulus

as soon as it was displayed. In anti-saccade trials, participants were told to look in the opposite direction of the stimulus as soon as it appeared. There were 60 pro-saccade and 60 anti-saccade trials; in total, a block of 120 trials was randomly displayed to subjects. Participants were unaware of the subsequent coming trials when they ran the task. The experiment was run two times with a short break time in between. For each subject, we collected 240 trials lasting 13.8 minutes. The time in which the task was explained and trained depended very much on the individual. Including calibration time, teaching time, and recording time, IPAST required nearly 20 minutes. This session was followed by the second experiment (FV) which will be explained in the next chapter. Approximately 35 minutes were dedicated to IPAST and FV. Additional time was required for completing clinical questionnaires.

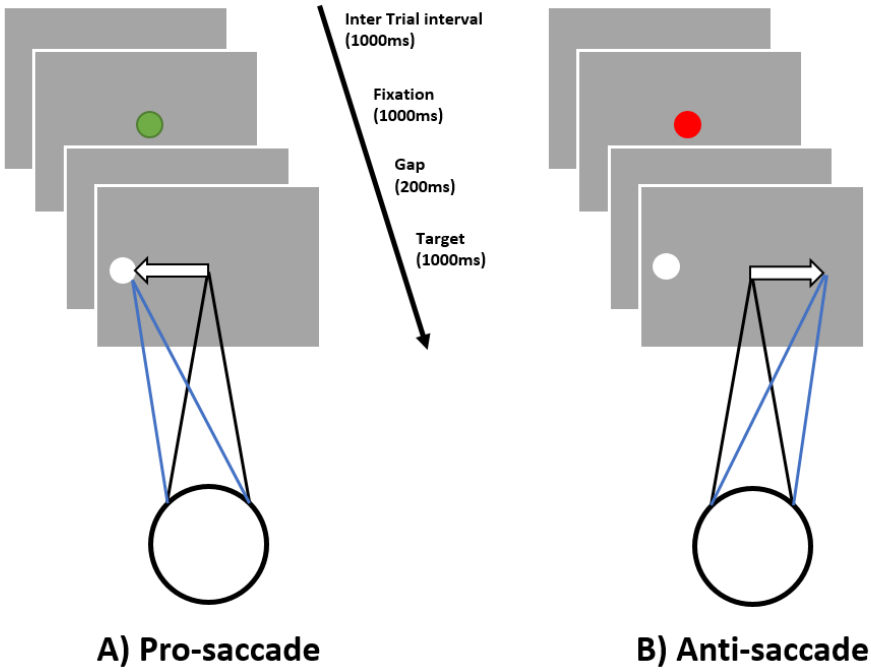

Figure II-1. Pro/Anti-Saccade Task A) Proceedings of a pro-saccade trial and B) Proceedings of an anti-saccade trial. There is a 1000 ms inter-trial interval during which the screen is blank. Then on a black background (0.1 cd/m2), a central fixation point appears (FIX: 0.5° diameter, 44 cd/m2) that lasts 1000 ms. The FIX color revealed the task type (green: pro-saccade; red: anti-saccade). After then, FIX disappeared, followed by a blank screen around 200 milliseconds (gap period). Following the gap interval, a peripheral white stimulus (0.5° diameter, 62 cd/m2) emerged 10° horizontally to the left or right of the FIX location. Arrows represent the direction of the saccade from the center screen to the screen edges.

II.1.3. Eye tracker

A video-based monocular eye tracker was used to monitor eye location, pupil size, and blink rate at a rate of 500 Hz (Eyelink-1000 Plus, SR Research Ltd, Osgoode, ON, Canada). Stimuli were displayed on a 17-inch LCD monitor with a screen resolution of

1280 x 1024 pixels (60 Hz refresh rate), corresponding to a viewing angle of 32° x 26°, and the distance between the eyes and the monitor and infrared camera was adjusted at 60 cm (an optimum distance between the camera and the eye). All recordings and calibrations were done monocularly, using the right eye as the reference point. The location of the eye was first calibrated using a nine-point grid (eight around the periphery and one central). Stimuli were flashed randomly over the screen, and participants were required to focus on each one until the next appeared. Following calibration, the procedure was repeated to ensure that the average error between fixation and stimulus was less than 1° and that there was no loss of eye tracking. Video-based eye-tracking devices were regularly tested to verify that observed significant differences were not attributable to differences in location. This study used a spectrometer to ensure that the eye-trackers displays emitted an identical amount of brightness, which had no impact on pupil baseline, constriction, or dilation levels. All data were taken in a windowless testing room inside the department of neurology, Marburg, with all lights, turned off to ensure that the only illumination source was the computer display.

II.1.4. Model schematic

Figure II-2 depicts the epochs during which saccade and blinks were measured. Further sections describe the procedures in more detail.

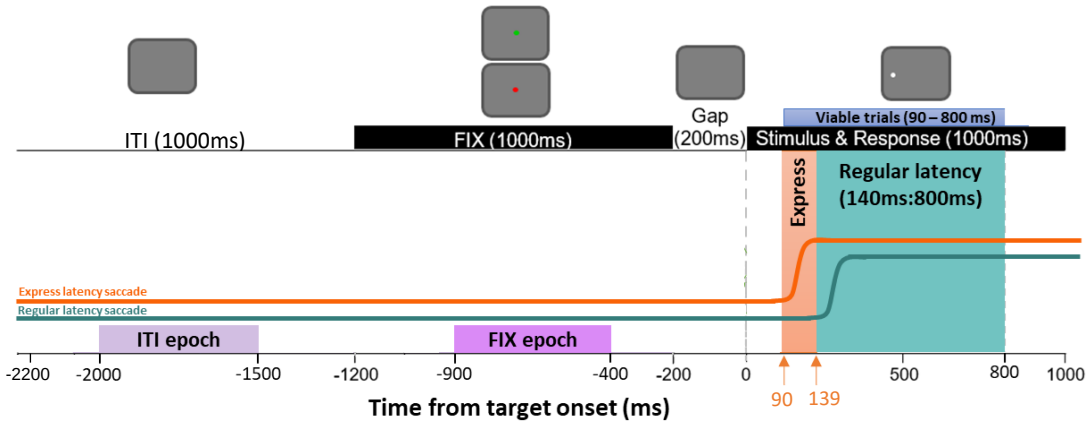

Figure II-2. A time-lapse depiction of IPAST. The top panel depicts the experiment paradigm; the task begins with a 1000 ms inter-trial interval (-2200:-1200 ms; time zero is considered as the time that the target stimulus appears) and is followed by the fixation dot in the middle of the screen that is either red (anti-saccade) or green (pro-saccade) and lasts 1000 ms (-1200 ms:-200 ms). The Gap period lasts 200 ms before the target stimulus appears. The target stimulus arrives at time zero and remains on the left or right side of the screen for about 1000 ms. At the bottom panels, eye traces are depicted. The express saccades happen from 90 to 139 ms, and regular latency saccades from 140 to 800 ms. Viable trials are the combination of both express and viable trials. The range of ITI blink epochs (-2000: -1500 ms) and FIX blink epochs (-900: -400 ms) are shown by the purple shaded boxes. ITI: Inter-trial Interval; FIX: Fixation.

II.2. Saccade analyses

All saccades were marked for direction, amplitude, peak velocity, and duration (Coe et al. 2021). Saccades were defined as eye movements with an instantaneous velocity greater than 20 deg/s and a duration longer than 10 ms. We computed the z-score of the velocity-amplitude relationship for each eye movement that was initially coded as a saccade in order to distinguish non-physiological data from real saccades. Specifically, initially coded saccades whose z-score was > ±3 standard deviation (SD) were considered outside the range of a normal saccade and were removed.

II.2.1. Saccade reaction time

The time elapsed between the time of stimulus onset and the beginning of the first saccade is referred to as saccade reaction time (SRT). Based on the latency, saccades could be categorized into different types. Some saccades could have relatively short latencies, but the others could last longer. We defined two different saccades comparative to the literature (detailed in the following sections): express (90 ms ≤ SRT ≤ 140 ms) and regular latency saccades (140 ms < SRT < 800 ms). Saccades occurring after 800 ms are extremely rare. These delayed saccades were excluded from analyses because they were outliers whose impacts would distort the interpretation of an individual's behavior and skills.

II.2.2. Express latency saccades

The term express latency saccade is derived from the study by Fischer and Boch in 1983 on monkeys and was studied by Fischer and Ramsperger in 1984 in human eye movements (Fischer and Boch 1983; Fischer and Ramsperger 1984). Based on the studies mentioned above by Fischer et al., monkeys were found to have a latency of about (70 - 120 ms) which is longer in humans (90-140 ms). Express latency saccades also depend on different factors like stimulus characteristics, target stimulus eccentricity, the amount of training, and different laboratory conditions (Fischer and Ramsperger 1984).

During fixations, the eyes are actively held in place by groups of neurons that inhibit saccades. Researchers have identified neurons in the substantia nigra pars reticulata (SNpr) which become silent during fixation, and this process does not depend on whether visual stimuli are present or absent (Munoz and Wurtz 1993). Munoz and Wurtz revealed in 1993 that the fixation neurons at the rostral pole of the intermediate layers of the SC

play a key role in maintaining fixation and preventing the involuntary execution of saccades (Munoz and Wurtz 1993). However, express latency saccades more occur in the gap scenario when the fixation point disappears about 200 ms before target stimulus onset and after intensive training. The SC is a critical area that has been found to contribute to the modulation and generation of express latency saccades (Dorris and Munoz 1995). Dorris and Munoz hypothesized that during the gap period, the monkey's saccadic generating system would be disinhibited by the lower activity of fixation cells in the SC (Dorris and Munoz 1995). This, in turn, will result in a faster saccadic reaction time (express latency saccades). These saccades have a high probability of being incorrect since they result from spontaneous judgments with little time for thought.

II.2.3. Regular latency saccades

Saccades that take longer to begin are typically more precise or undergo a further assessment prior to happening (Everling et al. 1997). According to previous studies, regular latency saccades were defined as those with a latency greater than 140 ms (Perkins et al. 2021; Coe and Munoz 2017). Generally, observers' responses to anti-saccades are slower (around 250-350 ms) than their responses to pro-saccades (mean around 150-250 ms) (Kandel et al. 2000).

II.2.4. Direction error

Direction error is defined as failing to make saccades in the direction they are supposed to gaze (toward the target stimulus in pro-saccade trials and away from the target stimulus in anti-saccade trials). Errors are more likely to occur as tasks become more complicated; anti-saccade trials are excellent candidates for driving direction errors. Other variables, such as lack of attention, forgetfulness, and cognitive difficulties, might all have an effect on the subjects' performance (Bahill and Stark 1975). As a result of the lower number of errors in pro-saccade trials, we only considered direction errors in anti-saccade trials.

II.3. Pupil analyses

Pupil analyses in the IPAST paradigm are intended to describe pupillary responses during the central fixation epoch (Figure II-3). The pupil light response (i.e., constriction) is momentarily elicited during this interval by the presence of the fixation point. Because pupil size is sensitive to the eye position, pupil analyses were conducted utilizing just pupil recordings from a 1200 ms window when the subject was fixating on the central

fixation point. The analysis window began with the onset of the fixation point and ended at the onset of the peripheral stimulus, as shown in Figure II-3.

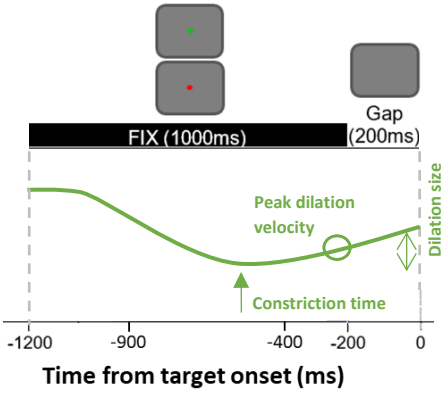

Figure II-3. Pupil response. The pupil constriction and dilation during the fixation period until the target stimulus onset have been shown. Time zero shows the target stimulus onset. Constriction time has been considered the time when the biggest constriction occurred. The biggest dilation velocity after constriction time until target appearance has been referred to as Peak dilation velocity. The pupil size difference between peak constriction size and target stimulus onset represents dilation size. FIX: fixation.

II.3.1. Identifying IPAST trials for pupil analyses

Pupil metrics are sensitive measurements that can be influenced by various circumstances, requiring active central fixation by the participants. As a result, not all trials are suitable for pupil analyses. If even one of the following conditions is not met, the trial is considered unsuitable for pupil analyses.

1) Fixation onset time

The presence of the fixation cue indicates the start of a trial, as it is also the stimulus that initiates pupil responses during the fixation period. During ITI, participants who gaze away from the center may be unable to initiate central fixation sufficiently early to provide adequate pupillary data to analyze. To be included in our pupil study, individuals had to initiate fixation within 150 ms of the fixation point's presentation.

2) Maintenance of fixation

As previously stated, deviations in eye position from central fixation can cause pupil measurements to be distorted. Thus, participants had to maintain fixation for a trial to be included in our pupil analyses. When participants look more than 2° away from the

fixation point over the 150 ms following the fixation point onset, and until the beginning of the peripheral stimulus, the trial has been removed.

3) Blinks

During the FIX window, eye blinks can disrupt pupil monitoring. Linear interpolation has been used to replace missing pupil values during eye blinks. Depending on how long the blink lasts, this interpolation may be inaccurate in capturing the accurate pupil response. Therefore, the number of trials was limited by considering the blink duration and frequency. A maximum of one blink within the fixation period, which lasts no longer than 200 ms, was taken into account for pupil analyses.

II.3.2. Pre-processing of pupil signal

Pupil size was reported as pupil area (pixels) within the range of (100 to 10000 units with a precision of 1 unit) by Eyelink 1000 plus. Cornea optical distortion can impact pupil size quantifications up to 10%, and therefore, we limited the noise by drift correction between the trials. Other camera factors could also affect pupil size, particularly when the eye gets away from the center of the screen.

We resampled the Pupil signal, which is noise-less and preprocessed and has consequently some lost data (including blinks too), by using the Shape-preserving piecewise cubic interpolation function of MATLAB.

We smoothed the zero-normalized pupil traces for each participant using a MATLAB smoothing tool (local regression using weighted linear least squares and a 1st-degree polynomial model with a 50-sample span). We next obtained the first derivative of the smoothed traces (pupil velocity). We reapplied the smoothing algorithm, as the derivative amplifies any residual noise and would otherwise result in false alarms when detecting trustworthy parameters from the traces. We used a running signed-rank test (1-tailed, i.e., negative velocity in the constriction condition and positive velocity in the dilation condition) to identify the moment at which the velocity curves were significantly different from a baseline velocity.

II.3.2.1. Fixation onset time

As previously mentioned, the fixation onset time was critical in pupil analyses. We considered a limited time window within that subjects were supposed to be around the

fixation point. Trials in which subjects were slower than 150 ms have been removed from the analyses of CTRL, RBD, and PD, and any subjects with the remaining trials equal to 5 have been involved. We had to allow a higher delay amount for the MSA and PSP patients as they had a considerable delay. Therefore, for these two diseases, the beginning of pupil responses, which was pupil constriction, could not be considered reliable. However, we considered the pupil dilation quantification reliable for these two groups.

II.3.2.2. Peak constriction time

The time that the pupil reached the highest amount of constriction was computed as the peak constriction time.

II.3.2.3. Dilation size and velocity

Following the maximum pupil constriction size, the pupil dilates until it reaches a stable state, which was not the objective of this study. We evaluated pupil dilation until the target appeared and computed the peak dilation velocity during the dilation phase.

The term "dilation size at the target onset" refers to the difference between the size of the pupil at its maximum constriction and the size of the pupil at the time of the target onset.

II.4. Blink rate

There can be several data losses during video-based eye tracking due to blinking or other circumstances that may cause the pupil to be covered. It was important to distinguish true blinks from other data lost. Pupil area data was further clarified to determine when specific blinks were responsible for data loss. The maximum allowed blink (or data lost) in every trial was less than 40 times. Blinks with more than 600 ms lengths were ignored and not considered in the blink rate or blink duration analyses.

The pupil area has been normalized for each trial using equation (1). Pixels represented pupil area, and they spanned between 100 and 10000 pixels. Therefore, the numbers below 10 were removed. Throughout all of the trials, the non-zero mean of the pupil area was maintained at 300 (It was chosen arbitrarily, more details here: (Coe et al. 2021)).

$$A300 = \frac{A}{mean(A(A > 10))} * 300 \quad (1)$$

The pupil data was smoothed to find the part of the data that was indicative of eye loss. The abnormal pupil area was considered as the A300 < 200 or A300 > 400.

To calculate the high-velocity data (>1000), a smoothed velocity profile of A300 (with the help of a three-point kernel) was calculated. This data was then cleaned up by removing high-velocity data and abnormal pupil areas.

In order to replace the removed data, linear interpolation was applied between the preceding and following data points. In order to smooth this signal, a large 50-point kernel was applied to the low-frequency model. After smoothing, the signal was subtracted from the A300.

With the low-frequency modulation removed from A300, a flattened A300 was created with bolded high-velocity changes, which made it easier to identify the beginning and end of lost data. After a blink, it takes some time for the eyelid to completely cover the pupil, so the camera continues to collect pupil data until it loses it. This results in a change in pupil size around the data loss, which is recorded prior to and after a blink. As a result, the blink appears shorter than it really is. Consequently, these variations were thought to be part of the blink.

The beginning and ending times of the data loss were then used to calculate the beginning and ending times of a complete blink using the A300's smoothed absolute velocity, with a trial-by-trial dynamic threshold. We differentiated between data loss due to a blink and data loss due to some other interference based on data loss duration. Only real blinks were considered in this dissertation.

II.5. Statistical analyses

Based on Ethic application, using variance analysis (ANOVA, single factorial, fixed effects) it was determined that an effect size of f = 0.355, with an applied significance level of 5% and a test power of 80%, would require a sample size of n=23 participants in each group.

The minimum sample size was considered 20% more (n=28) to compensate for an approximated failure rate of 20%, taking into account at least 10% dropout during execution (e.g., as a consequence of insufficiently possible calibration) and, in addition, a technical data loss during the later data analysis of about 10% (often due to too strong interfering artifacts/missing values within the recorded eye-track, such as too pronounced blinking or a pupil obscured by the eyelid).

However, two patient groups, MSA and PSP, were too rare and difficult to recruit, and the minimal sample size was not met. Nevertheless, the power analysis using G*Power software of the two indicated groups revealed that both groups were statistically distinguishable from other groups (details in appendix).

We examined the normality distribution of the data using the One-sample Kolmogorov-Smirnov test (in MATLAB); however, because the data were not distributed randomly, a non-parametric test was selected. The significant statistical differences were determined using a pairwise non-parametric test, Mann-Whitney-U-test (Mann and Whitney 1947), in MATLAB. In view of the exploratory nature of the study, multiple comparison adjustments were not considered. Nevertheless, a comparison of the results before and after the Bonferroni adjustment is presented in Table II-6, which demonstrates that most of the results remained unchanged.

II.6. Results

II.6.1. The cumulative saccade reaction time (SRT) distribution

To illustrate a general overview of the data, we calculated the histogram of SRT during the pro and anti-saccade trials for both correct and error saccade types. The cumulative distribution of SRT for correct and direction error trials in both pro- and anti-saccade tasks is depicted in Figure II-4, A, and B. Saccades before target onset (time zero) have no label of correct or error because there is no target on the screen yet, and all the saccades are randomly initiated. In Figure II-4, A, and B, Pro saccade trials across groups are represented by curves that extend above the baseline line, whereas direction error is represented by lines that extend below the baseline line.

There was no clear difference in reaction time between CTRL and RBD, PD, and PSP groups in pro-saccade trials, as all patients showed almost the same pattern as CTRL.

However, MSA displayed much faster reaction times than CTRL, as indicated by the MSA curve going higher than CTRL's (Figure II-4, A). While CTRL and RBD early peaks in anti-saccade trials were almost aligned, patients and CTRL peaks differed dramatically. Therefore, anti-saccade trials are well suited to revealing the differences between patients and CTRL.

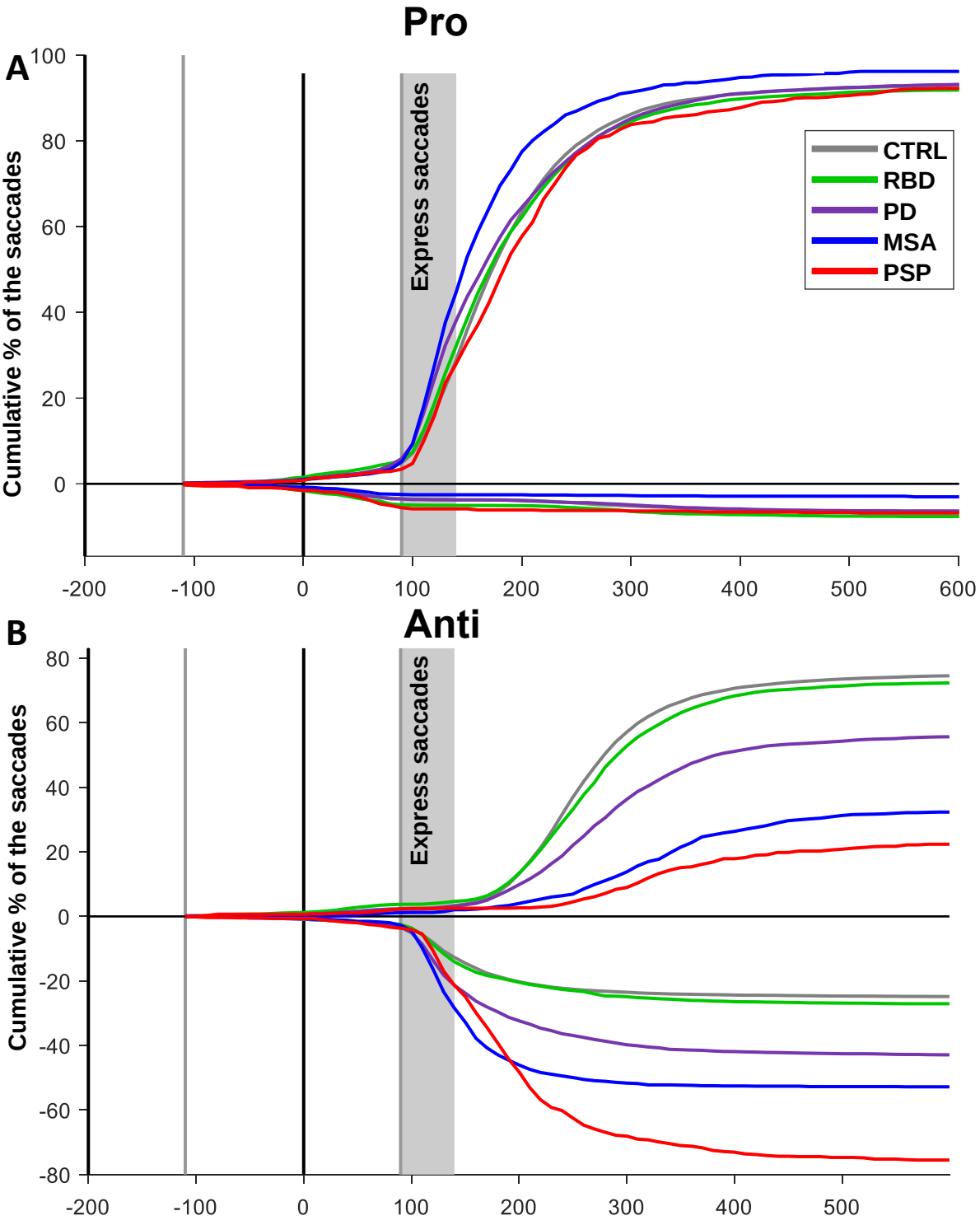

Figure II-4. Cumulative saccadic reaction time (SRT) distribution of group responses from -200 to 600 ms for the (A) pro-saccade and (B) anti-saccade trials. At a particular reaction time, each distribution reflects the cumulative percentage of correct and incorrect responses. The range of express latency saccades is shown by the broad gray-shaded boxes in (A) and (B) (90:140 ms). Responses shown above the zero line are correct, while those shown below are errors. SRTs were binned into 10 ms epochs to construct the curves. CTRL: Control group; RBD: REM sleep behavior disorder; PD: Parkinson's disease; MSA: Multiple system atrophy; PSP: Progressive supranuclear palsy.

II.6.2. Correct median saccade reaction time (SRT)

Figure II-5 depicts the SRT of the viable trials (regular plus express latency saccades). CTRL (178 ms) exhibited a significantly longer reaction time than MSA (158 ms, U=572.5, z=-2.39, $P<.05$) in pro-saccade trials that were correctly performed (towards the target). In pro-saccade trials, although other patient groups (RBD: 174 ms, PD: 172 ms, PSP: 189.5 ms) also showed longer reaction time than MSA, none of the comparisons was significant.

During anti-saccade trials, CTRL (262 ms) had a faster SRT than PD (275 ms, U=1862.5, z=-2.09, $P<.05$), MSA (314 ms, U=528.5, z=-2.33, $P<.05$), and PSP (386 ms, 176.5, z=-3.18, $P<.01$). Additionally, RBD (279 ms) and PD had faster reaction times than PSP (U=60, z=-2.71, $P<.01$ and U=71.5, z=-2.20, $P<.05$, respectively).

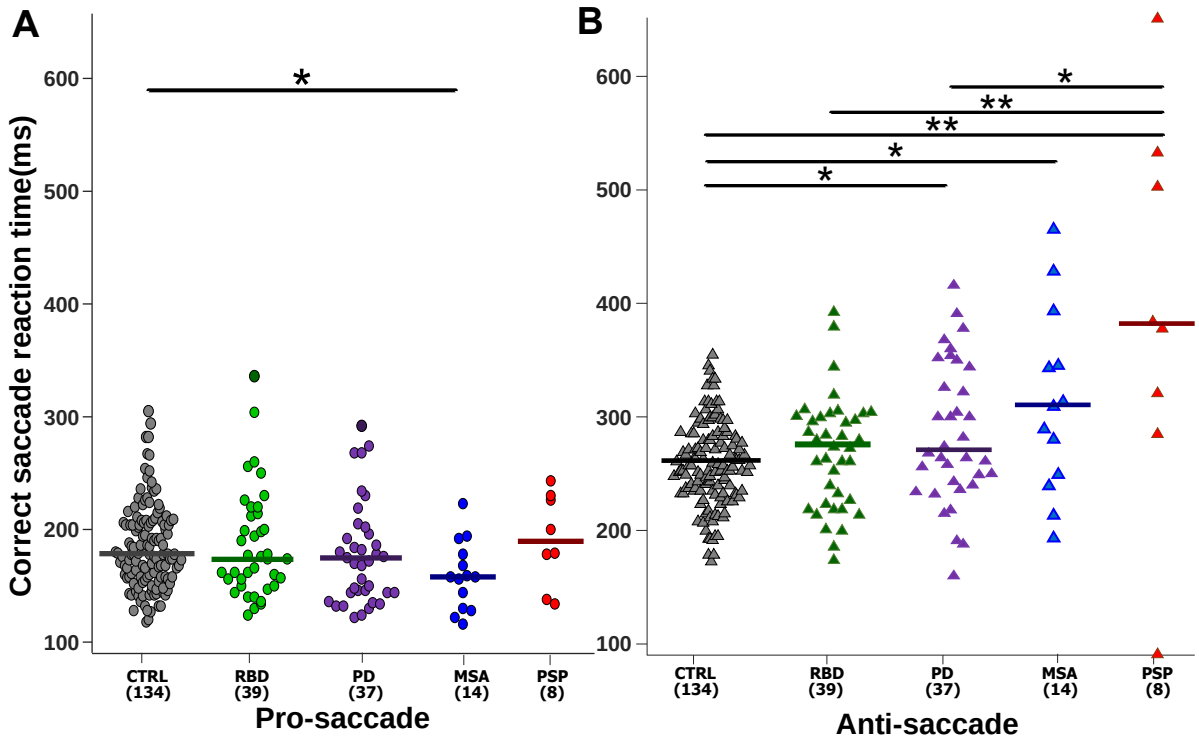

Figure II-5. The reaction time of the correct saccade for each group during (A) pro-saccade and (B) anti-saccade trials. The horizontal solid line on each group's data points shows the median. CTRL: Control group; RBD: REM sleep behavior disorder; PD: Parkinson's disease; MSA: Multiple system atrophy; PSP: Progressive supranuclear palsy.

II.6.3. Express latency saccades

CTRL showed express latency saccade rates of 14,64 %, while RBD, PD, MSA, and PSP showed respective express latency saccade rates of 12.5 %, 25 %, 21.25 %, and 13.47 % (Figure II-6). In pro-saccade trials, CTRL had a significantly lower express latency saccade rate than PD (U=1925, z=-2.07, $P<.05$) and MSA (U=596.5, z=-2.23, $P<.05$).

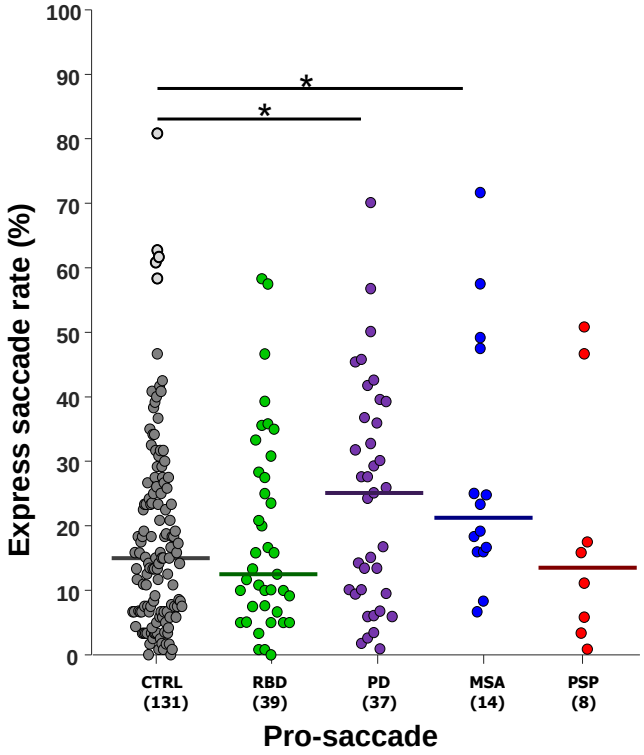

Figure II-6. Express latency saccades for each group during pro-saccade trials. The horizontal solid line on each group's data points shows the median. CTRL: Control group; RBD: REM sleep behavior disorder; PD: Parkinson's disease; MSA: Multiple system atrophy; PSP: Progressive supranuclear palsy.

II.6.4. Regular latency saccades

Patients with more express latency saccades had fewer regular latency saccades. In pro-saccade trials (Figure II-7), CTRL (72.77 %) displayed higher regular latency saccade rates than both PD (U=1558.00, z=-3.455, 54.16 %, $P<.001$) and MSA (41.36 %, U=386.00, z=-3.61, $P<.001$). The regular latency saccade rate of MSA was even lower than that of RBD (69.74 %, U=161.50, z=-2.25, $P<.05$). PSP displayed to have a regular latency saccade rate of 57.91 %.

In anti-saccade trials, CTRL had more regular latency saccades (82.08 %) than PD (69.16 %, U=1713.00, z=-2.87, $P<.01$), MSA (53.33 %, U=337, z=-3.93, $P<.001$), and PSP (72.08 %, U=298.5, z=-2.10, $P<.05$). This is because there were more correct anti-saccades in CTRL compared to other PD, MSA, and PSP but not RBD. MSA had a lower regular latency saccade rate than both RBD (83.33 %, U=117.5, z=-3.11, $P<.01$) and PD (U=161.5, z=-2.05, $P<.05$).

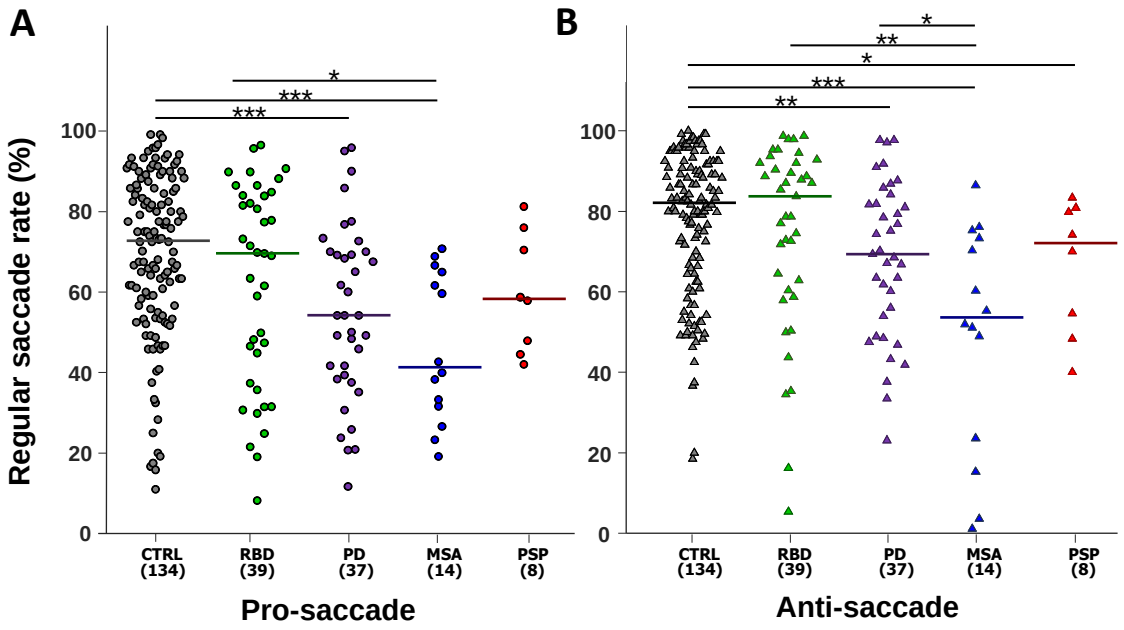

Figure II-7. Regular saccades for each group during (A) pro-saccade and (B) anti-saccade trials. The horizontal solid line on each group's data points shows the median. CTRL: control group; RBD: REM sleep behavior disorder; PD: Parkinson's disease; MSA: Multiple system atrophy; PSP: Progressive supranuclear palsy.

II.6.5. Anti-saccade direction error

The percentage of anti-saccade errors over all viable, express, and regular latency saccades have been represented in Figure II-8, A to C.

Figure II-8, A shows the anti-saccade direction error rate in viable trials (express + regular). There was no difference between CTRL (15.06) and RBD (19.16), but CTRL showed a lower direction error rate compared to PD (31.09, U=1264.5, z=-4.55, $P<.001$), MSA (41.66, U=499, z=9544, $P<.01$), and PSP (57.36, U=59, z=-4.2, $P<.001$). RBD had a lower direction error than PD (U=385, z=,-3.49 $P<.001$), MSA (U=148, z=-2.52, $P<.05$), and PSP (U=17, z=-3.93, $P<.001$). PD patients had a smaller direction error than PSP (U=55, z=-2.76, $P<.01$) patients. PSP patients nearly had the greatest direction error.

Task 1: Interleaved Pro/Anti Saccade Task (IPAST)

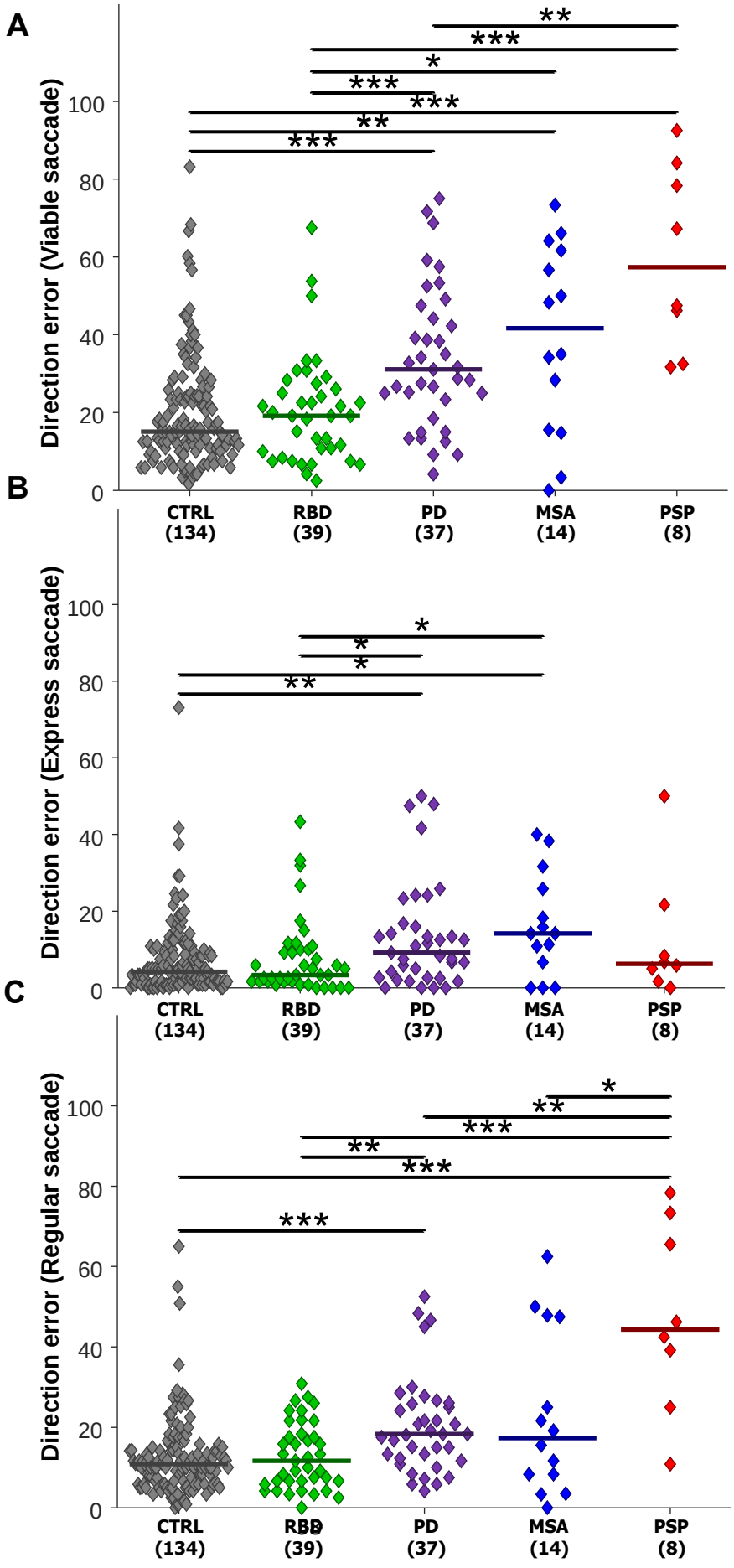

Figure II-8. Direction error in viable (A), Express (B), and regular (C) latency saccades. The horizontal solid line on each group's data points shows the median. CTRL: Control group; RBD: REM sleep behavior disorder; PD: Parkinson's disease; MSA: Multiple system atrophy; PSP: Progressive supranuclear palsy.

In express latency saccades, PD (9.1 %) patients had a higher direction error rate than CTRL (4.2 %, U=1726, z=-2.82, $P<.01$) and RBD (3.3 %, U=505.5, z=-2.24, $P<.05$) (Figure II-8, B). MSA (14.1 %) also showed more direction error than CTRL (U=574.5, z=-2.38, $P<.05$) and RBD (U=170, z=-2.08, $P<.05$). No other comparisons were significantly different (PSP: 6.25 %).

As shown in Figure II-8, C, the anti-saccade direction error rate in regular epochs was lower in CTRL (10.83 %) compared to PD (18.33 %, U=1333, z=-4.29, $P<.001$) and PSP (44.35 %, U=88.5, z=-3.96, $P<.001$). RBD (11.66 %) also showed lower errors compared to PD (U=445.5, z=-2.87, $P<.01$) and PSP (U=24, z=-3.74, $P<.001$). PSP with the highest direction error also showed more errors than PD (U=51, z=-2.88, $P<.01$) and MSA (17.34%, U=25.5, z=-2.08, $P<.05$).

II.6.6. Correct saccade amplitude

Figure II-9 shows the pro-saccades amplitude for all groups and all saccades (viable trials). Comparisons only happened between pro-saccade trials because of the high amplitude variations in anti-saccade trials. During correct pro-saccade trials, CTRL (9.36 degrees) displayed a bigger saccade amplitude than PD (8.79 degrees, U=1184, z=-4.85, $P<.001$), MSA (8.37 degrees, U=410, z=-3.46, $P<.001$), and PSP (7.58 degrees, U=104, z=-3.82, $P<.001$). RBD saccade amplitude (9.23 degrees) was also bigger than PD (U=364, z=-3.71, $P<.001$), MSA (U=119, z=-3.1, $P<.01$), and PSP (U=30, z=-3.56, $P<.001$). Moreover, PD showed a bigger saccade amplitude than PSP (U=65, z=-2.46, $P<.05$).

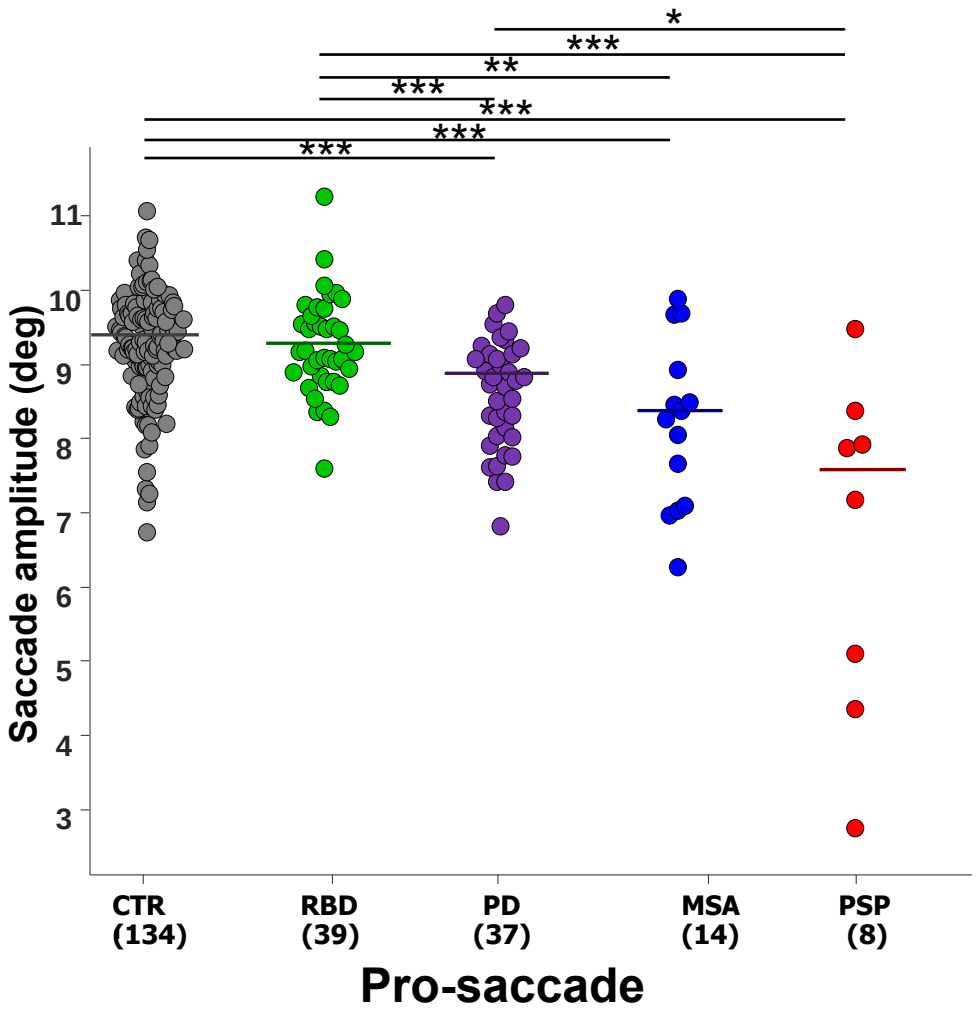

Figure II-9. The median amplitude of viable saccades for each group during pro-saccade trials. The horizontal solid line on each group's data points shows the median. CTRL: Control group; RBD: REM sleep behavior disorder; PD: Parkinson's disease; MSA: Multiple system atrophy; PSP: Progressive supranuclear palsy.

II.7. Blink results

II.7.1. Blink rate during the inter-trial interval

Blinks that occurred during the ITI period (Figure II-10), in CTRL (14.16) were insignificantly higher than in RBD (10.83, U=2092, z=-1.89, *P*=0.06) and PD (11.66, U=2162.5, z=-1.18, *P*=0.23) but significantly higher than in MSA (5.83, U=532, z=-2.66, *P*<.01), and in PSP (zero, U=32.5, z=-4.45, *P*<.001). PSP showed the lowest blink rate and was lower than in RBD (U=34, z=-3.47, *P*<.001), PD (U=16, z=-3.92, *P*<.001), and MSA (U=17.5, z=-2.68, *P*<.01) too.

During anti-saccade trials, CTRL (14.16) had more blinks than RBD (8.33, U=2066, z=-1.98, *P*<.05), MSA (7.5, U=540.5, z=-2.6, *P*<.05), and PSP (0.41, U=53.5, z=-4.27, *P*<.001). PSP appeared to have lower blink than RBD (U=37, z=-3.38, *P*<.001), PD (13.33, U=33, z=-3.42, *P*<.001), and MSA (U=24.5, z=-2.179, *P*<.05). In conclusion, PSP had the lowest blink rate during the ITI period in both anti and pro-saccade trials.

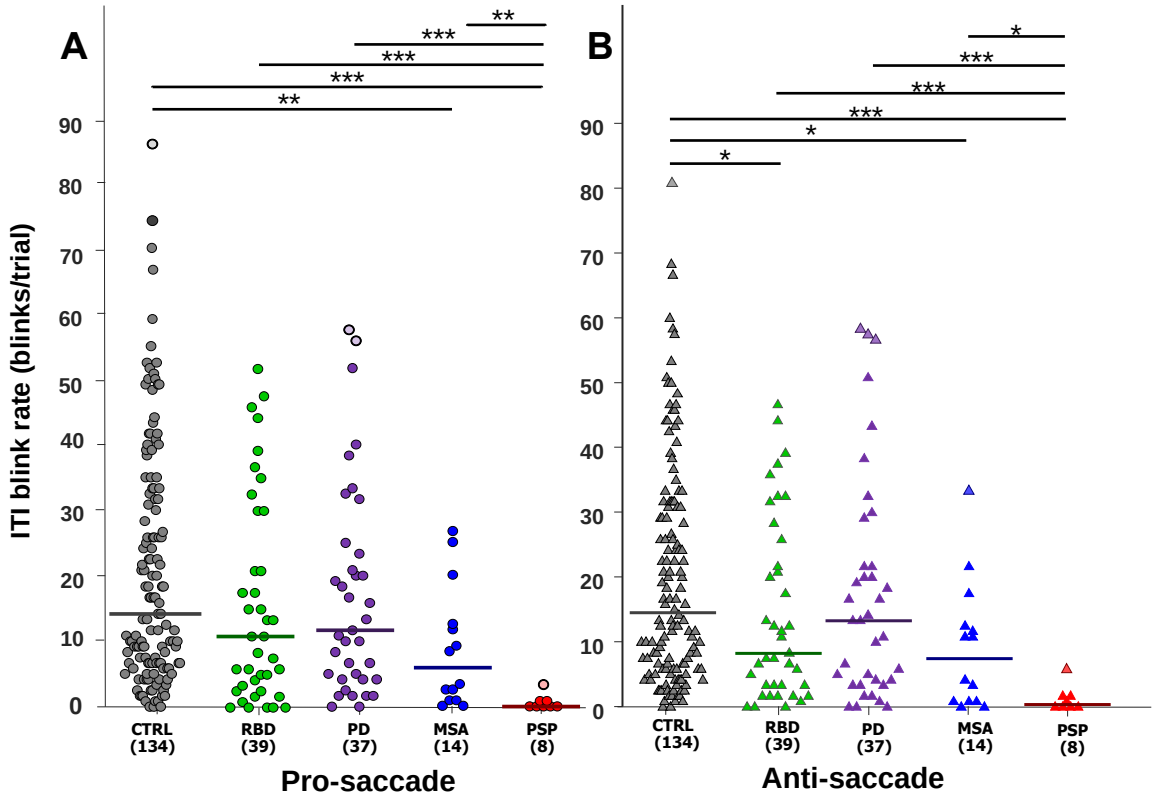

Figure II-10. Blink rate during the inter-trial interval for each group during (A) pro-saccade and (B) anti-saccade. The horizontal solid line on each group's data points shows the median. CTRL: Control group; RBD: REM sleep behavior disorder; PD: Parkinson's disease; MSA: Multiple system atrophy; PSP: Progressive supranuclear palsy.

II.7.2. Blink rate during the fixation period

The fixation period is the time that fixation is on the screen (see method). Blinks that occurred during the fixation period in anti and pro-saccade trials have been measured and compared across the groups (Figure II-11). During pro-saccade trials, CTRL (11.6) showed a higher blink rate than RBD (5, U=2004, z=-2.21, *P*<.05), MSA (1.25, U=474.5, z=-3.03, *P*<.01), and PSP (zero, U=66.5, z=-4.15, *P*<.001). RBD showed a higher blink rate compared to PSP (U=38.5, z=-3.34, *P*<.001). PD (14.1) also represented more blinks than MSA (U=142.5, z=-2.46, *P*<.05) and PSP (U=27.5, z=-3.59, *P*<.001).

During anti-saccade trials, CTRL (8.33) had more blinks than MSA (1.25, U=642, z=-1.94, *P*<.05) and PSP (zero, U=130, z=-3.59, *P*<.001). PSP had lower blinks than RBD (3.3, U=57.5, z=-2.81, *P*<.01), PD (10.83, U=41, z=-3.19, *P*<.01), and MSA (U=27.5, z=-2.01, *P*<.05).

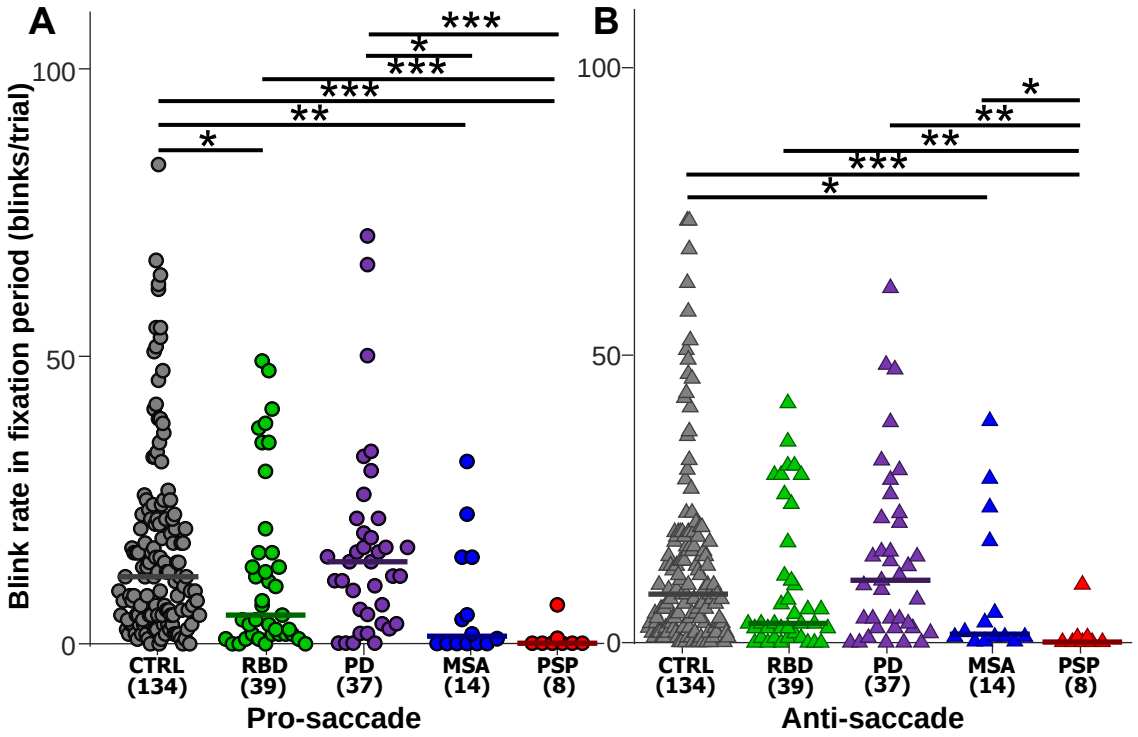

Figure II-11. Blink rate during the fixation period for each group during (A) pro-saccade and (B) anti-saccade. The horizontal solid line on each group's data points shows the median. CTRL: Control group; RBD: REM sleep behavior disorder; PD: Parkinson's disease; MSA: Multiple system atrophy; PSP: Progressive supranuclear palsy.

II.8. Pupil results

Pupil traces for all cohorts are shown in Figure II-12, and since there was no variation in pupil baseline across groups, they have been normalized to baseline. Each trace represents the average of all potential trials that met the inclusion criteria. The time stamp ranges from 150 to 1200 ms following the fixation point onset. A rebound follows a brief period of constriction. We next compared the pupil traces using multiple metrics gained from these traces.

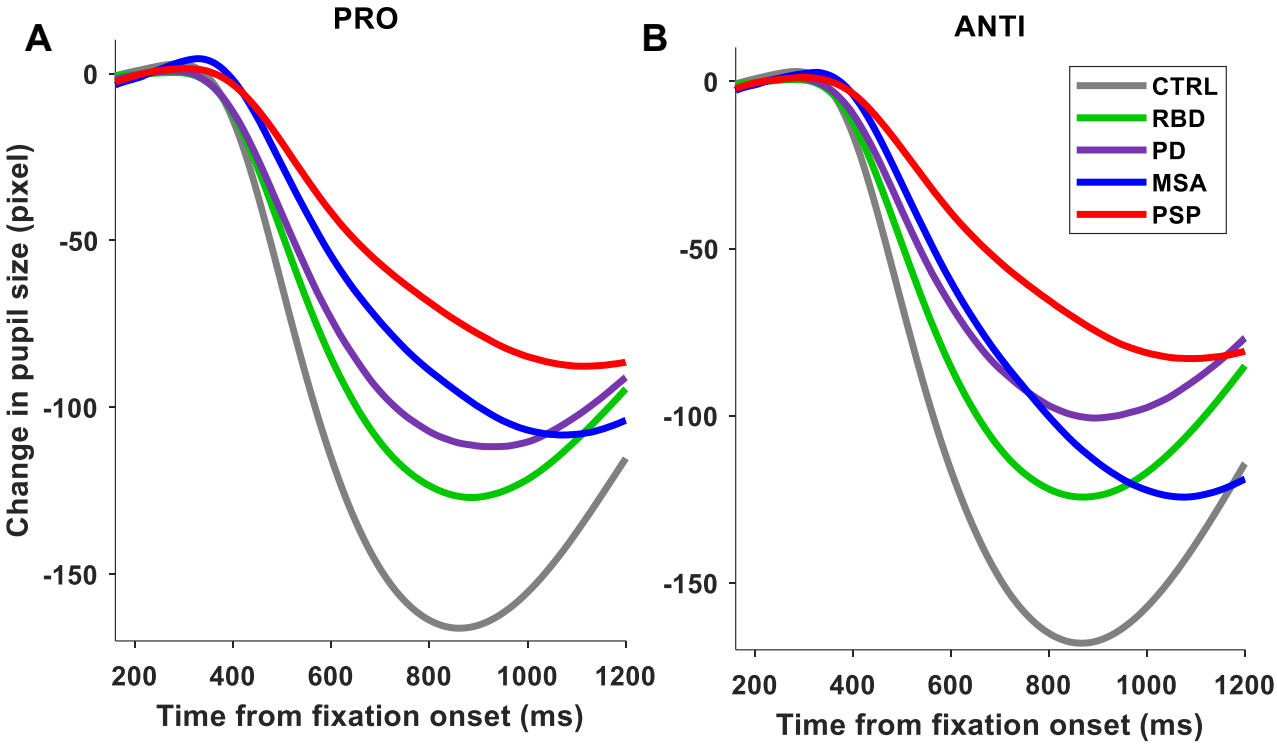

Figure II-12. Mean pupil traces for each patient group are represented during the pro- (A) and anti- (B) saccade trials over time. To generate the curves, pupil size was binned into 10 ms epochs. CTRL: Control group; RBD: REM sleep behavior disorder; PD: Parkinson's disease; MSA: Multiple system atrophy; PSP: Progressive supranuclear palsy.

II.8.1. Fixation onset time

The fixation onset time for all patients has been shown in Figure II-13. CTRL had a median reaction time of 47.26 ms which was comparable to RBD (52.86 ms) but faster than PD (124.36 ms, U=1349, z=-4.23, *P*<.001), MSA (225.32 ms, U=159, z=-5.1, *P*<.001), and PSP (276.74 ms, U=30, z=-4.47, *P*<.001). RBD was faster than PD (U=409, z=-3.24, *P*<.01), MSA (U=50, z=-4.49, *P*<.001), and PSP (U=9, z=-4.16, *P*<.001).

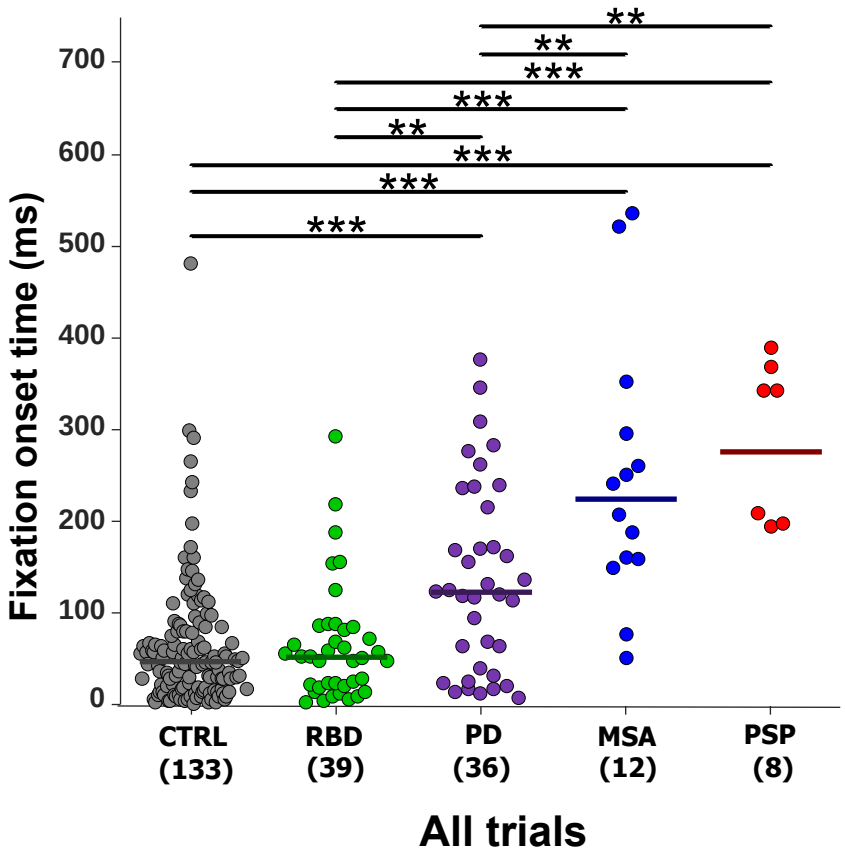

Figure II-13. Fixation onset time. When each patient group looks at the fixation point after it appears is shown in the vertical axis. The horizontal solid line on each group's data points shows the median. CTRL: Control group; RBD: REM sleep behavior disorder; PD: Parkinson's disease; MSA: Multiple system atrophy; PSP: Progressive supranuclear palsy.

Furthermore, PSP was slower than PD (U=45, z=-3.05, *P*<.01) and MSA (U=42, z=-0.95, *P*<.01).

In order to assess constriction time, fixation onset time was taken into account. The results of pupil constriction for PSP patients were unreliable due to their considerable delay, but they have not been removed from the figures.

II.8.2. Pupil peak constriction time

The time that maximum pupil constriction has achieved has been shown in Figure II-14. CTRL had a peak constriction time of 853 ms which was not different from RBD (861 ms) but was lower than PD (895.5 ms, U=1850.5, z=-2.087, $P<.05$) and MSA (1006 ms, U=312, z=-3.48, $P<.001$). Additionally, RBD was faster than MSA (U=92.5, z=-3.14, $P<.01$). PSP had a constriction time of 1017.5 ms; however, as mentioned earlier, these results were not considered reliable. Therefore, in Figure II-14, the comparisons between other groups and PSP are presented in red color.

During anti-saccade trials, CTRL had a lower constriction time (856 ms) than MSA (1056 ms, U=333, z=-3.33, $P<.001$). Moreover, RBD (847 ms) showed a lower constriction time than MSA (U=93, z=-3.13, $P<.01$). PD constriction time (862 ms) was comparable to CTRL.

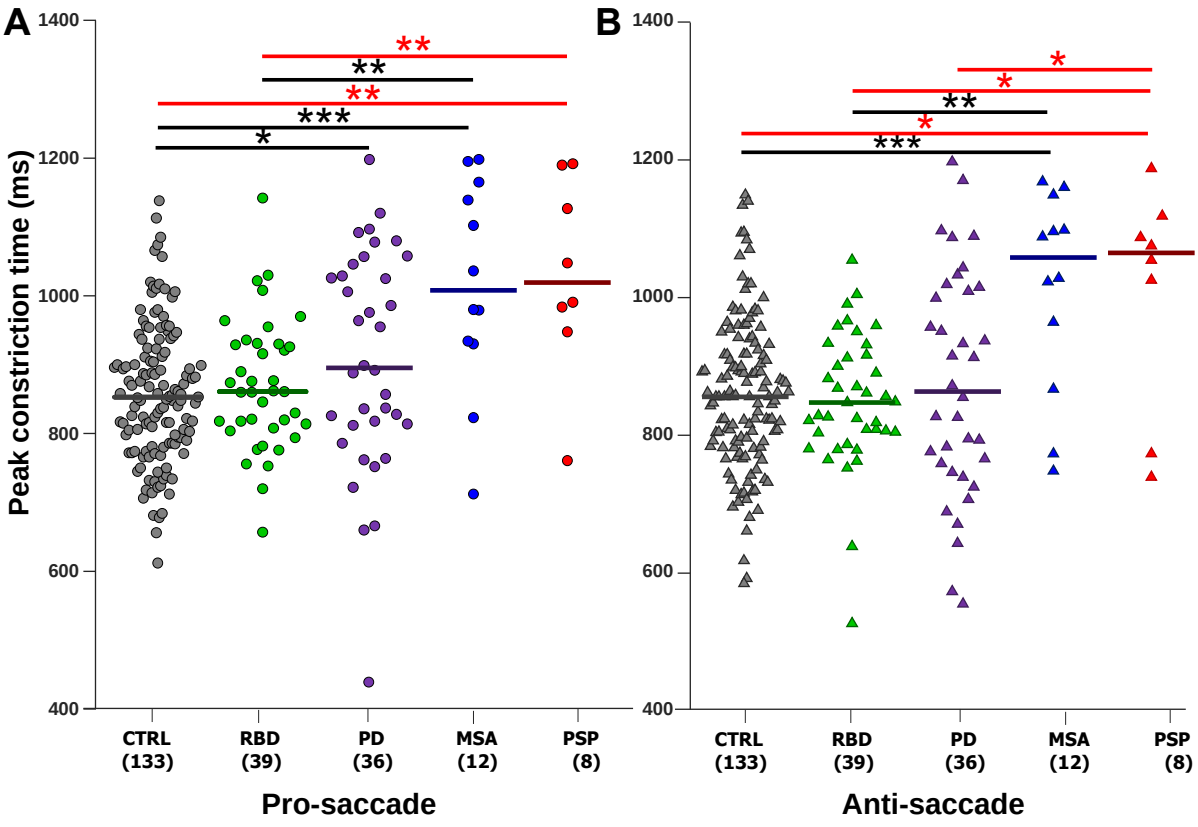

Figure II-14. Peak constriction time for each group during (A) pro-saccade and (B) anti-saccade. The horizontal solid line on each group's data points shows the median. The red lines and asterisks indicate unreliable results. CTRL: Control group; RBD: REM sleep behavior disorder; PD: Parkinson's disease; MSA: Multiple system atrophy; PSP: Progressive supranuclear palsy.

II.8.3. Dilation size

After the pupil caught its maximum level of constriction, it started to dilate, and the difference between the peak pupil constriction size and the size of the pupil at the target onset was calculated as the dilation's size. Figure II-15, A shows that in pro-saccade trials, CTRL (Pro: 56.5, anti: 60) had a bigger dilation size than RBD (Pro: 36, anti: 41), PD (Pro: 22, anti: 30.25), MSA (Pro: 8, anti: 9), and PSP (Pro: 6.25, anti: 8).

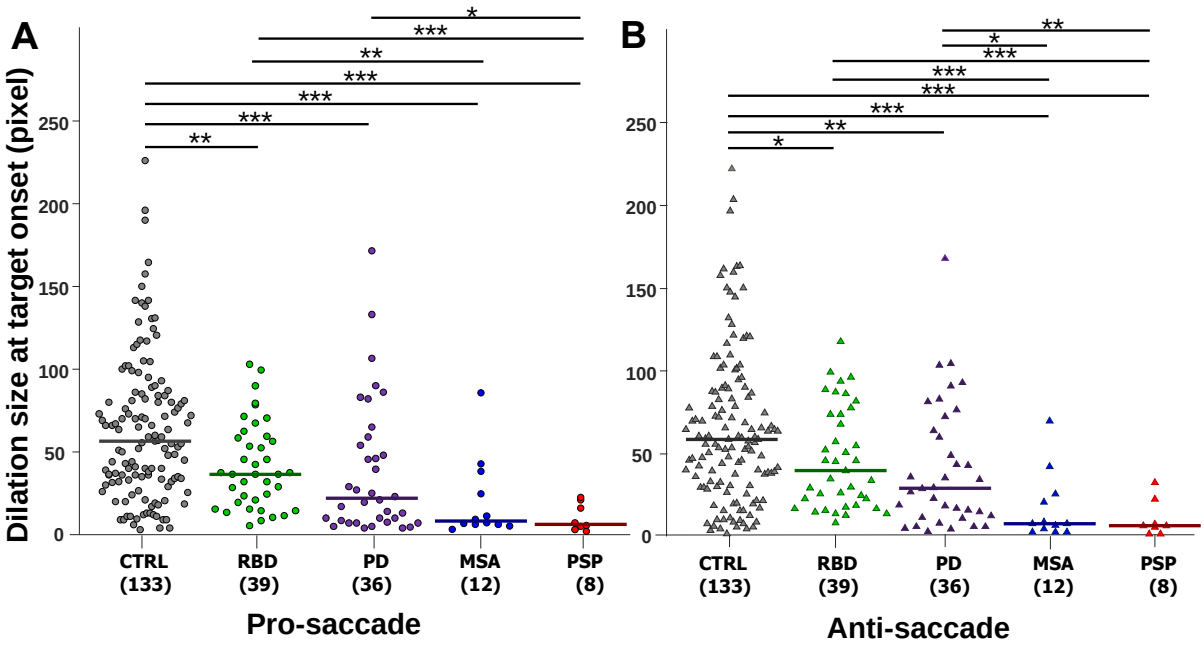

Figure II-15. Dilation size at target onset for each group during (A) pro-saccade and (B) anti-saccade trials. The horizontal solid line on each group's data points shows the median. CTRL: Control group; RBD: REM sleep behavior disorder; PD: Parkinson's disease; MSA: Multiple system atrophy; PSP: Progressive supranuclear palsy.

Table II-2 provides the details of the statistical analysis for comparing CTRL subjects with other patients.

Table II-2. Pupil dilation size in CTRL versus patients. CTRL: Control group; RBD: REM sleep behavior disorder; PD: Parkinson's disease; MSA: Multiple system atrophy; PSP: Progressive supranuclear palsy

Mann-Whitney-U-Test		Pro	Anti
CTRL-RBD	**U**	1835	2031.5
	Z	-2.77	-2.05
	P-value	0.006	0.039
CTRL-PD	**U**	1446	1600.5
	Z	-3.64	-3.04
	P-value	0.0002	0.002
CTRL-MSA	**U**	258.5	219.5
	Z	-3.87	-4.15
	P-value	0.0001	0.00003
CTRL-PSP	**U**	74	88.5
	Z	-4.08	-3.95
	P-value	0.00004	0.00007

There was no difference between RBD and PD in pupil dilation size (Table II-3). However, RBD had a bigger dilation size than MSA in pro ($P<.01$) and anti-saccade trials ($P<.001$). Furthermore, RBD had a bigger dilation size than PSP in both pro and anti-saccade trials (both $P<.001$). PD in pro-saccade trials was bigger than PSP ($P<.05$), while in anti-saccade trials, PD appeared to have a bigger dilation size compared to both MSA ($P<.05$) and PSP ($P<.01$).

Table II-3. Pupil dilation size at target onset: comparing patient groups together. CTRL: Control group; RBD: REM sleep behavior disorder; PD: Parkinson's disease; MSA: Multiple system atrophy; PSP: Progressive supranuclear palsy.

Mann-Whitney-U-Test		Pro	Anti
RBD-PD	**U**	554	574
	Z	-1.57	-1.35
	P-value	0.11	0.17
RBD- MSA	**U**	104	72.5
	Z	-2.88	-3.58
	P-value	0.003	0.0003
RBD- PSP	**U**	33.5	28
	Z	-3.46	-3.62
	P-value	0.0005	0.0002
PD- MSA	**U**	143	110
	Z	-1.72	-2.52
	P-value	0.08	0.011
PD-PSP	**U**	66	57.5
	Z	-2.37	-2.63
	P-value	0.018	0.008
MSA-PSP	**U**	33.5	38
	Z	-1.12	-0.77
	P-value	0.26	0.43

II.8.4. Peak dilation velocity

The maximum (Peak) velocity in the dilation period revealed a significant difference between CTRL and all other groups (Figure II-16).

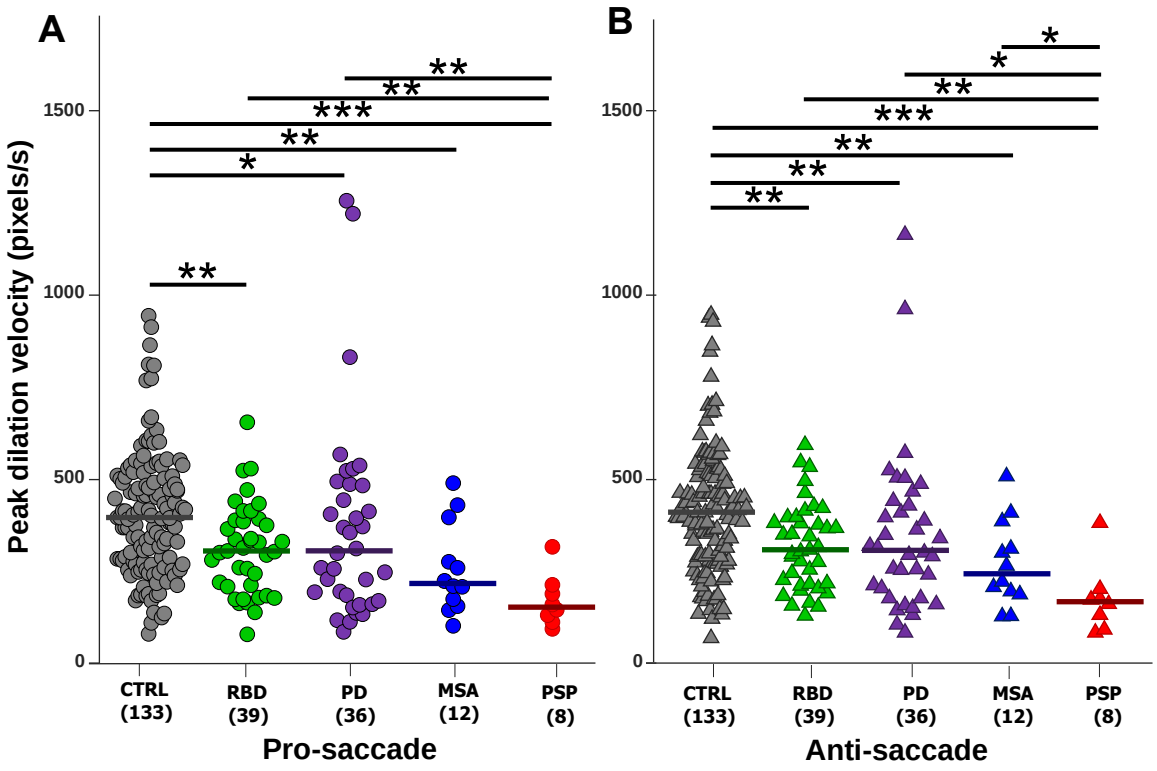

Figure II-16. Peak dilation velocity for each group during (A) pro-saccade and (B) anti-saccade trials. The horizontal solid line on each group's data points shows the median. CTRL: Control group; RBD: REM sleep behavior disorder; PD: Parkinson's disease; MSA: Multiple system atrophy; PSP: Progressive supranuclear palsy.

Table II-4 shows that in pro-saccade trials CTRL (395) had faster dilation velocity compared to RBD (308, $P<.01$), PD (304.5, $P<.05$), MSA (220, $P<.01$), and PSP (150.75, $P<.001$). In anti-saccade trials, CTRL (421) had faster dilation velocity compared to RBD (321, $P<.01$), PD (314.75, $P<.01$), MSA (253, $P<.01$), and PSP (181, $P<.001$).

Table II-4. Pupil dilation velocity in CTRL versus patients. CTRL: Control group; RBD: REM sleep behavior disorder; PD: Parkinson's disease; MSA: Multiple system atrophy; PSP: Progressive supranuclear palsy.

Mann-Whitney-U-Test		Pro	Anti
CTRL-RBD	**U**	1716.5	1745.5
	Z	-3.20	-3.10
	P-value	0.0013	0.0019
CTRL-PD	**U**	1834	1701
	Z	-2.15	-2.66
	P-value	0.031	0.007
CTRL-MSA	**U**	372.5	392.5
	Z	-3.05	-2.91
	P-value	0.002	0.003
CTRL-PSP	**U**	84.5	108.5
	Z	-3.98	-3.77
	P-value	0.00006	0.0001

RBD revealed significant differences with PSP in both pro (P<.01) and anti-saccade trials (P<.01) (Table II-5). Other comparisons between the groups demonstrated a smaller dilation size in PSP than PD in both pro (*P*<.01) and anti-saccade trials (*P*<.05). In anti-saccade trials, PSP had a smaller dilation size than MSA (*P*<.05).

Table II-5. Pupil dilation velocity: comparing Patients together. CTRL: Control group; RBD: REM sleep behavior disorder; PD: Parkinson's disease; MSA: Multiple system atrophy; PSP: Progressive supranuclear palsy.

Mann-Whitney-U-Test		Pro	Anti
RBD-PD	**U**	684.5	683.5
	Z	-0.18	-0.19
	P-value	0.853	0.84
RBD- MSA	**U**	172	177.5
	Z	-1.37	-1.25
	P-value	0.16	0.21
RBD- PSP	**U**	45.5	48.5
	Z	-3.128	-3.04
	P-value	0.0017	0.002
PD- MSA	**U**	169	175.5
	Z	-1.11	-0.96
	P-value	0.26	0.33
PD-PSP	**U**	57.5	63.5
	Z	-2.63	-2.45
	P-value	0.008	0.01
MSA-PSP	**U**	22.5	63.5
	Z	-1.96	-2.45
	P-value	0.049	0.014

II.9. Results summary

An overview of the findings can be found in Table II-6. Arrows indicate the statistic findings, whereas one arrow indicates a single asterisk, two arrows indicate two asterisks, and three arrows indicate three asterisks. The arrows point downward and upward, indicating a drop and a rise in the relevant metric, respectively. This table provides only a summary of the findings plus the possible results if considering multiple comparisons correction (by removing red arrows).

Table II-6. IPAST results summary. The arrows indicate how the parameter mentioned in the first column changed between the first and second groups. Upward: increase, downward: decrease. A NO symbol indicates there were no comparisons made. The red arrows indicate that these arrows would have been removed if the Bonferroni correction had been applied (new alpha level for comparisons between CTRL and patients: 0.05/4, 0.01/4, 0.001/4, and for comparisons between patients: 0.05/6, 0.01/6, 0.001/6). CTRL: Control group; RBD: REM sleep behavior disorder; PD: Parkinson's disease; MSA: Multiple system atrophy; PSP: Progressive supranuclear palsy.

Variable		RBD ↔ CTRL	PD ↔ CTRL	MSA ↔ CTRL	PSP ↔ CTRL	RBD ↔ PD	RBD ↔ MSA	RBD ↔ PSP	PD ↔ MSA	PD ↔ PSP	MSA ↔ PSP
Correct median SRT	Pro			↓							
	Anti		↑	↑	↑↑			↓↓		↓	
Express latency saccades	Pro		↑	↑							
Regular latency saccades	Pro		↓↓↓	↓↓↓			↑				
	Anti		↓↓	↓↓↓	↓		↑↑		↑		
ITI blink rate	Pro			↓↓	↓↓↓			↑↑↑		↑↑↑	↑↑
	Anti	↓		↓	↓↓↓			↑↑↑		↑↑↑	↑
Fixation blink rate	Pro	↓		↓↓	↓↓↓			↑↑↑	↑	↑↑↑	
	Anti			↓	↓↓↓			↑↑		↑↑	↑
Direction error viable	Anti		↑↑↑	↑↑	↑↑↑	↓↓↓	↓	↓↓↓		↓↓	
Direction error express	Anti		↑↑	↑		↓	↓				
Direction error regular	Anti		↑↑↑		↑↑↑	↓↓		↓↓↓		↓↓	↓
Correct amplitude	Pro		↓↓↓	↓↓↓	↓↓↓	↑↑↑	↑↑	↑↑↑		↑	
Fixation onset time	All		↑↑↑	↑↑↑	↑↑↑	↓↓	↓↓↓	↓↓↓	↓↓	↓↓	
Peak constriction time	Pro		↑	↑↑↑	⊘		↓↓	⊘		⊘	⊘
	Anti			↑↑↑	⊘		↓↓	⊘		⊘	⊘
Dilation size at target onset	Pro	↓↓	↓↓↓	↓↓↓	↓↓↓		↑↑	↑↑↑		↑	
	Anti	↓	↓↓	↓↓↓	↓↓↓		↑↑↑	↑↑↑	↑	↑↑	
Peak dilation velocity	Pro	↓↓	↓	↓↓	↓↓↓			↑↑		↑↑	
	Anti	↓↓	↓↓	↓↓	↓↓↓			↑↑		↑	↑

II.10. IPAST discussion

Across RBD patients, saccade reactions, express latency saccades, direction errors, saccade amplitudes, and peak constriction times were normal. However, there were abnormalities in the blink rate and pupil dilation profiles in RBD. Due to LC's involvement in both pupil and blink pathways in the brain (Joshi et al. 2016), these results suggest that LC may be affected in RBD patients earlier than the dopaminergic system- in line with the Braak hypothesis (Braak et al. 2003). Therefore, blinks and pupil behavior can serve as biomarkers for PD and MSA.

A comparison of RBD with manifest αSYN has been performed in order to predict whether RBD would convert to MSA or PD. A lower express latency saccade rate and direction error rate were observed in RBD than in both PD and MSA. Additionally, a greater amplitude was observed in RBD when compared to PD and MSA. Blink rate, however, did not differ between RBD, PD, and MSA. In conclusion, direction error and express latency saccade rate could not help in any prediction of RBD phenoconversion to PD or MSA. However, saccade amplitude was more reduced in MSA than in PD, which may be more characteristic of MSA disease rather than PD. Also, blink rate reductions in both RBD and MSA with no difference implicated that maybe blink can assist the phenoconversion prediction.

When it came to pupil responses, RBD almost did not differ from PD, but it did differ significantly from MSA. There was a longer pupil constriction time and a smaller pupil dilation size in MSA compared to RBD. In fact, the pupil abnormalities seen in αSYN groups were pronounced in MSA. Whether the pupil could predict the phenoconversion needs to be studied more.

There was a similar pattern of abnormalities between the Tauopathy and αSYN groups except for express saccades. However, the abnormalities in PSP were more severe than those in RBD and PD. Blink rate was the main difference between PSP and MSA. Overall, it was not easy to distinguish PSP from MSA. By looking at IPAST parameters such as express saccades and blink rate, PSP may be diagnosed earlier in the disease course.

In most cases, our findings were consistent with those in our lab's previous report (Perkins et al. 2021). Even though Perkins et al. found that RBD had shorter SRT than PD in anti-saccade trials, we found no difference between RBD and PD in the current

study. RBD and PD did not exhibit blink rate reductions in pro-saccade trials, contradicting Perkins et al findings. We found more RBD abnormalities than in the previous study, but our overall results were consistent. Aside from that, we also included cohorts from the MSA and PSP.

The results of our recent paper (Habibi et al. 2022) indicated that saccade amplitude was reduced in both PD and MSA during free-viewing (FV), as well as in RBD in certain saccade directions. The fact that the IPAST task complexity differed from FV might explain why RBD had a normal saccade amplitude in the current study. As for pupil dilation, we found abnormal pupil dilation in RBD in IPAST, but normal pupil dilation in FV. In the next chapter, FV will be described in detail, but briefly, FV stimulates the whole retina, whereas IPAST only stimulates the fovea. This might explain the lower MSA dilation size in IPAST, contrary to FV, which shows a larger MSA dilation. In addition, the dilation size of RBD decreased in IPAST, while it remained unchanged in FV.

II.10.1. Saccade reaction time

Consistent with the literature, PD patients could not generate voluntary responses easily, so their reaction time in anti-saccade trials has increased (Briand et al. 1999; Chan et al. 2005; Amador et al. 2006; Terao et al. 2016; Lu et al. 2019; Perkins et al. 2021). Some studies have demonstrated that the FEF activity level is related to SRT in anti-saccade tasks (Everling and Munoz 2000). Furthermore, it has been found that the variability in saccadic reaction times can also be attributed to the level of activity in the FEF and SC saccade neurons after the target appearance (Dorris et al. 1997; Everling and Munoz 2000). Studying saccadic reaction time can help us better understand the function of the cortical area and how it has been affected in these neurological disorders (Amador et al. 2006).

Here we showed that PD latency in Anti-Saccade trials differed from CTRL, which is similar to other studies which found that PD latency differed from CTRL (Briand et al. 1999; Chan et al. 2005; Amador et al. 2006; Terao et al. 2016; Lu et al. 2019; Perkins et al. 2021). In a recent paper published in our group, it has been reported that there is a significant difference in SRT between RBD and PD in anti-saccade trials (Perkins et al. 2021). However, we discovered no difference between RBD and PD in our current

investigations (Figure II-5), which might be attributed to the fact that we used early PD patients who had an average illness duration of fewer than two years, while in Perkins et al., there were more elderly PD patients. More research is required to determine the differences in SRT between PD and RBD, especially when the PD group is stratified in the PD with RBD and PD without RBD.

In pro-saccade trials, it has been reported that the MSA group had a longer SRT than CTRL and PD (Brooks et al. 2017). This was not the same as our findings, which revealed that MSA had a quicker reaction time in pro-saccade trials. However, in anti-saccade trials, MSA had a longer reaction time than CTRL (Figure II-5). Another research assessed the latency of saccades toward a jumping stimulus on the screen and found that MSA patients had a shorter saccadic latency than CTRL subjects, although the difference was not significant (Rottach et al. 1996). Meanwhile, according to the study above, PD patients had a much greater delay than CTRL individuals.

Brooks et al. found that MSA patients had slower SRT than CTRL individuals over a short period of time (Brooks et al. 2017). Initiating a saccade with a prolonged latency may be caused by the disease's impact on the frontal cortex and systems regulating attention and target selection (Everling and Munoz 2000; Terao et al. 2016). The fact that PD and MSA had slower reaction times in anti-saccade trials (voluntary movement) than CTRL might be utilized as a biomarker in the diagnosis of the early stages of the disease to diagnose it more quickly.

We found that PSP had a significantly longer SRT than CTRL, PD, and RBD. According to Perneczky et al., saccade latency is related to frontal and parietal eye field volume (Perneczky et al. 2011). The FEF contributes to the transfer of visual signals into saccadic commands. In PSP patients, deficiencies in the FEF and other midbrain areas responsible for saccadic generation might explain the latency of saccades.

II.10.2. Express and regular latency saccades

While visual processing of an item begins immediately upon its appearance in the visual field, this process takes time to mature (Munoz et al. 1998). When an eye movement occurs prior to adequate visual processing, it is called anticipatory and not visually prompted (Dorris and Munoz 1998). Visually guided saccade needs at least 90 ms to be initiated and occurs 90 ms after target onset (Munoz et al. 1998). Other saccades

before 90 ms can be anticipatory saccades toward one of two possible target locations. There is a 50% chance that anticipatory saccades go correctly towards one of two targets, while the visually-driven saccades are almost triggered successfully (Heeman et al. 2019).

Increased express latency saccade rate was not presented in RBD patients (Figure II-6), but we found that PD and MSA had more express latency saccades than CTRL in pro-saccade trials, in line with the other studies (Perkins et al. 2021; Chan et al. 2005). In this experiment, it was impossible to determine whether express latency saccades increase in the prodromal stage of the αSYN diseases, RBD. Nevertheless, the significant differences in express latency saccade direction error between RBD, PD, and MSA led us to hypothesize that express latency saccade deficiency is associated with the later course of the disease. To be more precise, the high frequency of express latency saccades may indicate an underlying pathology in the system controlling fixation or saccadic suppression (Biscaldi et al. 1996; Cavegn and Biscaldi 1996). Accordingly, the well-known deficits in PD and MSA basal ganglia might describe the higher number of express latency saccades in these diseases (Wenning et al. 2004).

The presence of express saccades was not significantly different between PSP and any other group. PSP patients showed regular latency saccades (Figure II-7) rather than express saccades due to the impaired frontal lobe (Brown et al. 2010). PD and MSA had fewer regular latency saccades, which made sense because they had more express saccades. A significant difference was found between MSA and RBD in terms of regular latency saccades. The MSA group had fewer regular latency saccades in anti-saccade trials than the PD group. As compared with other αSYN groups, MSA exhibited more difficulty with saccade initiation, suggesting that saccadic generating systems are more impaired.

II.10.3. Anti-saccade direction error

Direction errors are caused by an inability to suppress the automatic response and make a voluntary response (anti-saccade). A top-down control should be applied to the saccade-generating neurons in the FEF and SC in order to prevent the saccade from initiating before the stimulus appears (Coe and Munoz 2017). Although this controlling system deteriorates with age, patients with frontal lobe and basal ganglia deficiency demonstrate higher direction errors (Coe and Munoz 2017). This provides valuable insight into the

neural mechanism underlying saccadic inhibition as a result of the direction error. Consequently, comparing αSYN groups with Tauopathy might be helpful, since these are groups that have well-known deficits in the basal ganglia and frontal lobes.

In our study, RBD did not show any direction errors, contrary to a recent study showing RBD has a higher rate of direction errors than CTRl subjects (Hanuška et al. 2019). Their experiment design was more complex than ours since they had no gaps between the trials and they had targets in horizontal and vertical directions. Brooks et al. in 2017 showed that the PD and MSA groups represented more direction error than CTRL, while there was no difference between MSA and PD. This was aligned with our study that differentiated PD and MSA groups from CTRL based on direction error (Figure II-8).

In Perkins et al. study, the percentage of direction error was higher in PD compared to CTRL and RBD (Perkins et al. 2021). We were able to replicate her findings, and moreover, we displayed that CTRL and RBD had fewer direction errors (viable saccades) not only than PD but also than MSA and PSP (Figure II-8). The high rate of direction error in both groups was related to deficits in prefrontal or basal ganglia circuitry, resulting in impaired inhibition of automatic responses and impaired voluntary responses (Chan et al. 2005; Brooks et al. 2017).

By looking at the direction error of the express latency saccades, which occurred between 90:140 ms after target appearance, PSP patients were not different from other groups, but in regular saccades, PSP represented very high direction error. Garbutt et al. showed that in anti-saccade trials, PSP patients show high direction error that may be explained by the very severe cognitive impairments as the results of frontal lobe dysfunction shown in PSP (Brown et al. 2010; Garbutt et al. 2008).

II.10.4. Saccade amplitude

Comparing the saccade amplitude between the groups indicated significant saccade amplitude decreases in PD, MSA, and PSP. Making larger saccades by RBD than those of two other αSYN groups, PD and MSA, was fascinating in terms of establishing if there is any change in amplitude along the course of the illness.

The length of the saccades is pretty much dependent on the excitatory and inhibitory burst neurons (EBN/IBN), and the saccade amplitude is determined based on the length

of the burst neurons' firing period (Leigh and Zee 2015; Scudder et al. 2002). On the other hand, OPN inhibits the burst neurons. Any damage to burst neurons brings the need for a more strong power to compensate for the inhibitory power coming from OPN. Therefore SC will produce enough drive for the remaining healthy burst neurons to overcome the OPN inhibitions. Rebuilding this circuit brings more fluctuations and more error saccades. Because initiating the first saccade is usually unsuccessful, there would be more small saccades trying to foviate the eye in the correct position. PSP is a good example to look at in this fashion because there is brainstem damage, and burst neurons are partly affected, while the brainstem and cerebellum are relatively spared in PD. However, projections from basal ganglia seem important because projections from the FEF and dorsolateral prefrontal cortex (DLPFC) reach the SC via SNpr, which is an inhibitory gateway (Munoz and Everling 2004).

Decreased saccade amplitude in PD and MSA patients was consistent with other studies (Terao et al. 2016; Perkins et al. 2021; Hanuška et al. 2019; Rottach et al. 1996) that have shown that MSA patients had hypometric saccades. IPAST only included saccades that were directed horizontally, whereas in our earlier research (Habibi et al. 2022) we distinguished saccades into two categories - horizontal and vertical. We found that RBD patients exhibited normal saccades in the horizontal direction but reduced saccade size in the vertical direction.

PD and MSA may have saccade amplitude disturbances because of impairments of the basal ganglia, whereas affected saccade amplitudes in RBD only in the vertical direction may reflect progressively deterioration as disease severity increases from prodromal (RBD) to manifest (PD and MSA)(Terao et al. 2016).

Different studies have shown that PSP had small saccades (Bhidayasiri et al. 2001; Chen et al. 2010; Marx et al. 2012). In general, it is difficult for PSP to generate self-paced saccades but also suppress unwanted saccades.

PSP represented the smallest saccades, significantly smaller than CTRL, RBD, and PD (Figure II-9). This shows that although αSYN groups had decreased saccade amplitude, PSP represented even smaller saccades.

Analyzing the amplitude and saccades of the PSP group is more challenging because we should consider the dynamic overshoot saccades as described by (Otero-Millan et al. 2013). Occasionally, the saccadic delay of a subsequent saccade is approximately zero after the preceding saccade, which is called dynamic overshoot. Neglecting the dynamic overshoot may lead to the wrong conclusion that PSP has short-latency saccades.

Given that we conducted a longitudinal probe experiment and discovered that RBD patients had a lower saccade rate during follow-up visits (Results are not published yet), more research on saccade amplitude alterations is necessary.

II.10.5. Blink rate

Blink rate is an important metric that can easily distinguish PSP from all other cohorts (Armstrong 2011). Furthermore, detecting reduced blink rates in RBD and MSA, but not in PD, suggests that those with lower blink rates in RBD may phenoconvert to MSA. In PD patients, we did not find a decreased blink rate, but it has been documented that PD patients have a decreased blink rate, which may be useful in diagnosing (Perkins et al. 2021; Fitzpatrick et al. 2012).

The stage of PD disease has been reported to have a strong effect on blinking, and PD patients in the advanced stages are reported to have strong blink reduction (Karson et al. 1982). We found that PD patients had no significant blink reduction in the ITI period compared to CTRL. Even though we expected a blink reduction in PD, we saw a blink reduction in RBD patients in anti-saccade trials. It is partly different from Perkins et al. study, in which has been reported that the Blink rate reduced in both RBD and PD (Perkins et al. 2021).

Given that advanced Parkinsonism is associated with severe impairment of the dopaminergic nigrostriatal pathways (Scherman et al. 1989), there may be a correlation between decreased blink rates and decreased dopamine activity. Considering that most of our patients had modest impairments and were in the earlier stages of the disease, these generally normal blink rates may be explainable. This may be proven by separating treated and untreated PD patients and comparing the blink rate.

PSP patients had the lowest blink rate among the groups, which is a very well-known disease characteristic and has been reported previously (Lubarsky and Juncos 2008). PSP

showed almost zero blink rate during the ITI and fixation period (Figure II-10 and Figure II-11).

MSA patients had a lower blink rate than CTRL patients but a higher blink rate than PSP patients. Displaying significantly fewer blinks in MSA than PD during fixation may aid in the early detection of MSA patients. This necessitates researching MSA patients at an early stage of the disease and performing longitudinal studies, which is very difficult given that the majority of MSA patients arrive at the hospital at an advanced stage of the disease.

II.10.6. Pupil constriction time

By distinguishing RBD from MSA based on pupil constriction time, we hypothesized that pupil constriction time could be a better predictor of phenoconversion to PD than MSA. In both pro and anti-saccade trials in another study, PD has been shown to have a longer constriction time than CTRL (Wang et al. 2016). This is consistent with our findings in pro-saccade studies (Figure II-14), which indicated that PD had a longer constriction time than CTRL. It has been suggested by Micieli et al. that the prolonged constriction time could be due to a reduced parasympathetic activation relative to an overactive sympathetic system (Micieli et al. 1991). Furthermore, pupil light reflex abnormalities can be caused by deficits in the LC, which is the main noradrenergic nucleus in the brain stem. Pupil responses are affected by LC activity (Joshi et al. 2016), which is one of several impaired brain areas in PD.

Additionally, we showed that MSA also had a longer constriction time than CTRL and RBD. Moreover, MSA patients have impaired parasympathetic innervations in the brain (Fanciulli and Wenning 2015), which may have an impact on the pupil's constriction phase. Another investigation has verified the association between parasympathetic dysfunction and the length and size of pupil constriction (Aydogmus et al. 2017). According to research by Park et al., pupil constriction is slowed down in the MSA while also correlating with the severity of the disease (Park et al. 2019). Additionally, because the pupil's constriction and dilation phases are linked, an abnormality in one phase is likely to result in an abnormality in the other.

II.10.7. Pupil dilation size

According to our findings, pupil dilation size was decreased in RBD patients compared to CTRL (Figure II-15). This is indeed an interesting result, showing that even though all saccadic metrics were intact in RBD, pupil metrics displayed abnormalities. This suggests that the start of saccadic impairments might happen later in the course of the disease while pupil impairments start earlier. This is in agreement with the Braak staging that says disease degeneration starts from the lower level of the brain stem going up and involving more area (Braak et al. 2003). Because LC involves earlier than the area controlling saccades (e.g. superior colliculus) in the Braak staging hypothesis, pupil abnormality is expected to be more pronounced than saccades in RBD.

Pupil dilation size was able to distinguish RBD and PD from MSA and PSP when compared (Figure II-15). Several investigations have shown that PD dramatically decreased pupil dilation when compared to CTRL (Perkins et al. 2021; Wang et al. 2016). Pupil dilation pathways are mediated by LC (Szabadi 2018). The LC transmits inhibitory projections to the Edinger-Westphal nucleus (EW), a cholinergic nucleus that suffers from 50% neurodegeneration in PD (Hunter 1985). As a result of neurodegenerative changes in both LC and EW, sympathetic and parasympathetic innervation circuits are not tuned in PD, suggesting differences between CTRL and PD.

Additionally, we demonstrated that MSA had decreased pupil dilation size compared to CTRL (Figure II-15). The MSA pupil dilation change in IPAST was contrary to our previous findings in FV (Habibi et al. 2022), that MSA had a larger pupil dilation size than CTRL. However, the task condition and its impact on the eye differed between IPAST and FV. Furthermore, RBD had a bigger dilation size than MSA. These findings imply that the dilation size might gradually decrease from CTRL to RBD (as the prodromal phase) and subsequently to the manifest stages, PD and MSA.

II.10.8. Pupil peak dilation velocity

All αSYN groups, RBD, PD, and MSA, demonstrated a slower pupil dilation velocity (Figure II-16) and PSP displayed the slowest pupil velocity. There was no difference within αSYN groups meaning the existence of common symptoms in prodromal and manifest stages. MSA patients, similar to those shown by other studies, displayed lower dilation velocity than CTRL (Park et al. 2019). According to Park et al., the Unified

Multiple System Atrophy Rating Scale (UMSARS) in MSA patients is inversely connected with Pupillometric characteristics, particularly average constriction and dilation velocities. The UMSRS part I scale measures the intensity of autonomic symptoms. The association between the UMSRS and pupil dilation velocity demonstrates a clear link between autonomic dysfunction and pupil alterations.

II.11. Conclusion

Besides finding biomarkers in the RBD group, we intended to compare the αSYN and Tauopathy groups together in order to identify underlying differences between the two groups. A brief overview of our results is provided in the following paragraph in order to determine how closely they support the initial hypothesis.

The RBD group showed regular saccades but altered fixation break, blink, and pupil behavior compared to the CTRL group. PD and MSA exhibited high indices of direction error, exaggerated express latency saccades, damaged saccade amplitude, and damaged pupillary profiles. In all patient groups, damage to pupil responses was greater than saccadic behavior, indicating that pupil brain circuits may be impacted before saccadic control areas. Compared to CTRL, the PD and MSA deficits were more severe than the RBD deficit. Furthermore, MSA involved more damage to saccadic and pupillometric functions along with a lower blink rate than CTRL. The lack of significant differences between PD and MSA showed that IPAST was not able to distinguish these two groups except in some cases (fixation blink rate, Fixation onset time, and dilation size at target onset). Finally, PSP, the group with the lowest number of participants, showed the most severe deficiency in all saccades, blinks, and pupil metrics. PSP showed more differences with RBD and PD rather than MSA.

To conclude, we demonstrated that pupil responses and blink rate changes might be suitable candidates for biomarker applications. Furthermore, PSP differed almost significantly from PD on all metrics, including blinks, pupil, and saccades. Therefore, IPAST is a valuable tool for monitoring the neural mechanisms associated with eye movements, pupil, and blinks that could be applied in clinical settings.

To confirm the data in MSA and PD we have to recruit more patients. In the next step, it will be necessary to determine when these deficits will start to emerge in patients with

RBD. Our future objective will be to determine if the markers progress along with the development of RBD toward phenoconversion.

III. Task 2: Free viewing

In the IPAST part, like in other studies, we tried to use a structured task to identify abnormal saccade responses in neurodegenerative diseases (Perkins et al. 2021; Hanuška et al. 2019; Chan et al. 2005). Here, we employ the simple FV paradigm in which patients are shown a series of short video clips on a computer screen, and they are free to view these clips however they choose (data has been published (Habibi et al. 2022)). This approach does not allow for a detailed assessment of saccade dysmetria, but it allows for a richer assessment of saccade and pupil behavior to be recorded in a dynamic visual setting with a high temporal and spatial resolution in order to reveal abnormalities. Most importantly, this setting does not require extensive preparatory instructions for the participant to perform the task. We use the FV paradigm for the investigation of oculo/pupillo-motor functions in the prodromal (RBD) and manifest stages of αSYN (in this study PD and MSA) in comparison to PSP which is a Tauopathy with well-known oculomotor deficits. We specifically address the following questions: 1) which saccade or pupil parameters – when captured with FV - are altered in patients with the manifest αSYN PD and MSA or the Tauopathy PSP? 2) using these parameters, does the FV paradigm allow us to differentiate between patients with αSYN and PSP? 3) are abnormal pupil and saccade responses observed in PD or MSA also detectable in the prodromal αSYN stage RBD?

III.1. Materials and methods

III.1.1. Participants

We included five different groups of participants. Patients diagnosed with PD, MSA, RBD, and PSP were recruited in the department of neurology Philipps-University Marburg. CTRL subjects were recruited as part of a large study within the Faculty of Health Sciences at Queen's University in Kingston, Canada (Yep et al. 2022). The study protocol was approved by the human research ethics board of the Faculty of Medicine, Philipps-University Marburg (Protocol ID: 147/16) and the Faculty of Health Sciences, Queen's University (Protocol ID: PHYS-007-97; CNS-005-10). Voluntary informed

consent was obtained from each participant after a verbal and written explanation of the study, following the Declaration of Helsinki (included in the appendix).

All patients recruited were 45 - 84 years of age. All patients underwent clinical testing with the MoCA (Nasreddine et al. 2005), UPDRS III, BDI-II (Beck et al. 1996), PDNMS (Storch et al. 2010), and the RBDSQ (Stiasny-Kolster et al. 2007).

III.1.1.1. Exclusion criteria

For FV, the same exclusion criteria as in IPAST mentioned in the section Exclusion criteria II.1.1.1) were applied.

III.1.1.2. Participant's characteristics

RBD. Forty-six patients (5 females, 41 males, age range: 50.6 - 76.4 years) with video polysomnography-confirmed RBD (Darien IL, AASM, 2014) had mean UPDRS-III, MoCA, and BDI-II scores equal to 1.61, 28.2, and 7.7, respectively. All RBD patients were interviewed for a medical and drug history in detail and received a complete neurological examination. This procedure was repeated by a neurologist twice over a period of 1 year to reduce the risk of including subjects with secondary RBD in the study. This was part of the routine clinical diagnosis conducted by a neurologist, independent of my project. After 6 months, we closely monitored the specialists' reports to exclude anyone who indicated the first diagnosis of RBD was incorrect (fortunately, this never happened to any of our participants). In addition, we excluded RBD patients with cognitive impairment (MoCA < 25), and this would presumably minimize the number of patients likely to convert to DLB (Miglis et al. 2021). Clinical and demographic data are provided in Table III-1.

Table III-1. FV participants' clinical data. CTRL: Control group; RBD: REM sleep behavior disorder; PD: Parkinson's disease; MSA: Multiple system atrophy; PSP: Progressive supranuclear palsy; MoCA: Montreal cognitive assessment; UPDRS: Unified Parkinson's disease rating scale; BDI: Beck's depression inventory; PDNMS: PD non-motor symptoms scale; RBDSQ: RBD screening questionnaire.

Group	Number of Participants	Age at time of measurement (yrs)	MOCA Score	UPDRS III Score	BDI-II	PDNMS	RBDSQ
CTRL	132 (86F, 46M)	All=62.52 ± 9.93	-	-	-	-	-
		F=62.30 ± 9.93					
		M=62.95 ± 10.03					
RBD	46 (5F, 41M)	All= 65.17 ± 5.81	28.2 ± 1.73	1.61 ± 1.41	7.7 ± 7.34	8.4 ± 4.43	10.07± 2.31
		F= 72.35 ± 2.97					
		M= 64.30 ± 5.47					
PD	27 (2F, 25M)	All=66.26 ± 9.20	27.8 ± 2.89	15.73 ± 12.43	8.40 ± 7.18	7.14 ± 4.81	6.42± 3.99
		F= 67.08 ± 3.65					
		M= 66.2 ± 9.54					
MSA	17 (7F, 10M)	All=62.82 ± 7.47	26.7 ± 3.07	27.45 ± 11.12	11.0 ± 6.76	10.36 ± 5.28	5.5± 3.33
		F= 61.84 ± 7.81					
		M= 63.51 ± 7.56					
PSP	10 (5F, 5M)	All=69.51 ± 5.10	20.8 ± 7.12	34.7 ± 19.57	16.5 ± 13.21	9.6 ± 5.04	3.2± 2.137
		F= 68.33 ± 1.86					
		M= 70.70 ± 7.19					

PD. All PD patients were diagnosed according to the United Kingdom Brain Bank Criteria. Twenty-seven PD patients (2 females, 25 males, age range: 45.7 - 84.1 years) were included: 7 PD patients were de novo PD patients, 3 PD patients were investigated under treatment with dopaminergic medication (on-state), 14 PD patients were at least 12 hours without medication (defined off-state), and three with unknown medication status. Given the relatively minor variation in saccadic behavior between on and off states, all three groups were pooled into a single PD group, as previously reported (Cameron et al. 2012). Mean UPDRS-III, MoCA, and BDI-II scores for PD were 15.7, 27.8, and 8.4, respectively.

MSA. Seventeen MSA patients (7 females, 10 males, age range: 51.6 - 73.8 years) were diagnosed according to the second consensus statement on the diagnosis of MSA (Gilman et al. 2008). Mean UPDRS-III, MoCA, and BDI-II scores in MSA were 27.4, 26.7, and 11.0, respectively.

PSP. Ten PSP patients (5 females, 5 males, age range: 62.5 - 82.2 years) were diagnosed according to the National Institute of Neurological Disorders and Stroke and the Society for PSP (NINDS-SPSP) and Höglinger et al. criteria (Höglinger et al. 2017). PSP patients showed severe motor and cognitive problems with mean UPDRS-III, MoCA, and BDI-II scores of 34.7 and 20.8, and 16.5, respectively.

Control participants (CTRL). One hundred thirty-two healthy age-matched CTRL participated in the study (86 female, 46 male, age range: 45.5 - 84.3 years). Age is known to influence many saccade parameters (e.g., increased saccade latency, decreased saccade frequency, decreased saccade amplitude, and velocity) (Coe and Munoz 2017; Munoz et al. 1998; Dowiasch et al. 2015). To control for age effects, we created a separate CTRL group for each patient group. For each group, we selected CTRL that had a maximum of ±1 year age difference with each patient (Figure III-1). We confirmed that each control group was matched in age to its corresponding patient group. The CTRL groups, therefore, had different numbers and overlapping individuals in each group. The control group in the FV project closely resembles the one from IPAST project, although there are also some differences in its composition.

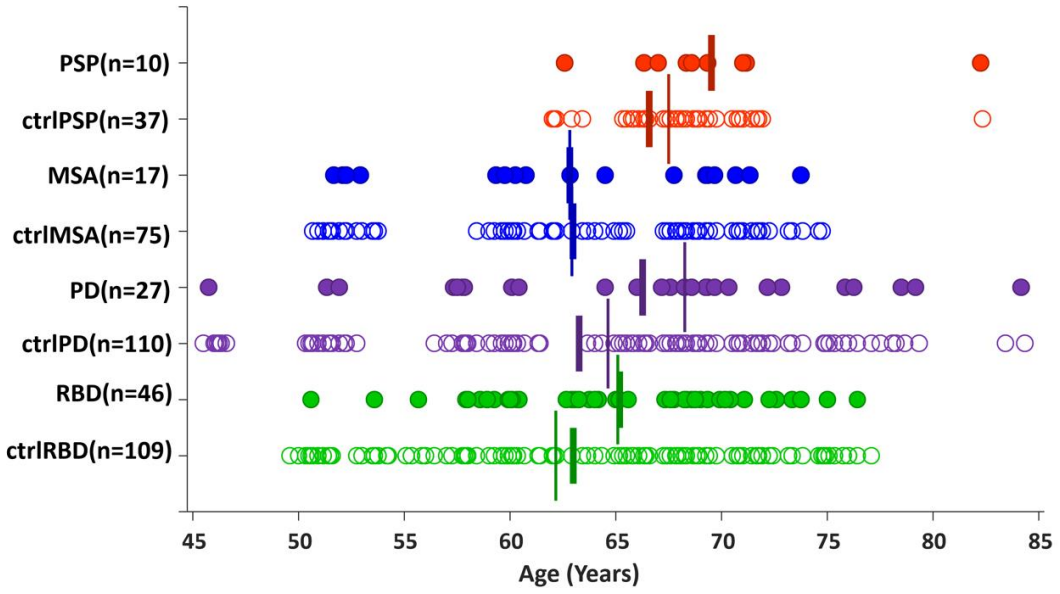

Figure III-1. Age distribution of all participants in each disease (filled circles) and control (empty circles) group. Thick and thin vertical lines represent the median and mean values, respectively, for each group. Numbers in the parenthesis show the number of subjects in each group. There was no statistical difference in age distribution between each patient group from the corresponding CTRL group (Mann-Whitney-U-test: all P<.05). CTRL: Control group; RBD: REM sleep behavior disorder; PD: Parkinson's disease; MSA: Multiple system atrophy; PSP: Progressive supranuclear palsy.

III.1.2. Eye-tracking task

Participants were seated with their heads resting on a headrest in a dark, windowless room, with a curtain drawn between them and the operator to limit any potential distractions. Despite this, PSP patients occasionally made a backward head movement during eye tracking. To prevent this from happening again, an experimenter used their hands to keep their head in a stable position on the chin and forehead rest. Additionally, the participants were seated in a chair that included a backrest to keep them from falling backward. Occasionally, we used a pillow to bridge the space between their neck and the backrest of the chair. We attempted to keep the amount of head motion to a minimum while collecting the data. Additionally, if participants pushed back, the eye tracker stopped recording, and the task was recalibrated.

A video-based monocular eye tracker was used to monitor eye position and pupil size at a rate of 500 Hz (Eyelink-1000 Plus, SR Research Ltd, Osgoode, ON, Canada). Stimuli were shown on a 17-inch LCD panel (1280 x1024 pixels, 32-bit color, 60 Hz refresh rate), corresponding to a viewing angle of 32° x 26° controlled by the operator through a Dell Latitude E7440 Laptop. Videos were delivered at 30 fps using custom software in Ubuntu 13 to interface with the eye tracker via the SR Research API. The distance between the eyes and the monitor and infrared camera was adjusted to 60cm, the optimum distance between the camera and the eye. All recordings and calibrations were conducted monocularly, using the right eye as the reference point. To begin, a nine-point grid was used to calibrate the eye location (eight around the periphery and one central). The stimuli were flashed in random patterns across the screen, and the participant was required to focus on each one until the next appeared. Following calibration, the procedure was repeated to ensure that the average error between fixation and stimulus was less than 1° and that there was no loss of eye tracking. To verify that observed substantial variations were not attributable to differences in location, both video-based eye-tracking devices were subjected to rigorous testing on a regular and recurring basis to assure consistency across machines. This study used a spectrometer to ensure that the eye-trackers displays emitted an identical amount of luminance, which had no impact on pupil baseline, constriction, or dilation levels.

III.1.3. Visual stimuli

Videos were displayed on the monitor, and all participants viewed a total of 10 movies (vertical boundary black lines in Figure III-2A). Each movie was approximately 1 minute in duration and consisted of 15-17 video clips that were ~2 – 5 s in duration (mean = 3.76, mode = 4). We made video clips of scenes with and without humans, animals, buildings, cars, and the clips were randomly assembled so that viewing was similar to watching television and changing the channel every few seconds. The clips were presented in a fixed sequence within each movie, but the order of the 10 movies was randomized between participants. The task required no instruction; the participants simply viewed the video clips. Clip changes produced a large visual perturbation that stimulated much of the central retina, producing a large visual transient signal (White et al. 2017) carried to all central visual areas that altered ongoing saccade and pupil behavior.

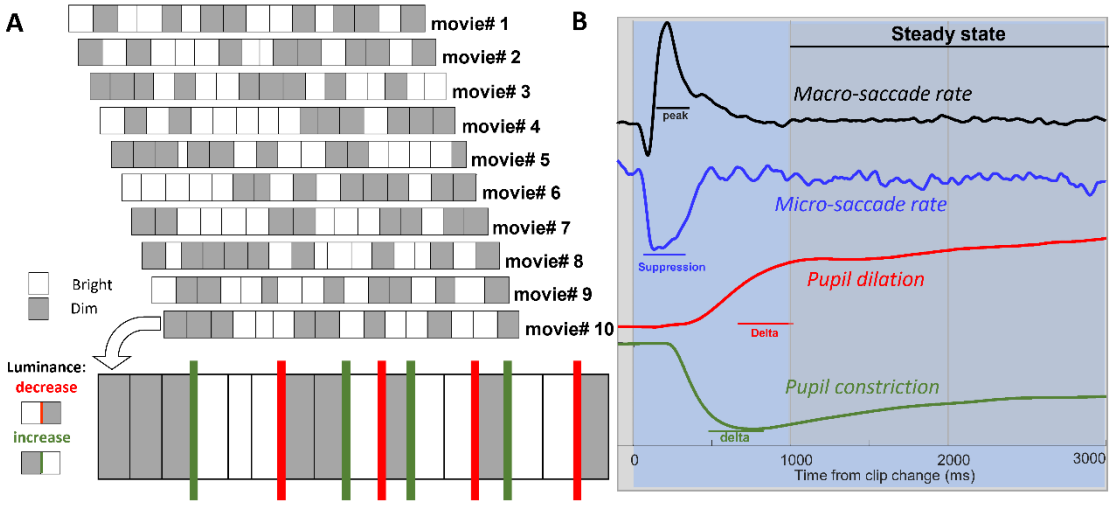

Figure III-2. Experiment paradigm (A) Illustration of all movie trials. Every movie consisted of ~17 different scenes (clips), which lasted ~60s in total. The gray boxes show the clip with lower luminance, and the white boxes show clips with higher luminance. The underneath panel shows an example of how we gain the appropriate clip changes to gain pupil analyses. Red lines show clip transitions with negative luminance delta (lead to pupil dilation), and green lines show clip transitions with positive luminance delta (lead to pupil constriction). (B) Different analyses of the data in each epoch of panel A. The black line shows the macro-saccade rate after the clip change (all rectangles in panel A), and the blue line shows the micro-saccade rate in the same epochs. Pupil dilation and constriction are the pupil responses of the trials indicated with red and green vertical lines, respectively, in panel A. The shaded area shows the time at which the steady state response was calculated. Other descriptions on the image pertain to the times when these parameters were collected.

We computed the luminance changes at each clip change that impacted pupil size. We defined "delta" as the change in luminance between the current frame and the previous frame. We then selected the top 20% of positive luminance deltas (clips with the greatest

increases in luminance; green vertical lines in Figure III-2A) and the top 20% of negative deltas (clips with the greatest decreases in luminance; red vertical lines in Figure III-2A). This resulted in 30 positive delta clips and 30 negative delta clips which were used to analyze pupil constriction and dilation responses.

III.2. Saccade analyses

We divided the analyses into: 1) low-level statistics independent of video content and 2) analyses aligned on clip changes (Figure III-2B). Auto-marking scripts developed in MATLAB were used to classify each trial and all eye movements (saccades, fixations, and pupil size). All saccades were marked for direction, amplitude, peak velocity, and duration (Coe et al. 2021). We computed the z-score of the velocity-amplitude relationship (main sequence (Baloh et al. 1975)) for each eye movement that was initially coded as a saccade to distinguish non-physiological data from real saccades. Specifically, initially coded saccades whose z-score was > ±3 SD were considered outside the range of a normal saccade and were removed. This resulted in the removal of 4% of the initially detected saccades. We then defined macro-saccades as all saccades ≥ 2° amplitude and micro-saccades (Otero-Millan et al. 2011; 2013; Susana Martinez-Conde et al. 2006; Alexander et al. 2019; Susana Martinez-Conde et al. 2004; S. Martinez-Conde et al. 2000) as all saccades < 2° amplitude.

III.2.1. Main sequence

As mentioned in I.8.2, the main sequence is a fundamental relationship between saccades' amplitude and peak velocity (Bahill et al. 1975a), which measures the integrity of the brainstem saccade premotor circuit (Luschei and Fuchs 1972). We measured the amplitude and peak velocity of all saccades > 2° and plotted peak velocity as a function of log amplitude for each participant, which produces a linear relationship (Bahill et al. 1975a). We then fit a linear function to the resulting data (Figure III-3). Analysis was repeated for each group participant to make comparisons (seeIII.6.1.5).

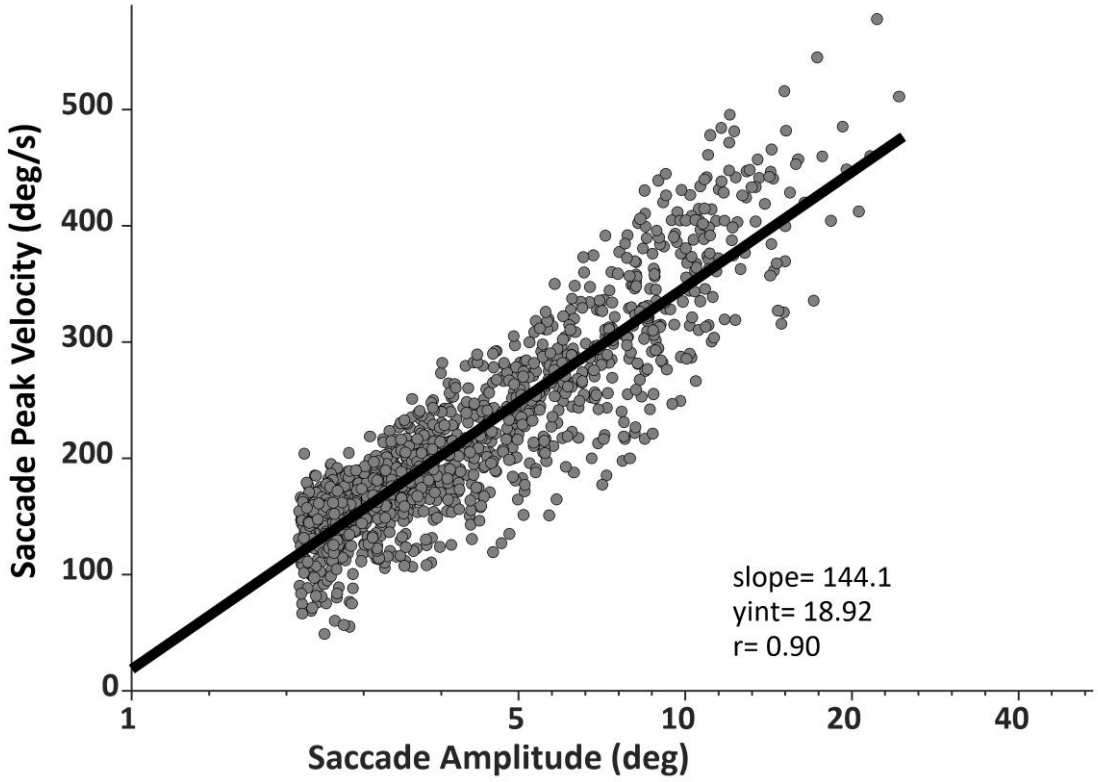

Figure III-3. Main sequence. The main sequence of saccade peak velocity against amplitude in one representative CTRL subject. The X-axis is the amplitude on a logarithmic scale. The linear fitting line is applied over all data points of the subject in 10 trials (Movies 1-10) in all directions.

III.2.2. Gaze distribution

We defined any period between successive saccades as a fixation period and quantified the fixation duration. Fixation durations < 50 ms were excluded because they have been shown to not activate the fixation system in the brainstem (Bergeron and Guitton 2001). The coordinates of each fixation were used to create gaze distribution maps. We created a 2D histogram with 32 x 26 bins (bin size: 1° of visual angle) of all fixations within a given movie. We then applied a Gaussian smoothing function (SD = 0.5 pixels) to the resulting image, which produced an average heatmap of the probability of gaze for each participant across all 10 movies. We also calculated the difference in gaze distribution for each patient group and its respective control group to generate "difference gaze probability" maps. To summarize these difference gaze probability maps, we extracted the data along the horizontal and vertical meridian (an averaged ±5^{o} strip across the meridian) of the difference maps to produce 2D line plots to illustrate the differences

better. Lastly, center bias, the excessive time gazing at the center of the screen (Tseng et al. 2009), was calculated for each participant and was defined as the mean ± 5º around the center of the probability map for each participant.

III.2.3. Saccade directions

We computed the frequency (saccade-count/viewing-duration) and average saccade amplitude in each of 60 different saccade directions (each bin was 6° polar angle). In subsequent analyses, we separated horizontal and vertical saccades because PSP patients have vertical gaze impairments specifically (Bhidayasiri et al. 2001). All saccades with direction ± 45° of the horizontal meridian were defined as horizontal, and all saccades ± 45° of the vertical meridian were defined as vertical.

III.2.4. Clip aligned analyses

The clip transitions produced transient changes in saccade and pupil behavior. We computed the macro- and micro-saccade rate (saccades / s) for each participant using a peri-stimulus time histogram (PSTH, 2 ms bin width due to the 500 Hz sample rate). We then smoothed these PSTH traces using a MATLAB smoothing function (local regression using weighted linear least squares and a 1st-degree polynomial model with a 50-sample span). For each participant, we extracted various parameters from these curves. The smoothing served to reduce the probability of false alarms in detecting meaningful dips and peaks in the curves and was verified by stepping through each participant's data and observing the detected parameters. For macro-saccades, we computed a baseline rate for each participant (-200 to +50 ms relative to the clip change) as well as the magnitude and timing of the dip in macro-saccade rate within an epoch from 70-200 ms post clip change ("saccade suppression", (Reingold and Stampe 1999)). Moreover, we calculated the peak macro-saccade rate after the clip transition (maximum value from the time of suppression to 300 ms post clip change), and the steady state macro-saccade rate (averaged from 1000-3000 ms after clip change).

A similar set of micro-saccade parameters was extracted for each participant. Micro-saccade PSTHs were created, and we computed a baseline rate (average rate from -200 to +50 ms relative to clip change). We computed the magnitude and timing of the suppression in micro-saccade rate in the epoch from 70-400 ms after clip change, and we

computed the steady state micro-saccade rate, which was the average over an epoch 1000-3000 ms after clip change.

III.3. Pupil analyses

We measured the mean global luminance of every frame of every movie by computing the luminance gamma functions of the red, green, and blue color gamuts at various output levels. We then used those functions to compute the luminance of every pixel in the frame and averaged across all pixels to get the mean screen luminance for that frame. Screen luminance changes drive the pupil to react, and it has a negative correlation with pupil size. To that purpose, we assessed all luminance changes in clip transitions to see how they affect pupil response, and we reported the mean pupil size of the entire group for each clip change. We correlated the mean pupil size with the mean screen luminance (cd/m2) across clips for each participant (Figure III-4).

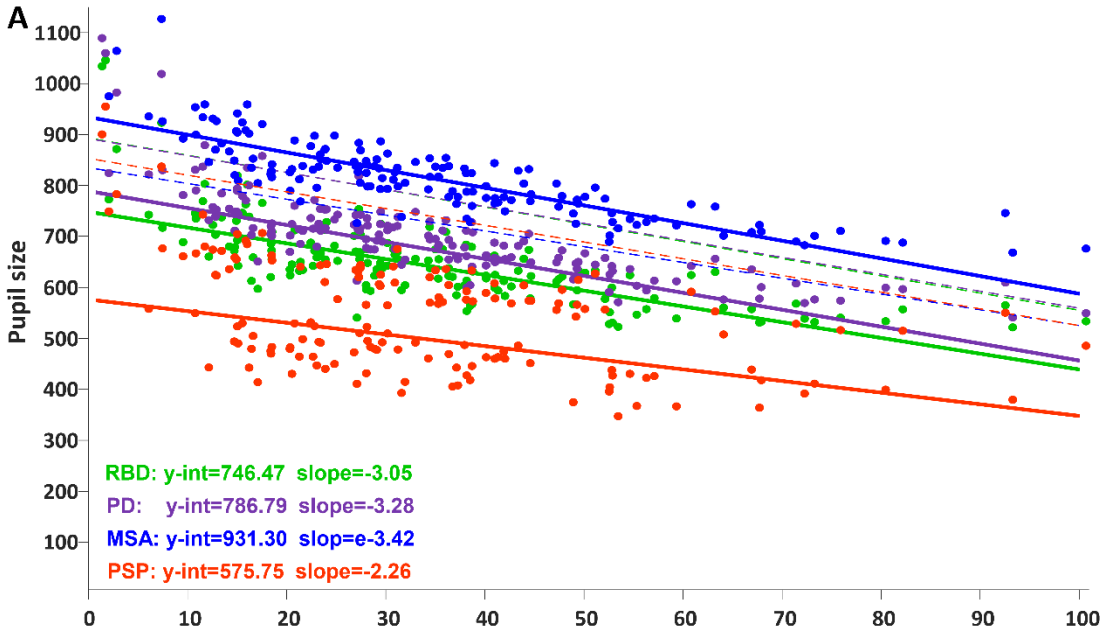

Figure III-4. Pupil sensitivity to luminance change. Pupil size change versus luminance. Each circle represents the average pupil size of the entire corresponding group at a clip change. The lines represent the linear fit across the data. The dashed lines represent the CTRLs of the same color-coded patient groups, while the solid line refers to patients. RBD: REM sleep behavior disorder; PD: Parkinson's disease; MSA: Multiple system atrophy; PSP: Progressive supranuclear palsy

We extracted various parameters from the clip-aligned pupil responses from the negative and positive luminance delta clip changes. For each participant, we smoothed each of the 30 zero-normalized clip-aligned pupil traces using a MATLAB smoothing

function (local regression using weighted linear least squares and a 1st-degree polynomial model with a 50-sample span). We then took the first derivative of the smoothed traces (pupil velocity). We applied the smoothing function again because the derivative amplifies any remaining noise and would otherwise result in false alarms in detecting reliable parameters from the traces. We determined the point where the velocity curves were significantly different from a baseline velocity (epoch ± 100 ms relative to the clip change) using a running signed-rank test (1-tailed; i.e., negative velocity in the constriction condition, and positive velocity in the dilation condition). Constriction/dilation latency for each participant was taken as the point where the curves were significantly different from baseline for at least 10 consecutive samples within an epoch from 100-500 ms post clip change in the constriction condition, and 200-600 ms post clip change in the dilation condition (to account for the slower dilation response). We also extracted the point where the velocity curves again became not significantly different from the baseline, and the difference in pupil size between that point and baseline was taken as the delta. The peak velocity for each participant was the maximum velocity in an epoch starting from the constriction/dilation latency (described above) for 200 ms. The time of peak velocity was also extracted. Finally, a steady state pupil parameter was extracted and was defined as the pupil size from 1000-3000 ms post clip change.

III.4. Unified Parkinson`s Disease Rating Scale (UPDRS III)

All patients underwent the UPDRS III and/or Movement Disorder Society (MDS)-UPDRS scores as part of their clinical routine. We converted all MDS-UPDRS scores to UPDRS scores for consistency. We utilized the formula proposed by Goetz et al. for this purpose (Goetz et al. 2012). On the same day as the eye movement assessment, UPDRS III and all other clinical data were collected.

Finally, we tested the correlation of all eye movement and pupil parameters versus UPDRS-III scores to examine the relationship between the severity of motor dysfunction and oculomotor and pupillometry parameters.

III.5. Statistical analyses

All statistical comparisons were performed in MATLAB and SPSS. The data distribution was tested using the One-sample Kolmogorov-Smirnov test and it was not

normally distributed. Therefore, we used a pairwise non-parametric test, Mann-Whitney-U-test (Mann and Whitney 1947), to determine the significant statistics. For the sake of simplicity, only P-values were reported in the test; other test statistics are included in the appendix. Multiple comparison adjustments were excluded due to the exploratory aspect of the study. However, a comparison of the results before and after the Bonferroni adjustment is provided in Table III-2. We performed different statistical comparisons to address our main questions. First, we compared patients to CTRL. We consistently report the patient values followed by CTRL unless stated otherwise. We then compared across patient groups to first determine if the prodromal αSYN group RBD started to reflect abnormalities that were already present in PD and MSA and then to identify which abnormalities reliably differentiated PSP from the αSYN groups.

III.6. Results

III.6.1. Low-level saccade statistics

III.6.1.1. Gaze distribution maps

We first analyzed the distributions of all fixations for the 10 minutes of free viewing from all participants, which produced gaze distribution maps. Patient groups (Figure III-5, top row) and their corresponding CTRL groups (Figure III-5, bottom row) all had a strong center bias (indicated in yellow), spending most of their time fixating on locations around the screen's center (Tseng et al. 2009). We subtracted the gaze distribution maps of CTRL groups from the patient groups to reveal the differences in the center bias (Figure III-5B). PD, MSA, and PSP groups all had a significantly greater center bias than CTRL (Figure III-5C; PD: 0.0043 average gaze/visual degree versus 0.0039, $P<.05$, MSA: 0.0042 versus 0.0039, $P<.01$, PSP: 0.0047 versus 0.0039, $P<.0001$). That means patient groups spent less time exploring the peripheral parts of the video clips than CTRL. We then compared the patient groups to one another. RBD and MSA had a significantly smaller center bias than PSP (RBD versus PSP: $P<.001$, MSA versus PSP: $P<.05$). We also looked at the difference in gaze distributions between patients and controls along the horizontal and vertical meridians (Figure III-5D; patient-CTRL). The PSP group had a greater center bias along horizontal and vertical meridians compared to all other groups.

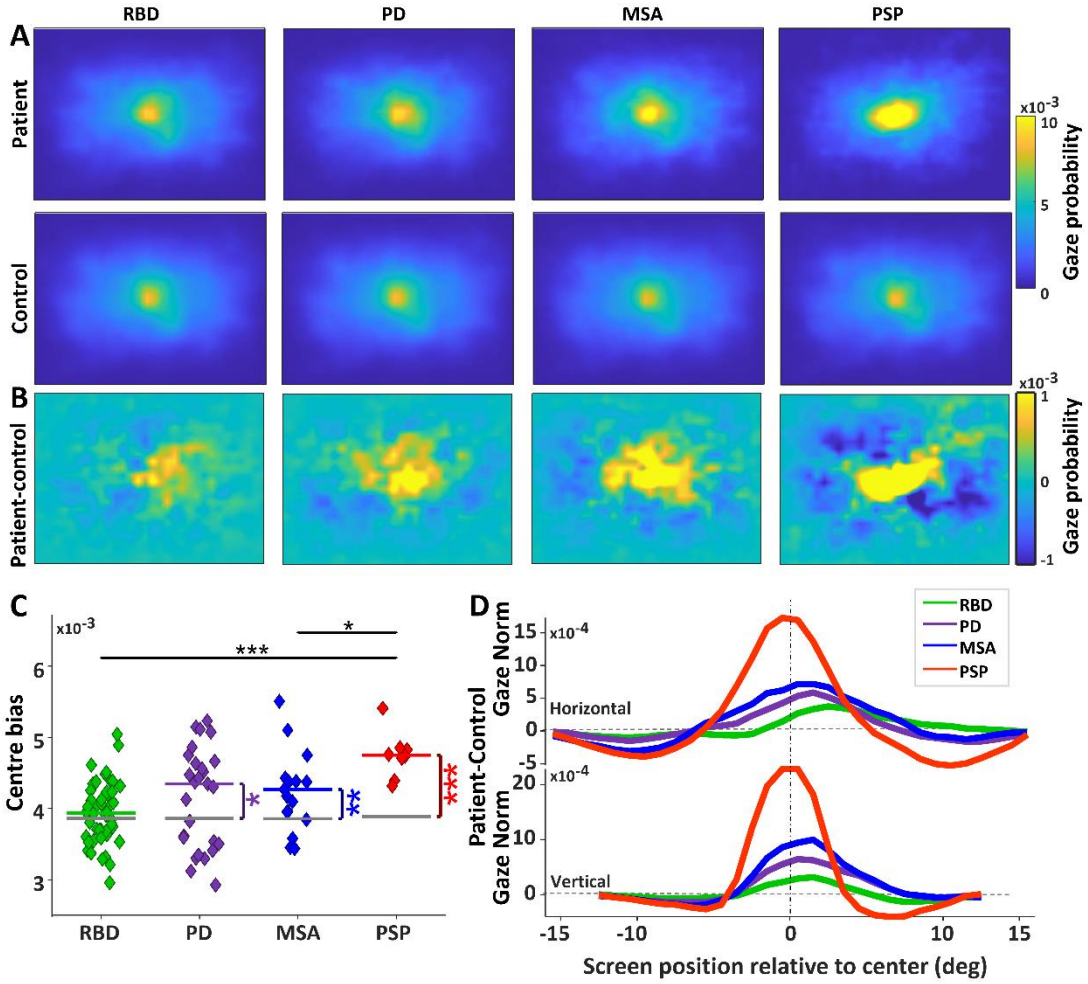

*Figure III-5. Characteristics of gaze distribution. (A) Gaze distribution for each group. The screen spanned 32 deg horizontally and 26 deg vertically. Higher gaze probability is represented by yellow. (B) Difference gaze probability maps of the patients minus controls, with yellow (positive values) indicating higher gaze probability for patients than controls. (C) Individual values of center bias, which was defined as the value at the center of the gaze probability map in A for each participant. The gray horizontal lines indicate the CTRL group's median, and the colorful horizontal lines indicate the patient group's median. Comparisons between the patients and CTRL were shown with vertical lines with asterisks if significant. Horizontal bares with asterisks indicate comparisons between the disease groups. (D) Difference in gaze probability between each patient group and their respective control group, extracted from a slice through the horizontal and vertical meridian of the difference gaze probability maps in C (positive values indicate higher gaze probability for patients relative to controls). Asterisks show a significance level of *P < .05 and **P < .01 and *** P < .001(same in all further figures). RBD REM sleep behavior disorder, PD Parkinson's disease, MSA Multiple system atrophy, PSP Progressive supranuclear palsy.*

III.6.1.2. Saccade and fixation duration distributions

We computed low-level statistics of saccade frequency, direction, and amplitude, as well as fixation durations. For these analyses, we separated macro-saccades from micro-saccades. All patient groups made fewer macro-saccades than CTRL (Figure III-6A; RBD: 1.74 saccades/s versus 1.89, $P<.05$; PD: 1.51 versus 1.87, $P<.0001$; MSA: 1.49 versus 1.92, $P<.0001$; PSP: 1.13 versus 1.94, $P<.0001$). Among patient groups, RBD had

a higher macro-saccade frequency, not only relative to PD ($P<.05$) and MSA ($P<.05$) but also relative to PSP ($P<.001$). Both PD and MSA had a higher macro-saccade rate relative to PSP (both $P<.05$). The overall micro-saccade rate (Figure III-6B) was not significantly different across groups. As a direct result of fewer macro-saccades, PSP and PD had longer fixation durations than CTRL (Figure III-6C; PD: 384 ms versus 357, $P< .05$; PSP: 416 versus 348, $P<.001$). PSP also had significantly longer fixation durations than RBD ($P<.01$) and MSA ($P<.05$).

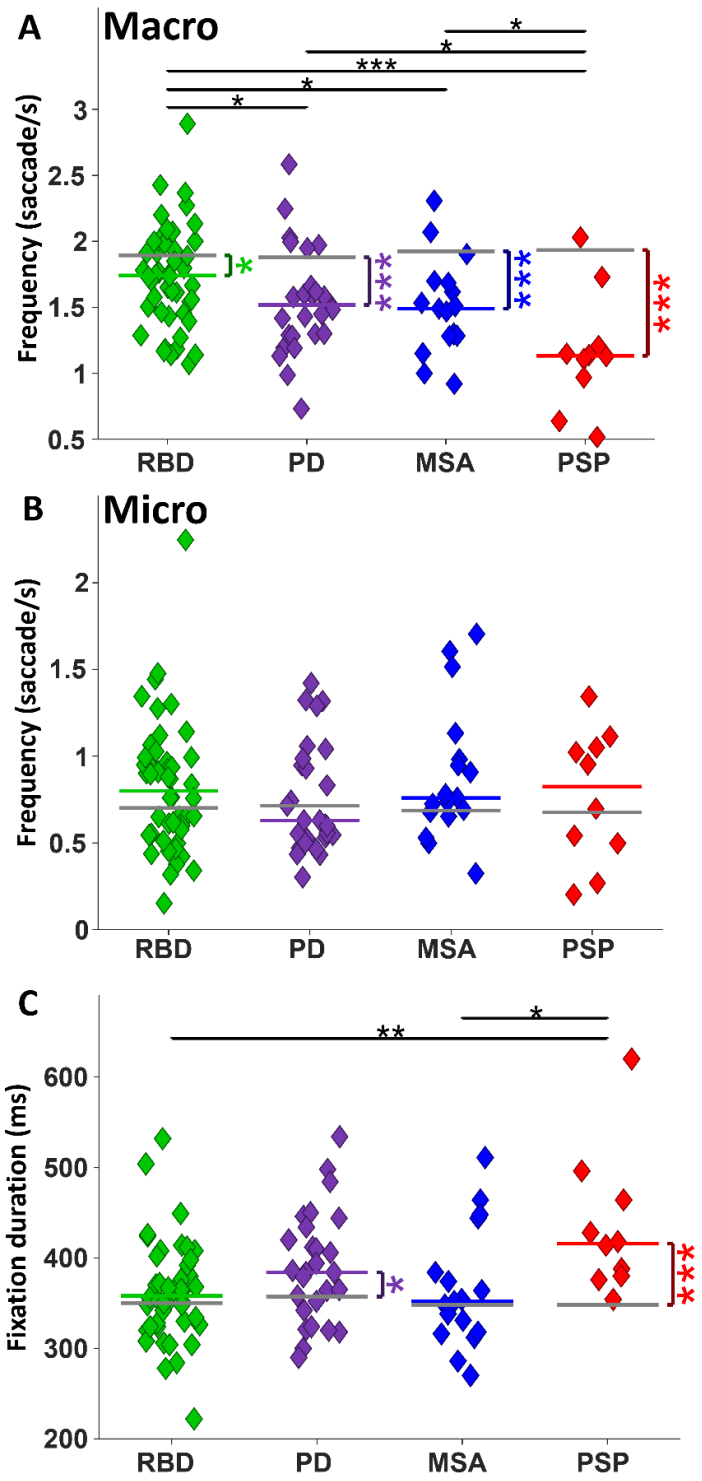

Figure III-6. Saccade frequency and median fixation duration. ***(A)*** *Macro-saccade rate per second for each group. The most important finding is the difference between RBD and PD. (B) Micro-saccade rate per second. (C) Median Fixation duration of each group.*

III.6.1.3. Distribution of macro- and micro-saccade directions

PSP patients develop vertical gaze palsy during disease progression (Armstrong 2011). To determine if there were directional biases in the distribution of saccade directions, we computed the frequency of macro-and micro-saccades in 60 different directions (Figure III-7A-B). PD, MSA, and PSP had reduced horizontal macro-saccade frequency compared to CTRL, but RBD did not differ (Figure III-7C; RBD: 1.21 saccades/s versus 1.28, P=0.08; PD: 1.05 versus 1.25, P<.01; MSA: 0.97 versus 1.28, P<.01; PSP: 1.05 versus 1.31, P<.05). Overall micro-saccade frequency in horizontal direction did not differ between patient groups and CTRL (Figure III-7D). Vertical macro-saccades were reduced in all patient groups relative to CTRL (Figure III-7E, RBD: 0.51 saccades/s versus 0.63, P<.001, PD: 0.44 versus 0.60, P<.0001, MSA: 0.42 versus 0.64, P<.0001, and PSP: 0.07 versus 0.62, P<.0001). Comparisons among αSYN groups revealed a significant difference between RBD and MSA (P<.05), while all αSYN groups had more vertical macro-saccades than PSP (all P<.001). PSP displayed lower vertical micro-saccade frequency than CTRL (Figure III-7F, 0.15 saccades/s versus 0.26, P<.05, all other comparisons of micro-saccades between patients and CTRL were not significant (all P>0.05)) and lower than all patient groups (all P<.05).

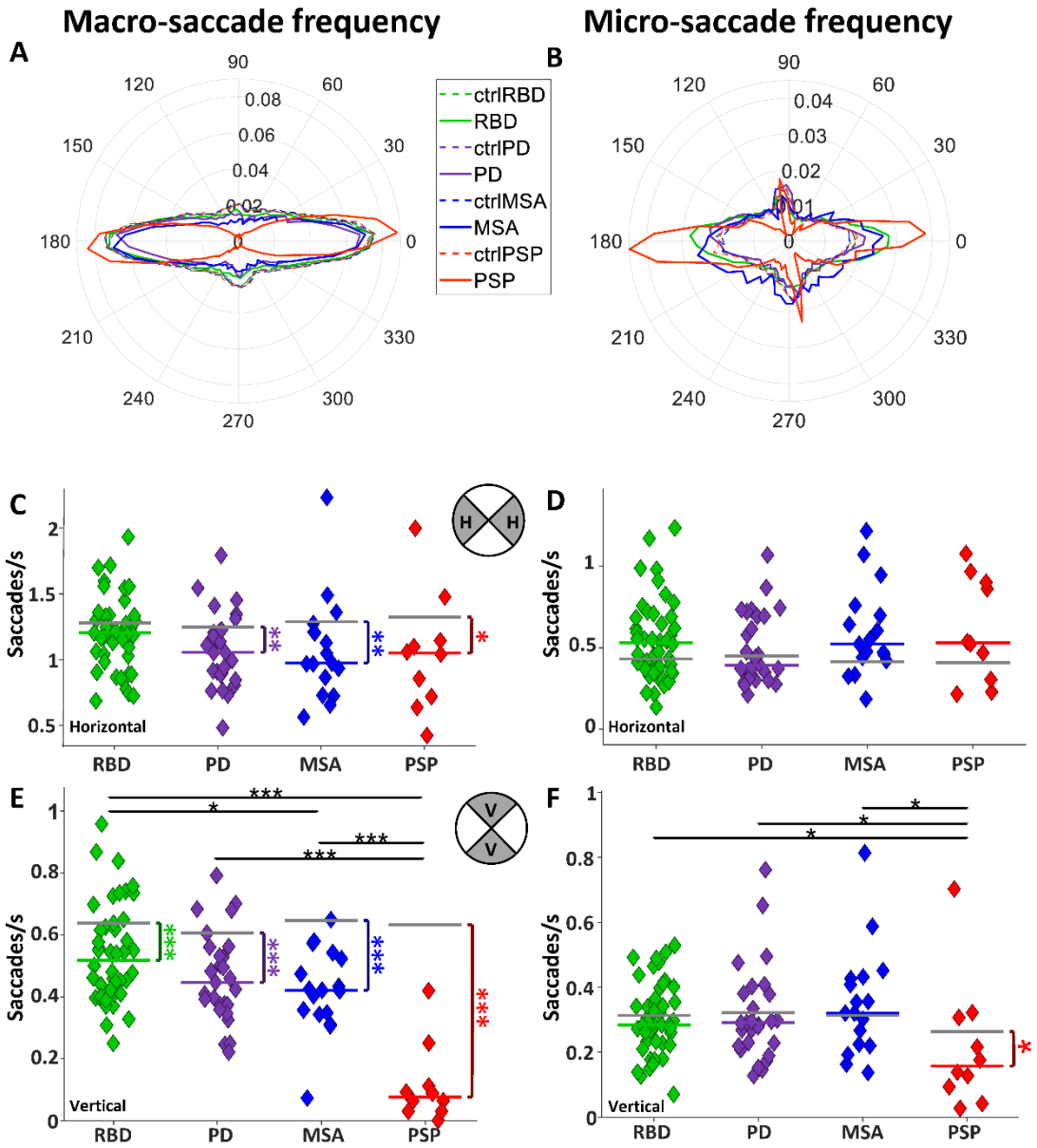

*Figure III-7. Saccade frequency in different directions. (**A**) Polar histogram of macro-saccade frequency and (B) Polar histogram of micro-saccade frequency for every group. Polar coordinates are saccade directions, and each circle represents the average macro/micro-saccade frequency within each group. (C) and (D) Horizontal macro and micro-saccade frequency, respectively. (E) and (F) vertical macro and micro-saccade frequency of each individual, respectively.*

III.6.1.4. Saccade amplitude

We determined the average saccade amplitude for each of the 60 directions (Figure III-8A-B). PSP participants made the smallest macro-saccade amplitude in all directions, followed by MSA, then PD, and finally RBD, while CTRL made the largest macro-saccades (Figure III-8A).

Horizontal macro-saccade amplitude was reduced in all patient groups compared to CTRL (Figure III-8C; RBD: 7.04 saccades/s versus 7.49, $P<.05$, PD: 6.76 versus 7.46, $P<.01$, MSA: 6.46 versus 7.60, $P<.0001$, and PSP: 4.30 versus 7.52, $P<.0001$). RBD made larger macro-saccades than MSA ($P<.01$) and PSP ($P<.0001$). All αSYN groups made larger horizontal macro-saccades than PSP (RBD/PD versus PSP: $P<.001$, MSA versus PSP: $P<.01$). Horizontal micro-saccade amplitude was significantly larger in PSP versus CTRL (Figure III-8D; 1.33 degree versus 1.2, $P<.01$, all other comparisons of patients to CTRL were not significant (all $P>0.05$)). PSP had a horizontal larger micro-saccade amplitude than RBD ($P<.05$) and PD ($P<.01$).

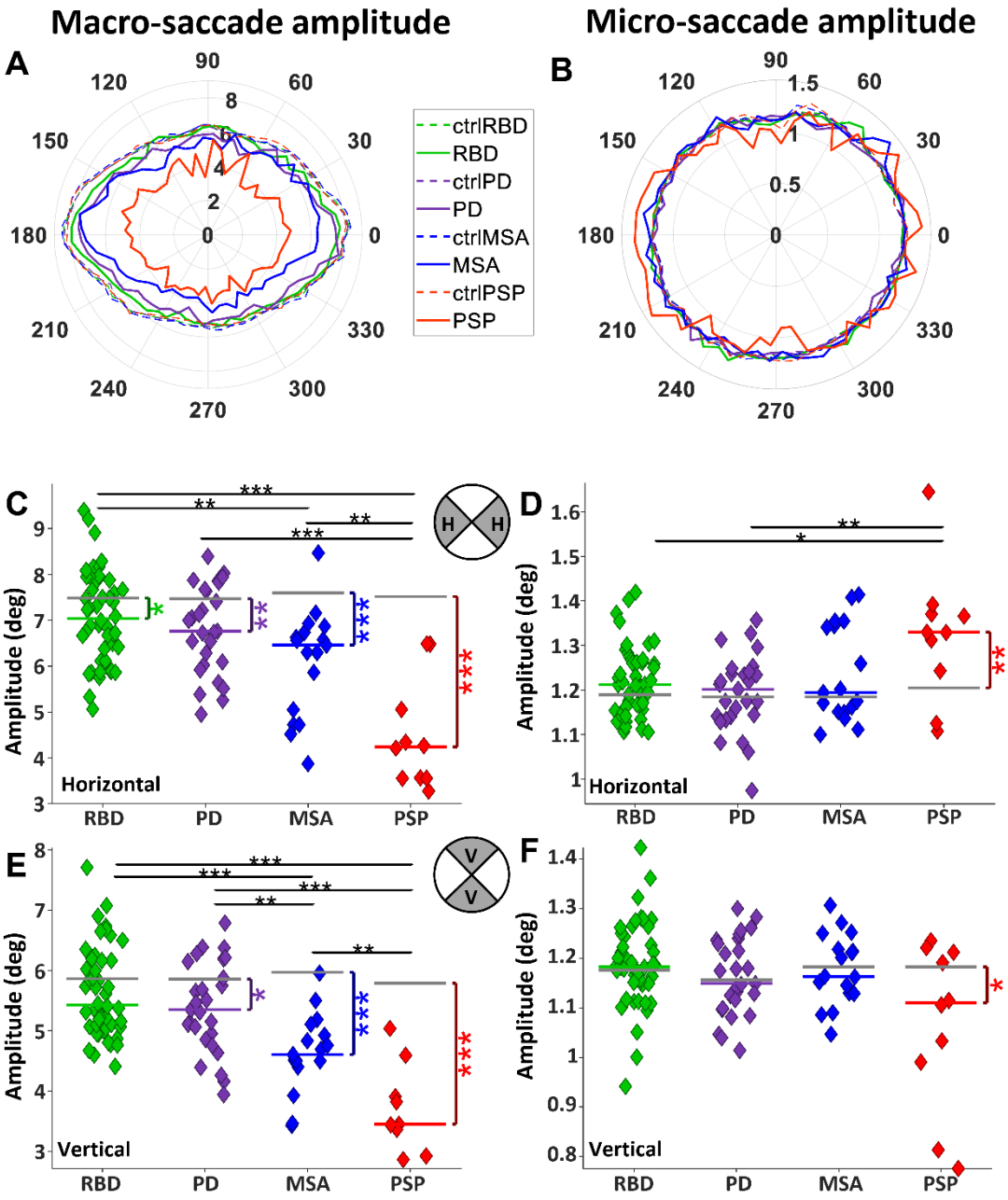

*Figure III-8. Characteristic of saccade amplitude in different directions. (**A**) Polar histogram of macro-saccade amplitude, (B) Polar histogram of micro-saccade amplitude for each group. Polar coordinates are saccade directions, and each circle represents the average saccade amplitude within each group. The bin angle was 10 degrees. (C) and (D) Horizontal macro- and micro-saccade amplitude, respectively. (E) and (F) Vertical macro- and micro-saccade amplitude, respectively.*

Vertical macro-saccades had reduced amplitude in PD, MSA, and PSP relative to CTRL (Figure III-8E; PD: 5.35 degree versus 5.86, $P<.05$, MSA: 4.60 versus 5.97, $P<.0001$, and PSP: 3.44 versus 5.82, $P<.0001$). Comparisons of αSYN groups showed that both RBD and PD had larger vertical macro-saccade amplitude than MSA (RBD versus MSA: $P<.001$, PD versus MSA: $P<.01$), while PSP had smaller vertical amplitude

compared to all groups (Versus RBD and PD: $P<.001$, versus MSA: $P<.01$). PSP had a smaller vertical micro-saccade amplitude than CTRL (VIII-8F; 1.11 versus 1.18, $P<.05$).

III.6.1.5. Saccade amplitude-velocity relationship

The average main sequence (saccade amplitude vs. velocity (Baloh et al. 1975); see Figure III-3 for single subject fit) of all groups showed that PSP patients had significantly slower saccades than CTRL and all other patient groups (Figure III-9A). The slopes of the individual participants' main sequence linear fits are shown in Figure III-9B. PSP had significantly slower saccades compared to CTRL (PSP: 118.37 degree/s versus 154.18, $P<.01$, all other comparisons of patients to CTRL were not significant (all $P>0.05$)). PSP also had significantly slower saccades compared to RBD ($P<.001$), PD ($P<.001$), and MSA ($P<.01$).

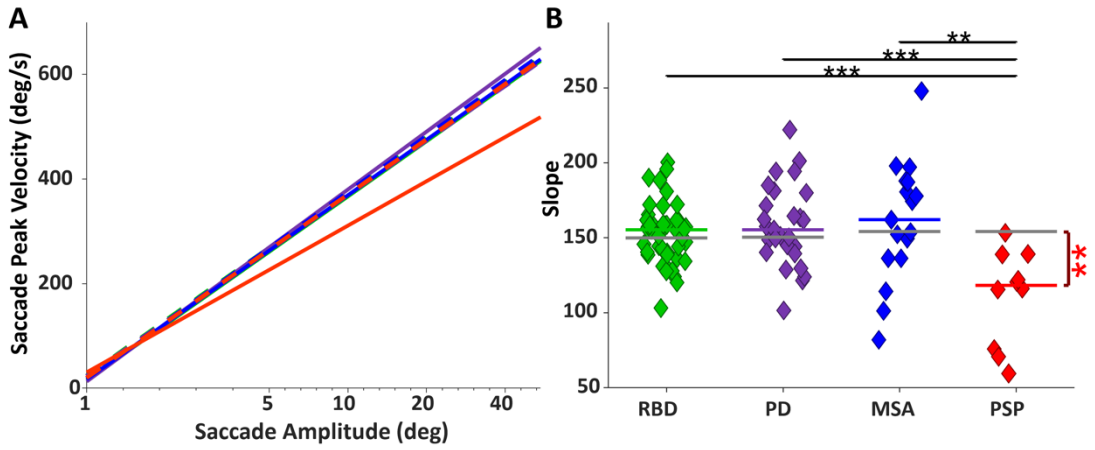

Figure III-9. Main sequence. ***(A)*** *The main sequence of all patient groups along with their matched CTRL. The X-axis is the amplitude on a logarithmic scale. The linear fitting line is applied over all data points of the subjects in 10 different movies in all directions.* ***(B)*** *The slope of the fit line for the main sequence of each individual.*

III.6.2. Clip-aligned changes in saccade rate

The clip transition represents a large perturbation in visual input to the brain. We examined the results of saccade and pupil responses that were influenced by these clip changes. About 65 ms after clip change, there was a momentary suppression in macro-saccade rate, followed by a rebound that started ~120 ms and peaked at approximately 200-250 ms (Figure III-10A). Finally, the saccade rate returned to a steady state rate of

about 400-500 ms after the clip change. The baseline saccade rate prior to clip change was reduced in all patient groups (Figure III-11A), but the depth of the suppression was not different across groups (Figure III-11B). Most importantly, although the start of the saccade rebound (120-170 ms after clip change) was similar in patients and controls, the peak of the rebound was significantly reduced in all patient groups relative to controls (Figure III-10B; RBD: 4.70 saccades/s versus 5.23, $P<.01$, PD: 4.20 versus 5.08, $P<.001$, MSA: 4.05 versus 5.2, $P<.001$, and PSP: 3.70 versus 5.16, $P<.0001$). RBD had a higher saccade peak than PD ($P<.05$) and PSP ($P<.001$). The average saccade rate in the epoch 1000-3000 ms (steady state) after the clip change was reduced in all patient groups relative to CTRL (Figure III-10C; RBD: 1.57 saccades/s versus 1.71, $P<.01$, PD: 1.36 versus 1.65, $P<.0001$, MSA: 1.29 versus 1.73, $P<.0001$, and PSP: 0.92 versus 1.79, $P<.001$). RBD had a higher steady state saccade rate compared to PD ($P<.05$), MSA ($P<.05$), and PSP ($P<.001$). PD also had a higher saccade rate compared to PSP ($P<.05$). When we separated the clips for high and low luminance, we did not observe differences in the saccade rate based on the luminance levels of the clips.

The micro-saccade rate was also affected by the clip change (Figure III-10D). In CTRL, the micro-saccade rate dropped ~70 ms after clip change, and this suppression persisted until ~500 ms before returning to a steady state. The magnitude of suppression of micro-saccade rate was reduced in PD and PSP relative to CTRL (Figure III-10; RBD: -0.67 saccades/s versus -0.67, $P=.54$, PD: -0.53 versus -0.67, $P<.05$, MSA: -0.70 versus -0.67, $P=.90$, and PSP: -0.42 versus -0.72, $P<.05$). RBD and MSA had larger suppressions than PSP (both $P<.05$). Steady state micro-saccade rate (1000-3000 ms after clip change) did not differ between the groups.

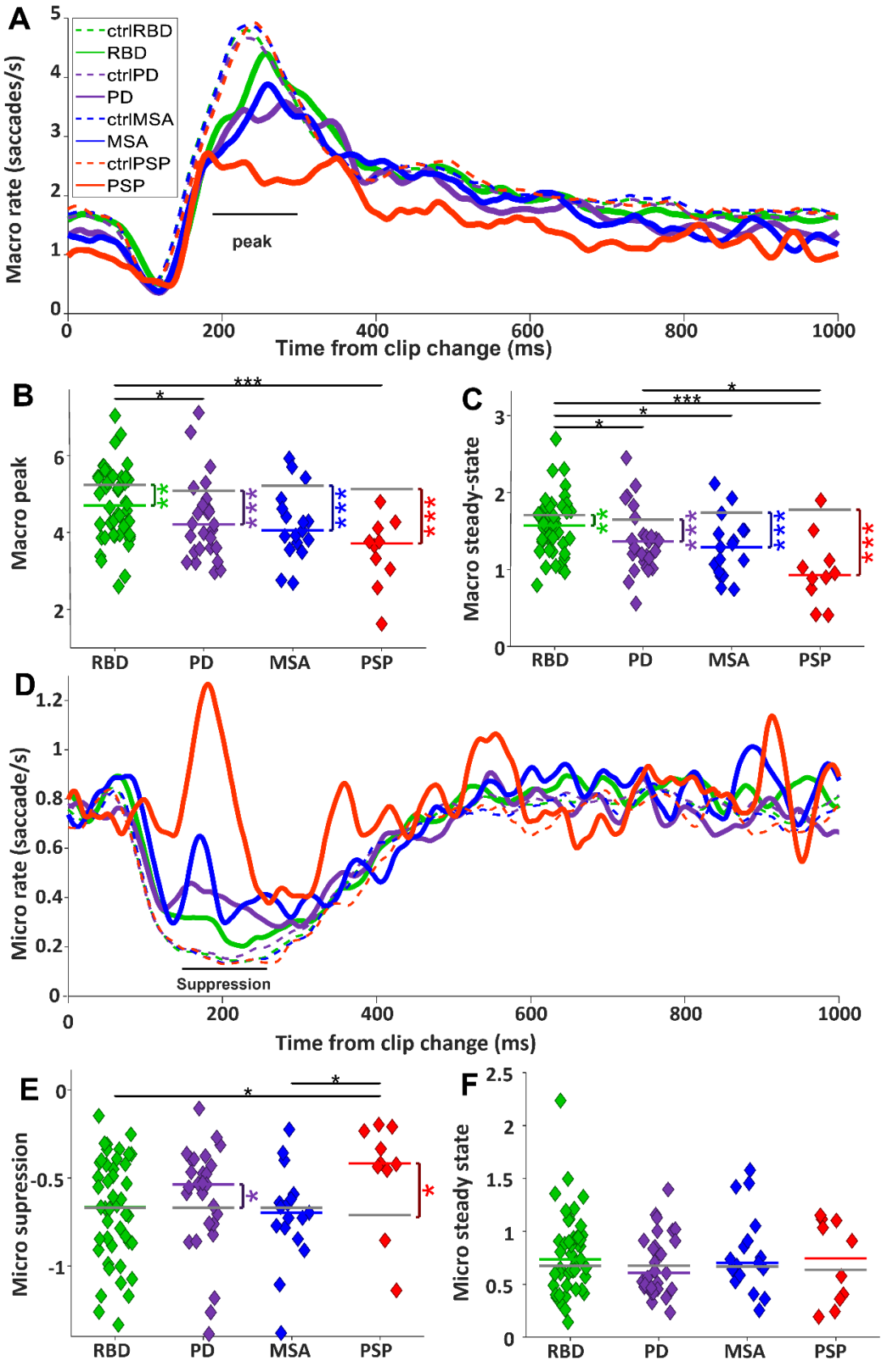
A
ctrlRBD
RBD
ctrlPD
PD
ctrlMSA
MSA
ctrlPSP
PSP
Macro rate (saccades/s)
peak
Time from clip change (ms)
B
Macro peak
RBD
PD
MSA
PSP
C
Macro steady-state
D
Micro rate (saccade/s)
Suppression
Time from clip change (ms)
E
Micro supression
F
Micro steady state

Figure III-10. Saccades rate after clip change. ***(A)*** *Macro-saccade rate after clip change. The black horizontal line shows the epoch in which the average macro-saccade peak was measured. Every trace represents the mean macro-saccades of all participants in all trials.* ***(B)*** *Median macro-saccade peak for each participant.* ***(C)*** *Median macro-saccade rate in steady state.* ***(D)*** *Micro-saccade rate after clip change. Every trace represents the mean micro-saccades of all participants in all trials. The black horizontal line shows the epoch in which the micro-saccade rate suppression has been measured.* ***(E)*** *Median of micro-saccade suppression magnitude.* ***(F)*** *Median micro-saccade rate in steady state.*

Figure III-11 shows the saccade rate baseline and suppression after clip changes (extracted from Figure III-10A). The saccade rate baseline was reduced in all patient groups compared to CTRL (Figure III-11A; RBD: 1.45 saccades/s versus 1.69, $P<.05$, PD: 1.16 versus 1.61, $P<.01$, MSA: 1.31 versus 1.73, $P<.001$, and PSP: 0.80 versus 1.73, $P<.001$). Furthermore, PSP had a lower saccade rate than RBD ($P<.01$), PD ($P<.05$), and MSA ($P<.05$).

Saccade rate suppression was intact in all patients relative to CTRL (Figure III-11B; RBD: 0.42 saccades/s versus 0.48, $P=.34$, PD: 0.33 versus 0.45, $P=.10$, MSA: 0.34 versus 0.49, $P=.06$, and PSP: 0.36 versus 0.49, $P=.21$). Therefore, all patient groups showed a reduction in the saccade rate baseline, but suppression did not change significantly.

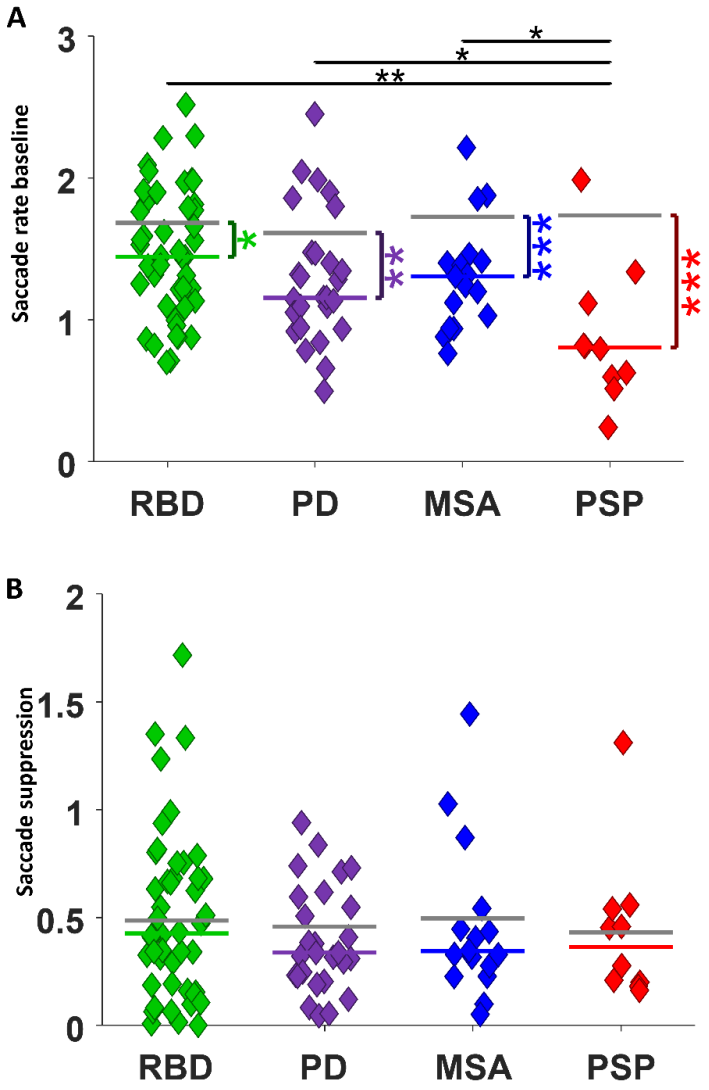

Figure III-11. Saccade rate baseline and suppression magnitude after clip change. ***(A)*** *Saccade rate baseline. The average saccade rate in the epoch from -200 to +50 ms relative to the clip change* ***(B)*** *Saccade suppression following clip change. The minimum saccade rate within an epoch from 70-200 ms post clip change.*

III.6.3. Clip-aligned changes in pupil size

Changes in global luminance evoke transient pupil responses (Loewenfeld 1993), and the clip changes included significant luminance changes on the screen that drive changes in pupil size. For the clip changes with the 20% most significant luminance increase (Figure III-12A), a robust constriction of the pupil was initiated ~300 ms after clip change and peaked at ~800 ms, followed by a gradual increase in pupil size over the next 2 s. The absolute pupil constriction change was smaller in PSP than in CTRL but failed to reach a significance level (Figure III-12B; PSP:-169.21 pixels versus -217.73, P=.19). MSA and

PD had a bigger pupil constriction delta than CTRL but failed to reach a significance level (PD: -262.9 pixels versus -235.26, *P*=.25, MSA: -273.96 versus -212.53, *P*=.06). RBD was very similar to CTRL in the size of pupil constriction delta (RBD: -236.53 pixels versus -245.25, *P*=.72). MSA had a significantly greater pupil constriction delta than PSP (*P*<.05). Relative pupil size in the steady state following luminance increase (Figure III-12C) was more constricted in MSA relative to CTRL (RBD: -154,16 pixels versus -168.11, *P*=.80, PD: -179.92 versus -153.62, *P*=.53, MSA: -231,52 versus -148.86, *P*<.01, and PSP: -116.27 versus -148.86, *P*=.11). In the steady state epoch, MSA had more constriction than RBD (*P*<.05) and PSP (*P*<.05).

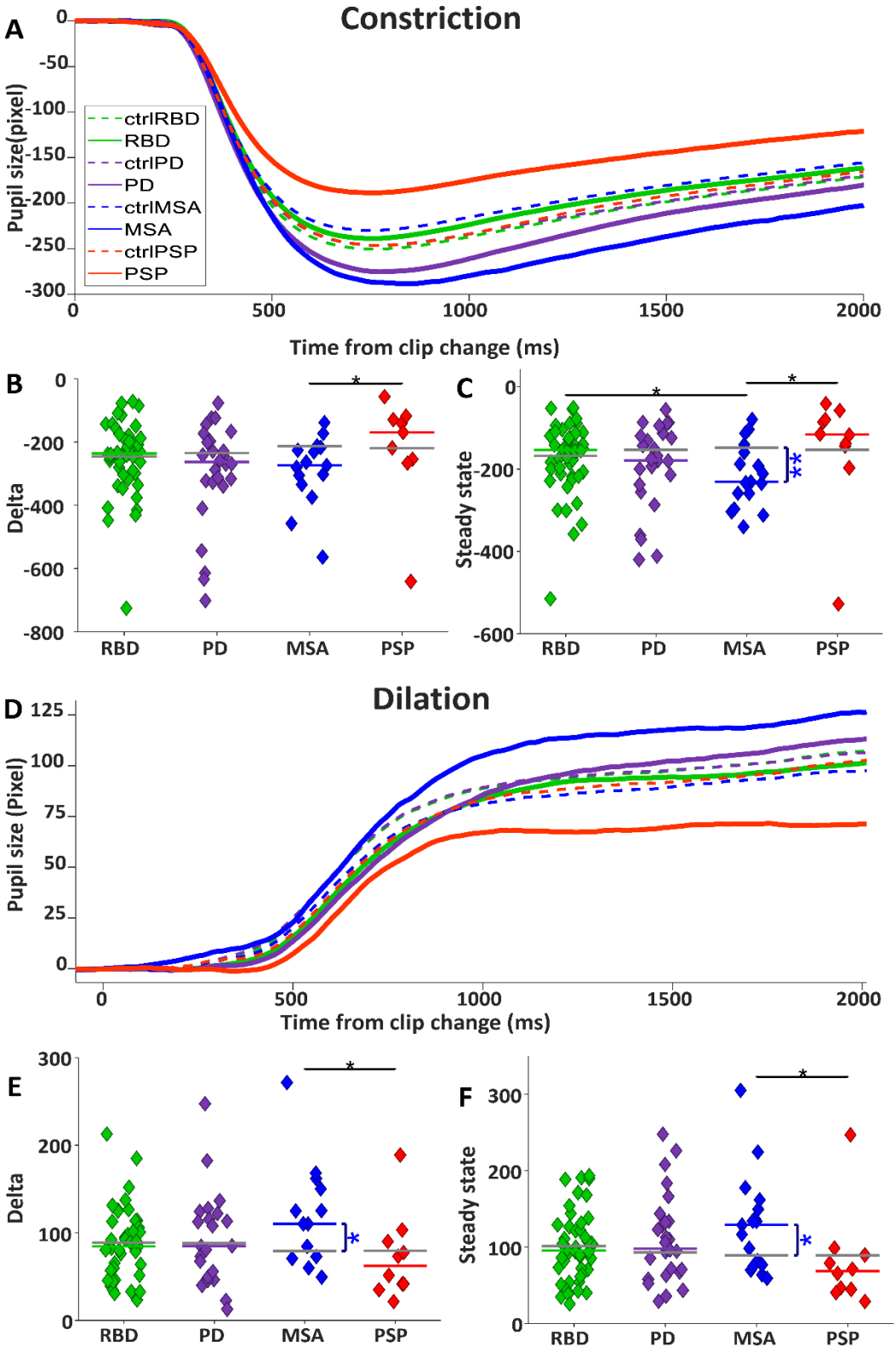

Figure III-12. Pupil response. ***(A)*** *Pupil constriction after clip change with positive luminance change. Time zero shows the onset of the clip change.* ***(B)*** *Median pupil constriction Delta and* ***(C)*** *median pupil size in steady state for each participant.* ***(D)*** *Pupil dilation after clip change with negative luminance change.* ***(E)*** *Median pupil dilation magnitude and* ***(F)*** *median pupil size in steady state.*

For the clip changes with the 20% greatest decrease in global luminance, there was a robust dilation of the pupil that began ~400 ms after clip change, followed by an increase in pupil size until a steady state was reached at approximately 1000 ms (Figure III-12D). However, there were significant differences in the magnitude of this dilation response across groups. MSA had larger pupil dilation compared to CTRL, while PSP elicited smaller dilation than CTRL, but this was not significant (Figure III-12E, RBD: 84.59 pixels versus 88.72, P=.51, PD: 84.85 versus 88.31, P=.80, MSA: 110.36 versus 79.18, P<.05, and PSP: 62.44 versus 78.87, P=.25). Pupil dilation was larger in MSA than PSP (P<.05). Relative to CTRL, median pupil size after dilation in steady state was bigger in MSA (Figure III-12F, 129.09 pixels versus 89.23, P<.05) while it was smaller (not significant) in PSP (68.46 pixels versus 86.85, P=.10). RBD and PD displayed a similar pupil dilation with CTRL (RBD: 95.75 pixels versus 101.38, P=.91, PD: 97.75 versus 92.95, P=.64). MSA had larger pupil dilation in steady state than PSP (P<.05).

Some of these changes in the dynamics of pupil responses following luminance changes could be the result of different baseline pupil sizes in the different disorders. It is intriguing that pupil baseline size was elevated in MSA, but slightly reduced in PD and RBD (Figure III-4). Baseline pupil size was greatly reduced in PSP, compared to CTRL and the αSYN groups.

III.6.4. Correlations between oculomotor and clinical assessment

A correlation analysis with the UPDRS-III scores of all patients from all groups and their saccade (Figure III-13; Figure III-14) and pupil (Figure III-15) parameters was performed. We also repeated the analyses without including the PSP patients to isolate the correlations for the αSYN groups.

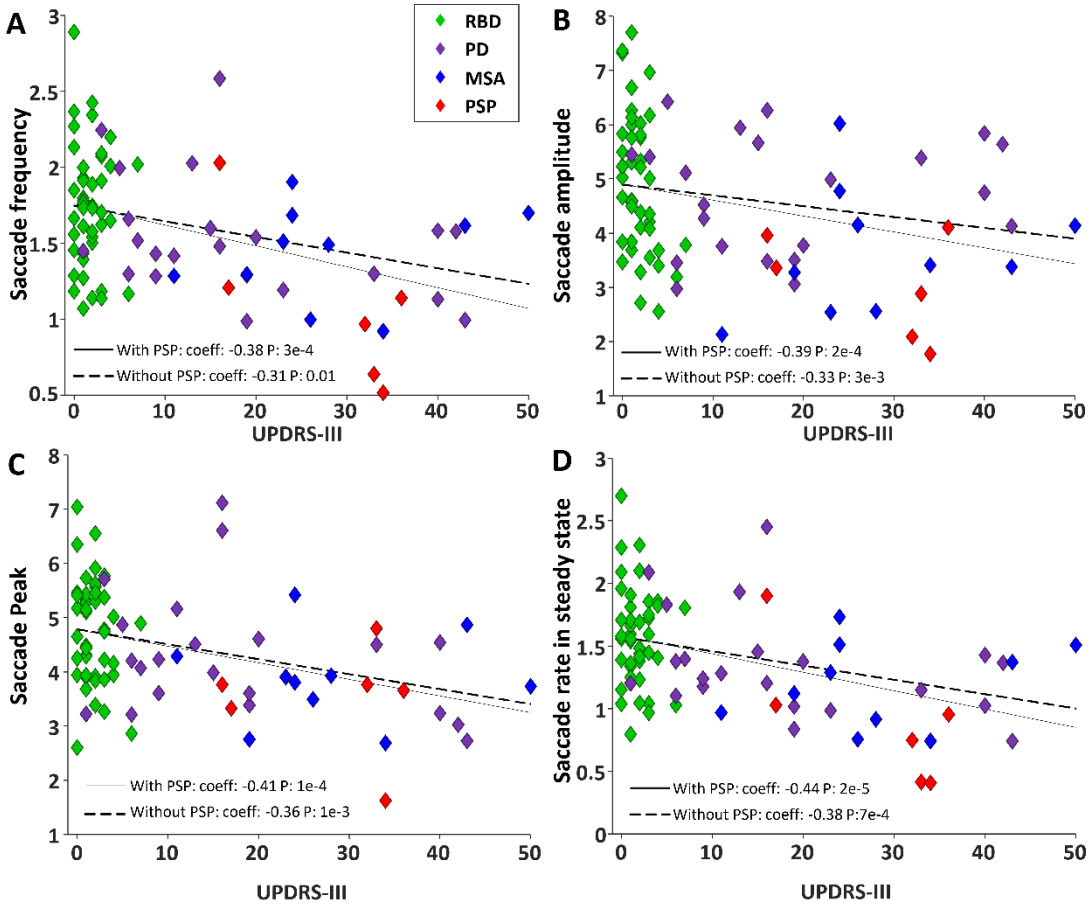

Figure III-13. Relation between UPDRS-III and saccade. ***(A)*** *Negative correlation between saccade frequency and UPDRS-III score.* ***(B)*** *Negative correlation between saccade amplitude and UPDRS-III score.* ***(C)*** *Negative correlation between saccade peak and UPDRS-III score.* ***(D)*** *Negative correlation between saccade rate in steady state and UPDRS-III. The solid and dashed black lines show the linear fit over data including PSP and without PSP, respectively.*

Spearman correlation revealed that macro-saccade frequency was negatively associated with the severity of motor symptoms in the combined patient group (Figure III-13A; with PSP: ρ= -0.38, $P<.001$; without PSP: ρ= -0.31, $P<.05$). Saccade amplitude was also negatively correlated with UPDRS-III (Figure III-13B, with PSP: ρ=-0.39, $P=.0002$, without PSP: ρ=-0.33, $P=.003$). The rebound in saccade rate following the clip changes was negatively correlated to the UPDRS score (Figure III-13C, with PSP: ρ=-0.41, $P<.0001$, without PSP: ρ=-0.36, $P<.001$), as well as the steady state saccade rate 1000-3000 ms after clip change (Figure III-13D, with PSP: ρ=-0.44, $P<.001$, without PSP: ρ=-0.37, $P<.001$).

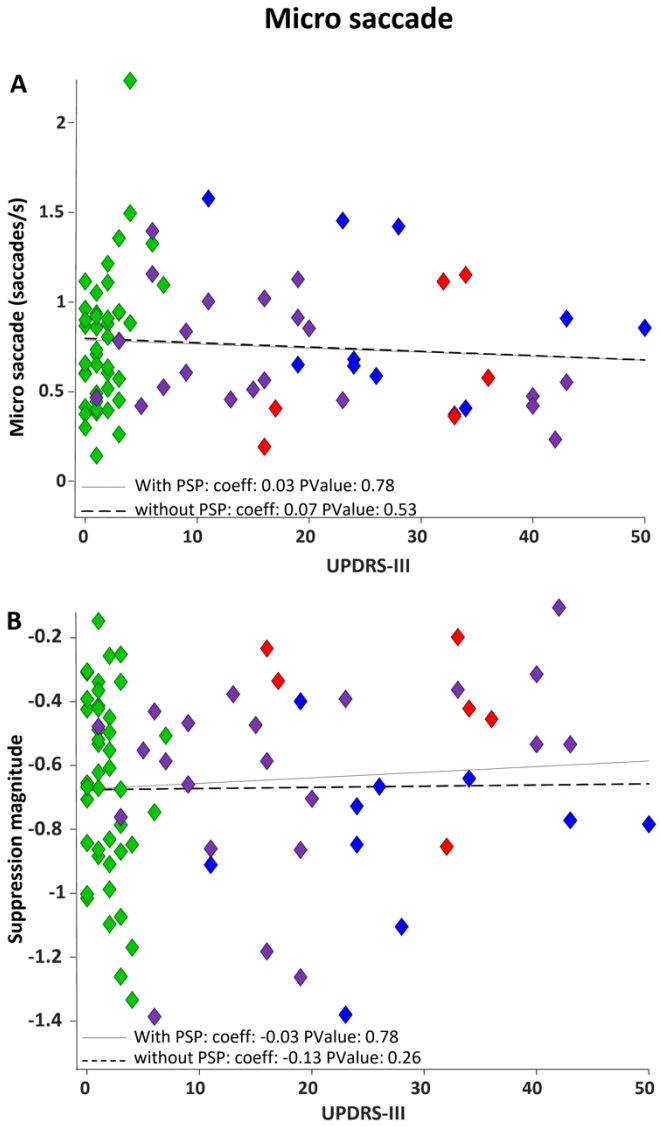

Figure III-14. UPDRS III correlation with micro-saccade. ***(A)*** *Relationship between micro-saccade rate after clip change and UPDRS III.* ***(B)*** *Relationship between micro-saccade suppression magnitude after clip change and UPDRS III.*

Neither micro-saccade rate (Figure III-14A) nor micro-saccade suppression magnitude (Figure III-14B) was correlated with the UPDRS score, either with or without PSP included.

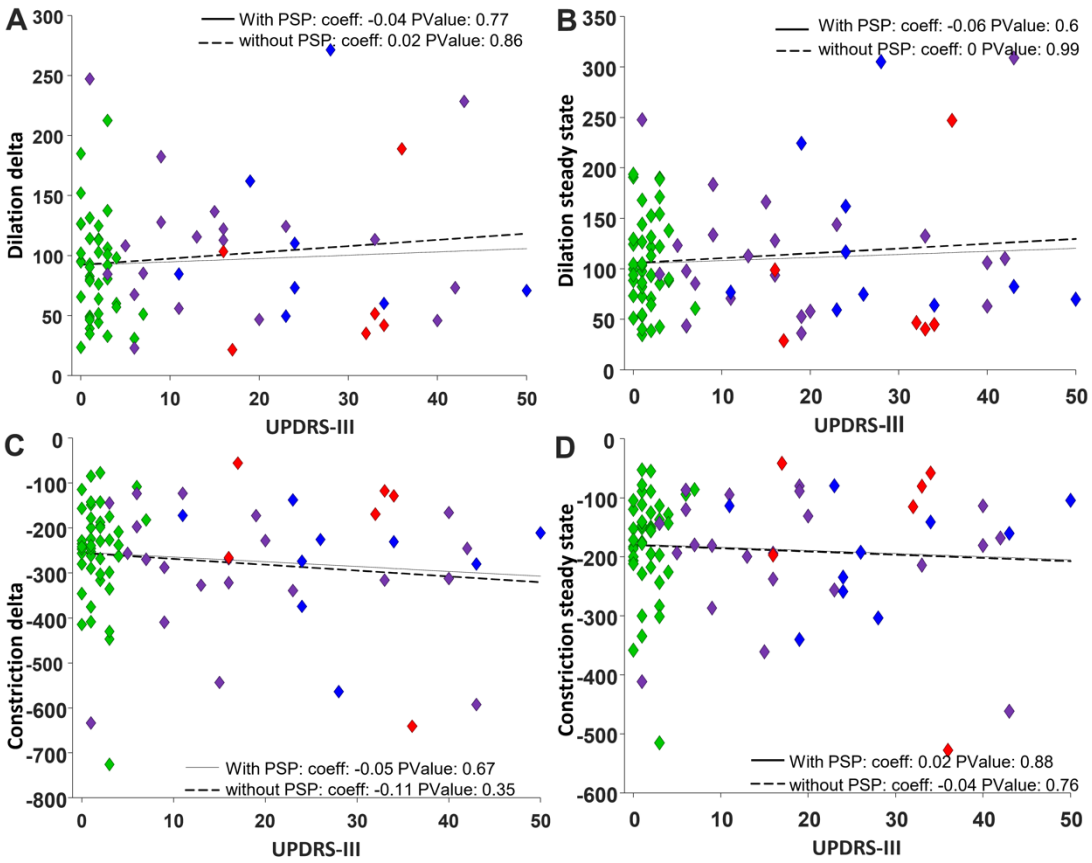

Figure III-15. Relation between UPDRS-III and pupil. ***(A) C****orrelation between pupil dilation delta and UPDRS-III score.* ***(B)*** *Correlation between pupil dilation in steady state and UPDRS-III score.* ***(C)*** *Correlation between pupil constriction delta and UPDRS-III score.* ***(D) C****orrelation between pupil constriction in steady state and UPDRS-III. The solid and dashed black line shows the 1st-degree polynomial fitting curve over data including PSP and without PSP, respectively.*

We did not identify any significant correlations between pupil parameters and UPDRS scores (Figure III-15A-D).

III.6.5. Results summary

Table III-2 provides an overview of the findings. Statistics are indicated by arrows, with one arrow representing one asterisk, two arrows representing two asterisks, and three arrows representing three asterisks. In each of the graphs, the arrows point downward or upward, indicating a decrease or an increase in the relevant metric, respectively. The fewer number of arrows beneath the column RBD versus CTRL indicated that there were fewer statistically significant differences between these two groups. On the other hand, other diseases exhibited more discrepancies with CTRL, especially PSP exhibited the most significant ones (more than one arrow). The other columns of importance are those that compared RBD to PD and MSA, and the results indicated that the differences were more between RBD and MSA than between RBD and PD. On the other hand, PD demonstrated only one significant difference when compared to MSA.

Table III-2. FV results summary. The arrows represent the difference between the first and second groups in terms of the parameter provided in the first column. For example, a downward arrow next to the group comparisons RBD ↔CTRL indicates that the corresponding variable has dropped in RBD relative to CTRL. The red arrows indicate that these arrows would have been removed if the Bonferroni correction had been applied (new alpha level for comparisons between CTRL and patients: 0.05/4, 0.01/4, 0.001/4, and for comparisons between patients: 0.05/6, 0.01/6, 0.001/6). CTRL: Control group; RBD: REM sleep behavior disorder; PD: Parkinson's disease; MSA: Multiple system atrophy; PSP: Progressive supranuclear palsy

Task 2: Free viewing

VARIABLE	RBD ↔ CTRL	PD ↔ CTRL	MSA ↔ CTRL	PSP ↔ CTRL	RBD ↔ PD	RBD ↔ MSA	RBD ↔ PSP	PD ↔ MSA	PD ↔ PSP	MSA ↔ PSP
Center bias		↑	↑↑	↑↑↑			↓↓↓			↓
Macro-saccade frequency	↓	↓↓↓	↓↓↓	↓↓↓	↑	↑	↑↑↑		↑	↑
Micro-saccade frequency										
Fixation duration		↑		↑↑↑			↓↓			↓
Horizontal macro-saccade frequency		↓↓	↓↓	↓						
Horizontal micro-saccade frequency										
Vertical macro-saccade frequency	↓↓↓	↓↓↓	↓↓↓	↓↓↓		↑	↑↑↑		↑↑↑	↑↑↑
Vertical micro-saccade frequency				↓			↑		↑	↑
Horizontal macro-saccade amplitude	↓	↓↓	↓↓↓	↓↓↓		↑↑	↑↑↑		↑↑↑	↑↑
Horizontal micro-saccade amplitude				↓↓↓			↑		↑↑	
Vertical macro-saccade amplitude		↓	↓↓↓	↓↓↓		↑↑↑	↑↑↑	↑↑	↑↑↑	↑↑
Vertical micro-saccade amplitude				↓						
Main sequence slope				↓↓			↑↑↑		↑↑↑	↑↑
Clip-aligned analyses:										
Saccade rate peak	↓↓	↓↓↓	↓↓↓	↓↓↓	↑		↑↑↑			
Saccade rate in steady state	↓↓	↓↓↓	↓↓↓	↓↓↓	↑	↑	↑↑↑		↑	
Micro-saccade suppression		↓		↓			↑			↑
Micro-saccade in steady state										
Saccade rate baseline	↓	↓↓	↓↓↓	↓↓↓			↑↑		↑	↑
Saccade rate suppression										
Pupil constriction delta										↑
Constriction steady state			↑↑			↑				↑
Dilation delta			↑							↑
Dilation steady state			↑							↑

III.7. Discussion

In this exploratory study, we investigated parameters of oculo/pupillo-motor function in the manifest αSYN PD, MSA, and the prodromal αSYN RBD in comparison to the Tauopathy PSP. We employed a Free Viewing paradigm (FV) - in combination with novel analysis methods of saccade and pupil behaviors- to study the above-mentioned movement disorders. Previous studies have used visually guided saccade tasks to quantify horizontal and vertical gaze abnormalities (Hanuška et al. 2019; Perkins et al. 2021). When uninstructed participants watched short video clips for only 10 minutes, this FV paradigm allowed us to answer the three questions lined out in the introduction as follows: 1) FV revealed qualitatively similar vertical gaze abnormalities as reported for the visually guided saccade task, but in addition, we describe several novel findings related to saccade and pupil behavior as detailed below; 2) the behavioral results from FV differentiated between patients with αSYN and PSP –in principle in line with the results obtained with the visually guided saccade task; and 3) in the αSYN prodrome RBD, the FV paradigm allowed us to identify already discrete, but distinct saccadic abnormalities, which however are less pronounced than in PD and MSA patients.

III.7.1. Saccade abnormalities in neurodegeneration

All patient groups had altered saccade behavior during the free-viewing task, including increased center bias (Figure III-5) and reduced saccade amplitude and frequency (Figure III-7-Figure III-8). These basic deficits mean patients with neurodegenerative disorders harvested less visual information from the peripheral visual display and instead focused their limited resources on the center of the screen, which would greatly reduce their ability to process the whole gist of any clip.

The clip transitions had a profound impact on saccade production (Figure III-10). Within ~70 ms of clip transition, the macro-saccade rate plunged to a nadir of ~120 ms before rebounding. This initial suppression in saccade rate was the result of large changes in the visual display at clip change (Reingold and Stampe 1999) and was likely produced by visual input passing through the SC to the brainstem OPNs (Büttner-Ennever et al. 1999) which gate all saccades via direct inhibition of EBNs and IBNs (King 1977;

Strassman et al. 1987) in the PPRF and the riMLF. OPNs have transient visual responses (Everling et al. 1998), and so the visual perturbation produced by the clip change, which is known to activate neurons in the SC (White et al. 2017), likely led to an increase in OPN discharge which would immediately inhibit saccade burst neurons in the riMLF and PPRF and lead to saccade suppression.

In structured oculomotor tasks, visually-triggered saccades are typically initiated more than 90 ms after target appearance and can be further characterized as express latency saccades or regular latency saccades (Fischer and Ramsperger 1984; Coe and Munoz 2017). Saccades with reaction times <90 ms are not visually triggered (Munoz et al. 1998). Analogous to the structured pro-saccade task, in FV, saccade triggered < 90 ms after clip change preceded the transient epoch of saccade suppression, and the ensuing rebound in saccade rate represents the shortest latency visually-triggered saccades, which could include both express (90-140 ms) and regular (>140 ms) latency saccades. Express latency saccades, the shortest latency visually-triggered saccades that humans can make (Fischer and Ramsperger 1984), are produced when transient visual signals traveling through the SC become the saccade command (Dorris et al. 1997; Dorris and Munoz 1998).

Following the clip transitions in the free-viewing task, the depth of the saccade suppression and initial part of the rebound was intact in all patient groups. However, the peak of the saccade rebound was significantly blunted in all patient groups (Fig. 6B), which is analogous to the time of regular latency saccades in the pro and anti-saccade tasks (SRT>140 ms) (Coe and Munoz 2017). The reduced frequency of saccades at this time was likely the result of cognitive impairments due to neurodegeneration in cortical/ basal ganglia circuits affecting or delaying key inputs to the SC, which is analogous to increased latency of correct saccades among PD patients performing the anti-saccade task (Chan et al. 2005; Amador et al. 2006; Cameron et al. 2012; Perkins et al. 2021). In contrast, the generation of automatic visually triggered pro-saccades remained relatively unimpaired in PD (Chan et al. 2005; Cameron et al. 2012), likely because these automatic saccades are driven by visual inputs from occipital and parietal cortex to the SC, regions of the brain that were less impacted in the diseases studied here.

The FV task provided an assessment of many saccade parameters. However, we were not able to determine subtle saccade abnormalities related to dysmetria because we did not define visual targets in the video clips. The visually-guided saccade task is ideal for investigating saccade dysmetria and the difference between vertical and horizontal saccades. The FV task is better for measuring ongoing and continuous saccade and micro-saccade behavior, and pupil behavior without having to introduce any complex instructions or task parameters.

The SC represents a competition map for the generation of saccades in a winner take all manner (Itti and Koch 2001) in which only one spatial location can issue a saccade burst at any one time. Likely due to the reduced macro-saccade rate following the clip change (Figure III-10A, B), the micro-saccade rate was less suppressed following the clip change in PD and PSP (Figure III-10D, E). However, the micro-saccade steady state was not increased in the patient groups (Figure III-10F), despite the significant reductions in macro-saccade steady state in all patient groups (Figure III-6C), so this inverse relation between macro- and micro-saccade rates was not consistent across the entire clip but was most evident immediately following clip transition (< 500 ms).

III.7.2. Vertical saccade deficits in neurodegeneration

All patients had a significant reduction in vertical saccade rate, which was greatest in PSP (Figure III-7E). PD patients make hypometric saccades in vertical and horizontal directions (Jung and Kim 2019) but do not exhibit downward vertical gaze paresis, which is typical in PSP (Otero-Millan et al. 2011). This dramatic vertical gaze palsy in PSP was likely the result of degeneration in the midbrain that impacted the riMLF, which houses the vertical saccade burst neurons that project directly to the pools of vertical extraocular muscle motoneurons in the oculomotor and trochlear nuclei (Moschovakis and Highstein 1994). Reduction in signals from these burst neurons in the riMLF will make it harder to initiate the vertical component of saccades, and those saccades will have a reduced amplitude and velocity. This is the pattern we observed in PSP, where it appears that these neurons were selectively damaged, leading to vertical gaze palsy. This hypothesis is supported by structural abnormalities in PSP that are known to often impact the midbrain

and hence riMLF (Leigh and Zee 2015), which may appear small and pathologic (Armstrong et al. 2007).

III.7.3. Pupil characteristics in neurodegeneration

Pupil responses were abnormal in the different patient groups but in dramatically different ways for the PSP versus the MSA group (Figure III-12) which suggest very different actions of pathophysiology. All participants showed a very robust center bias (Figure III-5), and pupil size is determined by global luminance. Therefore, the pupil differences we described cannot be attributed to local luminance differences based upon the location of fixation. Across the duration of the free-viewing of the video, pupil size for the PSP group was significantly smaller than for the MSA group (Figure III-4). Following the clip transition to darker or brighter clips, pupil dilation and constriction responses were attenuated in PSP but exaggerated in MSA (Figure III-12). Despite these large differences in the magnitude of the pupil responses between PSP and MSA, there were no differences in the onset latency of the constriction or dilation responses (not shown), suggesting that the deficits likely arise from central (i.e., brainstem) rather than peripheral (i.e., retinal), origin.

The dominant luminance pathway consists of retinal input to the pretectal olivary nuclei via intrinsically photosensitive retinal ganglion cells (Armstrong et al. 2007). Neurons in the pretectal olivary nucleus project directly to the EW (Szabadi 2018). Many different brainstem nuclei and pathways are responsible for the non-luminance modulations of pupil size (Wang and Munoz 2015). The LC in the pons is a key structure in pupil control (Szabadi 2018). The discharge of LC neurons is correlated to the slow changes in pupil size that are related to arousal (Joshi et al. 2016). More recently, another non-luminance pathway has been identified through the SC (Wang and Munoz 2015). The same SC neurons that project to riMLF and PPRF also collateralize into regions of the central mesencephalic reticular formation (cMRF) (Scudder et al. 1996), which then projects to EW (Szabadi 2018) to influence pupil size. As a result, cognitive control signals from the cortex that flow through the SC have a route to influence pupil size.

Pathophysiology of the LC has been implicated in the early stages of αSYN, typically at stage II (Braak et al. 2003). Thus, alterations in LC activity, which likely occur in αSYN would lead to altered pupil control. Consistent with our findings, previous studies have also identified exaggerated pupil responses in αSYN, including larger pupil diameter after light adaptation in PD (Micieli et al. 1991) and larger pupil size after both light and dark adaptation in MSA (Micieli et al. 1995). However, other studies have identified conflicting results regarding pupil dysregulation in αSYN, including finding similarities in pupil baseline between PD and CTRL (Giza et al. 2011), reduced constriction amplitudes in PD, and longer latency of the light reflex (Giza et al. 2011; Micieli et al. 1991). However, we observed no differences in constriction or dilation latency

PD patients have an autonomic imbalance and are more sensitive to light (Wang et al. 2016; Micieli et al. 1991). Previous studies have also identified additional abnormal pupil behavior in MSA; for instance, they lack a bigger pupil response to stress (Armstrong 2014), the average constriction and dilation velocities were considerably slower than controls (Park et al. 2019), and larger pupil size after both light and dark adaptation in MSA (Micieli et al. 1995). The above conflicting findings are likely the result of different stimulus manipulations on the retina. The pupil responses that we observed in the free-viewing task involved stimulation of much of the retina. Additional research will be required to determine what is the optimal visual stimulus required to reveal consistent pupil deficits in these patient groups.

Part of the hypothesis of the spread of pathophysiology in αSYN includes early involvement of the LC (Braak et al. 2003), which plays a critical role in regulating pupil size (Joshi et al. 2016). It has been shown in monkeys that LC discharge is tightly correlated to pupil size; greater discharge leads to increases in pupil size, and microstimulation of LC also increases pupil size (Joshi et al. 2016). It is hard to reconcile how the loss of neurons in LC leads to increased pupil size in αSYN. However, an animal model of αSYN revealed hyperactivity of the LC (Matschke et al. 2022), which could explain the increased baseline pupil size we observed in MSA (Figure III-4).

PSP is known to have a pathophysiology in the midbrain that may impact EW and cMRF, which are near riMLF (Armstrong et al. 2007; Litvan et al. 1996). Therefore, midbrain pathophysiology may impact either neurons within EW or afferents to this nucleus in the midbrain. EW receives both excitatory and inhibitory connections from the cMRF and could conceivably produce the opposite pupil effects we observed in PSP versus MSA (Figure III-12).

III.7.4. Discrete saccadic abnormalities in RBD are pronounced in PD and MSA

We specifically included the isolated RBD patient group in our study to determine whether this prodromal αSYN group started to reveal patterns of abnormality identified in PD and MSA. Although center bias was exaggerated in PD and MSA, RBD was similar to CTRL (Figure III-5). RBD made less macro-saccades than CTRL, but more than PD and MSA (Figure III-6 A, Figure III-10 A-C). All patient groups made smaller macro-saccades than CTRL, but this effect was very modest in RBD and much stronger in PD and MSA (Figure III-8 A, C, E). Pupil responses in RBD were not predictive of changes in PD and MSA. These results reveal that RBD patients already display some saccade control deficits (macro-saccade frequency and amplitude), which are intensified in PD and MSA. Our results suggest that saccade parameters were already changing in RBD, but pupil responses were not. These altered saccade responses in RBD could represent early predictive markers of αSYN, however, more studies are required to validate these findings. However, long-term studies, particularly including subjects who phenoconvert from RBD to PD or MSA during the study, are needed to confirm these findings.

Other studies have tried to identify early abnormalities in the prodromal RBD condition (Perkins et al. 2021; Hanuška et al. 2019) that could be used as predictive cues for early diagnosis of αSYN. Perkins et al. (Perkins et al. 2021) identified attenuated pupil responses for RBD and PD patients performing an IPAST following the appearance of a central fixation spot, but this visual stimulus was a tiny spot confined to the fovea. In our study, the clip change was a substantial visual stimulus, covering the entire screen in front of the participant that presumably activated most of the retina. In this situation, RBD and PD pupil responses were not different from CTRL; however, MSA had exaggerated

responses that were significant for dilation (Figure III-12E). Additional research is required to identify whether retinal disturbances contribute to the pupil abnormalities we have reported in these disorders and whether these disturbances are uniform across the retina or are confined to specific regions of the retina (e.g., fovea vs. extrafoveal).

III.7.5. Linking eye tracking to UPDRS

A standard procedure for diagnosing Parkinson's disease includes the UPDRS (Movement Disorder Society Task Force on Rating Scales for Parkinson's Disease 2003). We found that saccade frequency, average saccade amplitude, and the magnitude of the rebound burst of saccades after the clip change were all negatively correlated to UPDRS (Figure III-13). Other studies have also identified saccade parameters that correlated with clinical scores (Waldthaler et al. 2021; Kitagawa et al. 1994). None of our pupil measures were correlated to UPDRS (Figure III-15). Pupil assessment is not part of UPDRS (Goetz et al. 2008), but may provide some unique measures that may be altered in αSYN, at least for MSA. Pupil measures may also be sensitive for distinguishing PSP from PD and MSA. Our results suggest that pupillometry may tap into additional brainstem circuits and provide additional measures of dysfunction that are not captured with the UPDRS.

III.8. Conclusions

We used a simple FV paradigm to identify oculo/pupillo-motor abnormalities in various neurodegenerative movement disorders. We identified potential prodromal biomarkers in RBD and differences between αSYN and the Tauopathy PSP, suggesting that the FV task may be a tool to identify prodromal αSYN and help to distinguish early manifest αSYN from early PSP. Future intra-individual follow-up studies are required in RBD patients to determine whether the so far observed subtle changes in oculo/pupillo-motor measures will progressively increase over time and allow the prediction of the phenoconversion of RBD into manifest αSYN. These longitudinal studies will show whether oculo/pupillo-motor parameters can reliably classify neurodegenerative movement disorders in the manifest stage, and even more challenging, during their prodromal progression towards phenoconversion.

IV. Main findings of the dissertation

The objective of this dissertation was to investigate a variety of oculo/pupillo-motor changes in prodromal (RBD) and manifest αSYN (PD and MSA) using two different oculo- pupillomotor tests (IPAST and FV). As a novelty, FV was employed for the first time ever in the RBD cohort. Each method had its advantages and disadvantages; for instance, IPAST was challenging to learn for patients, but it was able to provide a comprehensive assessment of cognitive ability (e.g., direction error). As IPAST has been utilized in a wide range of research, we were able to interpret our results fairly well and attribute them to different circuits in the brain through the use of literature. Additionally, the majority of neurodegenerative diseases are old-onset diseases, and the process of learning and performing tasks is quite frustrating for participants. In contrast, FV is a very simple and easy-to-access method that does not require any instructions and can be performed by subjects without much difficulty. We were able to measure a wide range of parameters using FV. Both IPAST and FV were able to identify significant abnormalities in the diseases, enabling us to monitor the differences between them.

Saccade frequency was already found to be reduced in RBD patients, which might be a reliable biomarker for PD and MSA. There was a greater decrease in saccades in PD, MSA, and PSP. RBD patients' saccade reaction times were unchanged in anti-saccade trials, but all other patients' reaction times were longer than CTRL.

Both IPAST and FV revealed abnormal saccade amplitudes within RBD, PD, and MSA patients. In PSP, all abnormalities were more pronounced. IPAST was able to detect pupil abnormalities in RBD, which had a defective dilation phase. In all other patient groups, pupil dilation was impaired, and it was more problematic in MSA than in RBD. As a result, pupillary changes and differences between the prodromal and manifest stages of αSYN can be revealed through pupillary examination. PD and MSA had prolonged pupil constriction times, and MSA had even longer constriction times than RBD. It was possible to distinguish Tauopathy and αSYN diseases using pupil constriction.

Here are several potential clinical significances of eye movement studies in Parkinson's disease, including:

1- Early Diagnosis: The emergence of eye movement abnormalities as a first sign of Parkinson's disease may precede other motor symptoms. Monitoring eye movements employing the structured IPAST or/and the unstructured Free Viewing paradigm could enable doctors to diagnose the disease even in the prodromal stage, but most likely in the early manifest stage. Such prodromal or early manifest diagnosis would allow for initiating symptomatic and in the future early disease-modifying therapy.
2- Tracking the progression of Parkinson's disease: As Parkinson's disease progresses from the prodromal stage, “isolated REM Sleep Behavior Disorder (iRBD)”, towards conversion into the manifest motor Parkinson’s disease, eye movement abnormalities tend to become more evident. Consequently, eye movement tests may be useful for detecting Parkinson's disease at an early stage and should be further investigated in follow-up studies in iRBD patients. Such studies will clarify whether oculomotor and/or pupillomotor dysfunction is progressive and thus could be used as a prodromal progression marker. Such prodromal progression markers are urgently needed (at present only a single such marker is consented – the dopamine transporter ligand binding (DAT-) SPECT) in alpha synucleinopathies– in order to be employed as an outcome measure in future neuroprotection trials such as in iRBD patients.
3- Differentiating Parkinson's disease from other movement disorders: Various subtypes of Parkinson's disease manifest different abnormalities in eye movement, and such studies can be useful for identifying distinct subtypes of the disease and developing appropriate treatments for them. Alone the marked difference in pupil behavior in MSA versus PSP highlights the diagnostic potential of the methods we employed.

In general terms, testing of oculomotor and pupillomotor functions is non-invasive and is performed on a relatively simple, cheap machine, does not require an expensive infrastructure such as an MRI scanner, a SPECT or PET machine, is without radiation exposure, and the patient does not have to agree to a lumbar puncture or to take a blood sample. IPAST and Free Viewing are but simple to be carried out and can be repeated many times in a given time frame.

V. Limitations and strengths

PD patients were pooled into one cohort; thus, we did not differentiate between untreated de novo PD, PD with dopaminergic treatment, and PD patients in defined OFF. Future work will be required to determine if patients in these different subgroups have different responses in IPAST or FV. The sample sizes of the MSA and PSP patient groups were rather small when compared to the sample size of the RBD and also PD patient groups. This is due to the relative scarcity of patients with MSA and PSP relative to the abundance of patients with RBD and PD.

The CTRL participants are another limitation of this dissertation. Participants in the CTRL group have all been recruited in Canada and speak English while recruited patients in Germany speak German. In other words, the verbal explanation of the study was in two different languages but identical. Despite this, all equipment and set-ups were identical at both sites. Enough training was provided to operators who collected the data. They were instructed to perform the same experiment with the same instructions.

We are indeed testing our pipeline on a broad range of neurological disorders from several locations (thanks to the Ontario Neurodegenerative Disease Research Initiative (ONDRI) project: https://ondri.ca/). These ongoing projects have shown some intriguing results so far. For example, PD and PSP patients, which are also part of these other studies, have produced the same results (unpublished) as patients described in this dissertation. This could mean that there was no bias caused by geographical differences (or even language differences) in this dissertation.

There were no biases introduced throughout the data analyses because the data were completely processed automatically.

With free viewing (FV) eye-tracking behavior, we used a novel technique, which can be applied in many more cohorts than studies investigating visually guided saccades towards defined visual targets. As a result, beyond what can be obtained from more structured paradigms, IPAST, we provided a much richer set of results in FV. Hence, a strong advantage of this study is the relatively simple design of watching videos (FV) while capturing eye movements, the large number of patients, and the healthy control group.

In order for us to demonstrate convincingly that our paradigms can help differentiate diagnosis at early stages, it will require a longitudinal study design. This is the next step in our research that we are proceeding with. In the present dissertation, we demonstrated that oculo/pupillo-motor function changes could be identified in RBD patients with the IPAST and FV paradigms. It is currently impossible to predict with certainty which RBD patients would develop PD, DLB, or MSA using neither the clinical phenotype nor any recognized prodromal biomarker (Miglis et al. 2021).

Additionally, during the covid pandemic, recruiting patients was very difficult. This delayed the analyses and was another reason for the lower number of participants in PSP and MSA groups.

VI. Future directions

In a future study, longitudinal data will be beneficial. We hope to assess the changing rate of various pupil and saccade parameters with a particular focus on RBD subjects before and after conversion from RBD to PD or MSA during the course of the planned intra-individual follow-up study. This could help to correlate the analyses with the disease severity (disease duration). Nonetheless, the present study does reveal that this approach is possible.

We intend to increase the number of MSA and PSP cases for future studies. Furthermore, we are going to collect CTRL subjects in Germany to have a consistent database. There were significant constraints and problems associated with human research during the COVID-19 pandemic, which made it difficult to recruit patients on a regular basis for follow-up studies.

This method has been used for a variety of neurological and psychiatric disorders in ongoing projects of our lab, and the next step is to compare different neuropsychiatric disorders together.

VII. Dissertation abstract

VII.1. English

Isolated rapid eye movement (REM) sleep behavior disorder (iRBD) has been identified as the most specific and common prodromal stage of α-synucleinopathies (αSYN) such as Parkinson's disease (PD), dementia with Lewy bodies (DLB), and the sporadic disease multiple system atrophy (MSA). Within 10 to 20 years, patients with this dream-sleep disorder convert in up to 85 % of cases to a neurodegenerative disease of the type of αSYN. Hence, iRBD is an ideal group for testing a disease-modifying therapy to postpone or even prevent phenoconversion. The latency, however, from diagnosis to phenoconversion is prolonged, lasting years to decades. Therefore, identifying iRBD patients more likely to phenoconvert needs highly sensitive and specific prodromal biomarkers and progression markers.

The goal of this study was to contribute to the identification of biomarkers in manifest and prodromal αSYNs for their future selection as participants in protection trials. Furthermore, comparing patients with αSYN and Tauopathy is the second objective of this dissertation, aimed at identifying the underlying differences between the two disorders.

To date, most of the biomarkers and progression markers for manifest αSYN relate to the motor and cognitive dysfunctions and imaging of the central nervous system but less to sensory and autonomic dysfunction. For iRBD, a recent review paper has summarized the state-of-the-art that confirms the above statement that most of the works in the field of biomarkers are performed on motor and cognitive functions and imaging.

Until 2022, little has been published on oculomotor and pupillomotor dysfunctions in manifest and prodromal αSYN, but rather on the Tauopathy; progressive supranuclear palsy (PSP). The methodologies for studying eye movements and pupillary responses are highly developed. They offer a high resolution and precision in time and space for measuring sensory, autonomic, motor, and cognitive functions.

Therefore, we systematically investigated the saccade, pupil, and blink behaviors in the manifest αSYN PD and MSA and their prodrome iRBD compared to healthy age and

gender-matched controls. As a ”disease control” and for comparison, we also studied patients suffering from Tauopathy PSP.

PSP is well-known for its oculomotor abnormalities, particularly for its characteristic symptom of relative vertical gaze palsy. PSP is – like MSA – another atypical parkinsonian disorder with multiple brain tissue losses, for example, in the frontal cortex. Because the early diagnosis of PD and MSA from PSP is difficult, PSP patients have been recruited for this study.

As methods, we employed a structured saccade task that is called the Interleaved Pro/ Anti Saccade Task (IPAST) and a free viewing task (FV) to investigate oculomotor and pupillomotor function along with blink behavior in αSYN and PSP.

The IPAST is a structured saccade task that requires strong cognitive control, alertness, and attention. Previous studies on the manifest αSYN have shown that patients with PD have systemic abnormalities in oculomotor, pupillometric parameters, and blink behavior in the IPAST.

In order to simplify our method and broaden our ability to collect a wide range of eye movement parameters, we additionally employed another task, the unstructured free viewing of video clips (FV). Therefore, the research question is whether oculomotor and pupillomotor abnormalities and blinking during the IPAST and FV in iRBD patients differ from healthy controls, PD, MSA, and PSP.

This study represents the first use of FV for the investigation of eye movement and pupil responses in subjects suffering from prodromal and manifest αSYN. It is also the first study comparing prodromal and manifest αSYN (PD, MSA) with PSP in FV. This dissertation has been performed in the context of the evolving disease-modifying therapy trials for manifest αSYN, which are currently ongoing in patients with Parkinson’s disease.

The next challenge will be to test these therapies in people with iRBD to slow or even prevent the full manifestation of the αSYN. It will be essential to enrich prodromal populations with biomarkers of short-term conversion and to be able to monitor disease progression with serial measurements. Developing neurodegenerative disease treatments

is becoming increasingly important as the population ages and the burden on families and society increases.

In summary, we identified potential prodromal biomarkers in iRBD and differences between αSYN and the Tauopathy PSP, suggesting that the IPAST and especially FV task may be a tool to identify prodromal αSYN and help to distinguish early manifest αSYN from early PSP. The future goal is intra-individual follow-up studies in iRBD patients to determine whether the so far observed subtle changes in oculo/pupillo-motor measures will progressively increase over time and allow the prediction of the phenoconversion of iRBD into manifest αSYN. These longitudinal studies will show whether oculo/pupillo-motor parameters can reliably distinguish the different neurodegenerative movement disorders in the manifest stages, and even more challenging, during their prodromal progression towards phenoconversion.

VII.2. German

Die isolierte Rapid-Eye-Movement-(REM)-Schlafverhaltensstörung (iRBD) wurde als das spezifischste und häufigste Prodromalstadium von α-Synucleinopathien (αSYN) wie der Parkinson-Krankheit (PK), der Demenz mit Lewy-Körperchen (DLB) und der sporadischen Erkrankung Multisystematrophie (MSA) identifiziert. Innerhalb von 10 bis 20 Jahren entwickeln Patienten mit dieser Traumschlafstörung in bis zu 85 % der Fälle eine neurodegenerative Erkrankung vom Typ der αSYN (sogenannte Phänokonversion). Daher stellen Patienten mit iRBD eine ideale Gruppe für die Untersuchung einer krankheitsmodifizierenden Therapie dar, die diese Phänokonversion verzögern oder sogar verhindern soll. Die Zeitspanne von der Diagnose bis zur Phänokonversion ist jedoch sehr lang und kann Jahre bis Jahrzehnte dauern. Zur Identifizierung von iRBD-Patienten, bei denen die Wahrscheinlichkeit einer Phänokonversion höher ist, werden daher hochempfindliche und spezifische Biomarker für das Prodromalstadium und die Messung der prodromalen Krankheitsprogression benötigt.

Ziel dieser Studie war es, einen Beitrag zur Identifizierung von Biomarkern bei manifesten und prodromalen αSYN-Patienten zu leisten, damit diese künftig als Teilnehmer an neuroprotektiven Studien ausgewählt werden können. Darüber hinaus war der Vergleich von Patienten mit αSYN und Tauopathien das zweite Ziel dieser

Dissertation, um die zugrundeliegenden Unterschiede zwischen den beiden Erkrankungen zu identifizieren.

Bisher beziehen sich die meisten Biomarker und Progressionsmarker für manifeste αSYN auf die motorischen und kognitiven Störungen und die Bildgebung des zentralen Nervensystems, aber weniger auf sensorische und autonome Funktionsstörungen. Für iRBD hat eine kürzlich erschienene Übersichtsarbeit den Stand der Forschung zusammengefasst und bestätigt, dass sich die meisten Arbeiten im Bereich der Biomarker auf motorische und kognitive Funktionen und die Bildgebung beziehen.

Bis 2022 wurden vor allem Arbeiten zu Störungen der Okulo- und Pupillomotorik bei der Tauopathie, progressive supranukleäre Blickparese (PSP), veröffentlicht. Nur wenige haben sich mit Auffälligkeiten der Okulo- und Pupillomotorik bei manifesten und prodromalen αSYN beschäftigt. Die Methoden zur Untersuchung von Augenbewegungen und Pupillenreaktionen sind hoch entwickelt. Sie bieten eine hohe zeitliche und räumliche Auflösung und Präzision für die Messung sensorischer, autonomer, motorischer und kognitiver Funktionen.

Daher untersuchten wir systematisch das Sakkaden-, Pupillen- und Blinzelverhalten bei den manifesten αSYN, PK und MSA, sowie deren Prodromalstadium, der iRBD, im Vergleich zu gesunden, altersgleichen Kontrollen. Als "Krankheitskontrolle" und zum Vergleich untersuchten wir auch Patienten, die an der Tauopathie PSP leiden.

Typisch für die PSP sind Störungen der Okulomotorik, insbesondere das charakteristische Symptom der relativen vertikalen Blickparese. Die PSP gehört - wie die MSA – zu den atypischen Parkinsonsyndromen und ist durch multiple Hirngewebsverluste, zum Beispiel im frontalen Kortex, gekennzeichnet. Da die differentialdiagnostische Abgrenzung von PK, MSA und PSP in der Frühphase schwierig ist, wurden für diese Studie auch PSP-Patienten rekrutiert.

Als Methoden verwendeten wir eine strukturierte Sakkadenaufgabe, die Interleaved Pro/Anti Saccade Task (IPAST) genannt wird, und eine „FreeViewing“-Aufgabe (FV), um die okulomotorische und pupillomotorische Funktion sowie das Blinzelverhalten bei αSYN und PSP zu untersuchen.

Die IPAST ist eine strukturierte Sakkadenaufgabe, die eine starke kognitive Kontrolle, Wachsamkeit und Aufmerksamkeit erfordert. Frühere Studien zu manifesten αSYN haben gezeigt, dass PK-Patienten systemische Störungen der Okulomotorik, der pupillometrischen Parameter und des Blinzelverhaltens im IPAST aufweisen.

Um unsere Methode zu vereinfachen und unsere Möglichkeiten zur Erfassung eines breiten Spektrums von Augenbewegungsparametern zu erweitern, haben wir zusätzlich eine weitere Aufgabe eingesetzt: das unstrukturierte freie Betrachten von Videoclips (free viewing (FV)). Die Forschungsfrage lautete daher, ob sich okulomotorische und pupillomotorische Störungen und das Blinzelverhalten während der IPAST und des FV bei iRBD-Patienten von gesunden Kontrollen, PK-, MSA- und PSP-Patienten unterscheiden.

In dieser Studie wurde zum ersten Mal die FV zur Untersuchung von Augenbewegungen und Pupillenreaktionen bei Patienten mit prodromaler und manifester αSYN eingesetzt. Es war auch die erste Studie, die prodromale und manifeste αSYN (PK, MSA) mit der PSP in FV vergleicht. Diese Dissertation wurde im Kontext der sich entwickelnden krankheitsmodifizierenden Therapiestudien für manifeste αSYN durchgeführt, die derzeit bei Patienten mit PK durchgeführt werden.

Die nächste Herausforderung wird darin bestehen, diese Therapien bei Menschen mit iRBD zu testen, um die vollständige Manifestation der αSYN zu verlangsamen oder sogar zu verhindern. Es wird von entscheidender Bedeutung sein, Biomarker für die Prodromalphase zu haben, die Risikopatienten mit einem hohen Risiko der kurzfristigen Phänokonversion identifizieren und das Fortschreiten der Krankheit mit seriellen Messungen erfassen. Die Entwicklung von krankheitsmodifizierenden Therapien für neurodegenerative Erkrankungen wird immer wichtiger, da die Bevölkerung altert und die Belastung für Familien und Gesellschaft zunimmt.

Zusammenfassend haben wir potenzielle Biomarker für das Prodromalstadium der αSYN, der iRBD, und zur Messung von Unterschieden zwischen αSYN und der Tauopathie PSP identifiziert. Unsere Ergebnisse deuten daraufhin, dass die IPAST- und insbesondere die FV-Aufgabe ein Instrument zur Identifizierung von αSYN im Prodromalstadium und zur Unterscheidung zwischen früher, manifester αSYN und früher PSP sein könnte. Das zukünftige Ziel sind intra-individuelle Verlaufsstudien bei iRBD-

Patienten, um festzustellen, ob die bisher beobachteten subtilen Veränderungen der okulo- und pupillomotorischen Messungen im Laufe der Zeit zunehmen und die Vorhersage der Phänokonversion von iRBD in manifeste αSYN ermöglichen.

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IX. Appendix

IX.1. Publications

Journal of Neurology
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ORIGINAL COMMUNICATION

Eye tracking identifies biomarkers in α-synucleinopathies versus progressive supranuclear palsy

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Abstract

Objectives This study (1) describes and compares saccade and pupil abnormalities in patients with manifest alpha-synucleinopathies (αSYN: Parkinson's disease (PD), Multiple System Atrophy (MSA)) and a tauopathy (progressive supranuclear palsy (PSP)); (2) determines whether patients with rapid-eye-movement sleep behaviour disorder (RBD), a prodromal stage of αSYN, already have abnormal responses that may indicate a risk for developing PD or MSA.

Methods Ninety (46 RBD, 27 PD, 17 MSA) patients with an αSYN, 10 PSP patients, and 132 healthy age-matched controls (CTRL) were examined with a 10-min video-based eye-tracking task (Free Viewing). Participants were free to look anywhere on the screen while saccade and pupil behaviours were measured.

Results PD, MSA, and PSP spent more time fixating the centre of the screen than CTRL. All patient groups made fewer macro-saccades (> 2° amplitude) with smaller amplitude than CTRL. Saccade frequency was greater in RBD than in other patients. Following clip change, saccades were temporarily suppressed, then rebounded at a slower pace than CTRL in all patient groups. RBD had distinct, although discrete saccade abnormalities that were more marked in PD, MSA, and even more in PSP. The vertical saccade rate was reduced in all patients and decreased most in PSP. Clip changes produced large increases or decreases in screen luminance requiring pupil constriction or dilation, respectively. PSP elicited smaller pupil constriction/dilation responses than CTRL, while MSA elicited the opposite.

Conclusion RBD patients already have discrete but less pronounced saccade abnormalities than PD and MSA patients. Vertical gaze palsy and altered pupil control differentiate PSP from αSYN.

Keywords Parkinson's disease · REM sleep behaviour disorder · Multiple system atrophy · Progressive supranuclear palsy · Eye movement · Alpha-synucleinopathy · Biomarker

Abbreviations

αSYN	Alpha-synucleinopathy
BDI	Beck's depression inventory
cMRF	Central mesencephalic reticular formation
EW	Edinger westphal nucleus
H&Y	Hoehn and Yahr scale
LC	Locus coeruleus
MoCA	Montreal cognitive assessment
MSA	Multiple system atrophy
OPN	Omnipause neurons
PD	Parkinson's disease
PDNMS	PD-non-motor-symptom scale
PPRF	Paramedian pontine reticular formation
PSP	Progressive supranuclear palsy
REM	Rapid eye movement
RBD	Isolated REM sleep behaviour disorder

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RBDSQ	REM sleep behaviour disorder screening questionnaire
riMLF	Rostral interstitial nucleus of the medial longitudinal fasciculus
SC	Superior colliculus
UPDRS	Unified Parkinson's disease rating scale

Introduction

Assessment of the oculomotor system is an essential part of the neurological examination, especially for the differential diagnosis of neurodegenerative movement disorders such as alpha-synucleinopathies (αSYN) – Parkinson's disease (PD) [1], dementia with Lewy bodies (DLB), multiple system atrophy (MSA) [2] and the tauopathy (TAU) progressive supranuclear palsy (PSP). It is often difficult to clearly differentiate PSP from αSYN early in the disease process, particularly when atypical characteristics are present [3–5]. Video-based eye tracking can reliably and objectively measure different saccade, and pupil behaviour to assess the intactness of cortical and subcortical neural circuits and, therefore, potentially confirm clinical diagnosis and improve the oculomotor assessment and accuracy. With the advent of potentially neuroprotective therapies to treat αSYN and TAU, changes of saccade and pupil behaviour components in prodromal disease stages are of significant interest and may eventually qualify as prodromal biomarkers or even progression markers.

In this respect, isolated rapid eye movement (REM) sleep behaviour disorder (RBD) is a distinct prodromal stage of the manifest αSYN: within 15 years, up to 85% of RBD patients will convert to either PD, DLB, or more rarely MSA [6]. Therefore, RBD is suitable for looking for PD, DLB, and MSA prodromal markers. In the manifest stage, early autonomic dysfunctions are the key clinical parameters in MSA that differentiate MSA from PD [2, 8]. Various studies have compared the saccadic alterations in PD and MSA [9–12]. However, a comprehensive comparative study assessing changes in saccade and pupil behaviour in the two manifest αSYNs (PD and MSA) versus the prodromal αSYN RBD has not been done.

In this study, we not only compare various αSYNs, but also contrast them to the tauopathy PSP, an atypical parkinsonian disorder that is, for example, pathologically differentiable from PD by symmetrical tissue loss in the frontal cortex [13]. PSP is, in particular, characterized by impaired oculomotor control [14–16], which is a key symptom in many PSP patients [17]. Individuals with PSP show reduced vertical saccade frequency, saccade amplitude, and saccade velocity compared to age-matched controls (CTRL) [17–20]. Because of the difficulties with the early differential diagnosis between PD, MSA, and PSP, we devised a simple video-based eye tracking task, called Free Viewing (FV), to determine whether there are reliable differences in saccade and/or pupil control in PSP versus the αSYNs [7].

Previous studies have used structured tasks to identify abnormal saccade responses in neurodegenerative diseases [21–24]. Here, we employ the simple FV paradigm in which patients are shown a series of short video clips on a computer screen, and they are free to view these clips however they choose. This approach does not allow for a detailed assessment of saccade dysmetria, but it allows for a richer assessment of saccade and pupil behaviour to be recorded in a dynamic visual setting with a high temporal and spatial resolution to reveal abnormalities. Most importantly, this setting does not require extensive preparatory instructions for the participant to perform the task. We use the FV paradigm for the investigation of oculo- and pupillomotor function in the prodromal (RBD) and manifest stages of αSYN (in this study PD and MSA) in comparison to PSP which is a tauopathy with well-known oculomotor deficits. We specifically address the following questions: (1) which saccade or pupil parameters – when captured with FV—are altered in patients with the manifest αSYN PD and MSA or the tauopathy PSP? (2) Using these parameters, does the FV paradigm allow to differentiate between patients with αSYN and PSP? (3) Are abnormal pupil and saccade responses observed in PD or MSA also detectable in the prodromal αSYN stage RBD?

Materials and methods

Participants

We included five different groups of participants. Patients diagnosed with PD, MSA, RBD, and PSP were recruited in the Department of Neurology, Philipps-University Marburg, Germany. CTRL subjects were recruited as part of a large study within the Faculty of Health Sciences at Queen's University in Kingston, Canada. The study protocol was approved by the human research ethics board of the Faculty of Medicine, Philipps-University Marburg (Protocol ID: 147/16) and the Faculty of Health Sciences, Queen's University (Protocol ID: PHYS-007-97; CNS-005-10). Voluntary informed consent was obtained from each participant after a verbal and written explanation of the study, according to the Declaration of Helsinki.

All patients recruited were 45–84 years of age (see for Exclusion criteria Supplementary Material). All patients underwent clinical testing with the Montreal Cognitive Assessment (MoCA) [25], Unified PD Rating Scale (UPDRS-III and/or Movement Disorder Society (MDS)-UPDRS scale III), Beck's Depression Inventory-II (BDI-II) [26], PD Non-Motor Scale (PDNMS) [27],

and the REM Sleep Behaviour Disorder Screening Questionnaire (RBDSQ) [28]. Clinical and demographic data are provided in Supplementary Material (Supplementary Table 1).

RBD. Forty-six patients (5 females, 41 males, age range: 50.6–76.4 years) with video-polysomnography-confirmed RBD (Darien IL, AASM, 2014) had mean UPDRS-III, MoCA, and BDI-II scores equal to 1.61, 28.2, and 7.7, respectively. All RBD patients were interviewed for a medical and drug history in detail and received a complete neurological examination. This procedure was repeated twice over a period of 1 year to reduce the risk of including subjects with secondary RBD in the study. In addition, we excluded RBD patients with cognitive impairment (MoCA < 25), and this would presumably minimize the number of patients likely to convert to DLB [29].

PD. PD patients were diagnosed according to the United Kingdom Brain Bank Criteria. Twenty-seven PD patients (2 females, 25 males, age range: 45.7–84.1 years) were included: 7 PD patients were de novo PD patients, 3 PD patients were investigated under treatment with dopaminergic medication (on-state), 14 PD patients were at least 12 h without medication (defined off-state), and three with unknown medication status. Given the relatively minor variation in saccadic behaviour between on and off states, all three groups were pooled into a single PD group, as previously reported [30]. Mean UPDRS-III, MoCA, and BDI-II scores for PD were 15.7, 27.8, and 8.4, respectively.

MSA. Seventeen MSA patients (seven females, ten males, age range: 51.6—73.8 years) were diagnosed according to the second consensus statement on the diagnosis of MSA [8]. Mean UPDRS-III, MoCA, and BDI-II scores in MSA were 27.4, 26.7, and 11.0, respectively.

PSP. Ten PSP patients (five females, five males, age range: 62.5–82.2 years) were diagnosed according to the National Institute of Neurological Disorders and Stroke and the Society for PSP (NINDS-SPSP) and Höglinger et al. [15] criteria. PSP patients showed severe motor and cognitive problems with mean UPDRS-III, MoCA, and BDI-II scores of 34.7 and 20.8, and 16.5, respectively.

Control participants (CTRL). One hundred thirty-two healthy age-matched CTRL participated in the study (86 female, 46 male, age range: 45.5–84.3 years). Age is known to influence many saccade parameters (e.g., increased saccade latency, decreased saccade frequency, decreased saccade amplitude, and velocity) [31–33]. To control for age effects, we created a separate CTRL group for each patient group. For each group, we selected CTRL that had a maximum of ± 1 year age difference with each patient (Supplementary Fig. 1). We confirmed that each control group was matched in age to its corresponding patient group. The CTRL groups, therefore, had different numbers and overlapping individuals in each group.

Eye tracking task

Participants were seated with their head resting on a chin-rest and a forehead rest so that their eyes were positioned 60 cm away from a computer screen in a dark, windowless room, with a curtain drawn between them and the operator to limit any potential distractions. Despite this, PSP patients occasionally made a backward head movement during the eye tracking. To prevent this from happening again, an experimenter used their hands to keep their head in a stable position on the chin and forehead rest. Additionally, the participants were seated in a chair which included a backrest to keep them from falling backward. Occasionally, we used a pillow to bridge the space between their neck and the backrest of the chair. We attempted to keep the amount of head motion to a minimum while collecting the data. Additionally, if participants pushed back, the eye tracker stopped recording, and the task was recalibrated. All data were collected using a video-based eye tracker (Eyelink 1000 Plus; SR Research, Mississauga, Ontario, Canada), recording monocular right pupil size and eye position at 500 Hz (Details in Supplementary).

Visual stimuli

Videos were displayed on a 17-inch LCD monitor, and all participants viewed a total of ten movies (Supplementary Fig. 2A). Each movie was approximately 1 min in duration and consisted of 15–17 video clips that were ~ 2–5 s in duration (mean = 3.76, mode = 4). We made the video clips of scenes with and without humans, animals, buildings, cars, and the clips were randomly assembled so that viewing was similar to watching television and changing the channel every few seconds. The clips were presented in a fixed sequence within each movie, but the order of the ten movies was randomized between participants. The task required no instruction; the participants simply viewed the video clips. Clip changes produced a large visual perturbation that stimulated much of the central retina, producing a large visual transient signal [34] carried to all central visual areas that altered ongoing saccade and pupil behaviour.

Saccade analysis

We divided the analyses into: (1) low-level statistics independent of video content, and (2) analyses aligned on clip changes (see Supplementary Fig. 2B). Auto-marking scripts developed in MATLAB were used to classify each trial and all eye movements (saccades, fixations, and pupil size). All saccades were marked for direction, amplitude, peak velocity, and duration [35]. We defined macro-saccades as all saccades ≥ 2° amplitude and micro-saccades [36–41] as all saccades < 2° amplitude.

The coordinates of each fixation were used to create gaze distribution maps (see Supplementary Materials for details). Centre bias, the excessive time gazing at the centre of the screen [7], was calculated for each participant and was defined as the mean ± 5° around the centre of the gaze distribution map for each participant.

We computed the frequency (saccade-count/viewing-duration) and average saccade amplitude in each of 60 different saccade directions (each bin was 6° polar angle). In subsequent analysis, we separated horizontal and vertical saccades because PSP patients have vertical gaze impairments specifically [42]. All saccades with direction ± 45° of the horizontal meridian were defined as horizontal, and all saccades ± 45° of the vertical meridian were defined as vertical.

There is a fundamental relationship between the amplitude and peak velocity of saccades known as the main sequence [43], which measures the integrity of the brainstem saccade premotor circuit [44]. We measured the amplitude and peak velocity of all saccades > 2° and plotted peak velocity as a function of log amplitude for each participant, which produces a linear relationship [43]. We then fit a linear function to the resulting data (Supplementary Fig. 3).

The clip transitions produced transient changes in saccade and pupil behaviour. We computed the macro- and micro-saccade rate (saccades/s) for each participant using a peri-stimulus time histogram (PSTH, 2 ms bin width due to the 500 Hz sample rate; see Supplementary Materials). For macro-saccades, we computed a baseline rate for each participant (−200 to + 50 ms relative to the clip change), as well as the magnitude and timing of the dip in macro-saccade rate ("saccade suppression" [45]), the peak macro-saccade rate after clip transition (maximum value from the time of suppression to 300 ms post clip change), and the steady state macro-saccade rate (averaged from 1000 to 3000 ms after clip change).

A similar set of micro-saccade parameters was extracted for each participant. Micro-saccade PSTHs were created, and we computed a baseline rate (average rate from −200 to + 50 ms relative to clip change). We computed the magnitude and timing of the suppression in micro-saccade rate in the epoch from 70 to 400 ms after clip change. We computed the steady state micro-saccade rate, which was the average over an epoch 1000–3000 ms after clip change.

Pupil analysis

We measured the mean global luminance of every frame of every movie by computing the luminance gamma functions of the red, green, and blue color gamuts at various output levels. We then used those functions to compute the luminance of every pixel in the frame and averaged across all pixels to get the mean screen luminance for that frame. We correlated the mean pupil size with the mean screen luminance (cd/m^2) across clips for each participant (Supplementary Fig. 5A). For each participant, we extracted the y-intercept and slope (Supplementary Fig. 5B and 5C).

The clip changes produced luminance changes that impacted pupil size. We measured this luminance change and ranked all clip transitions to extract the 30 clip changes with the greatest increase in luminance and the 30 clip changes with the greatest decrease in luminance to measure the impact of clip change on pupil behaviour (see Supplementary).

Finally, we tested the correlation of all eye movement parameters versus UPDRS-III scores to examine the relationship between the severity of the motor dysfunction and oculomotor and pupillometry parameters.

Statistical analysis

All statistical comparisons were performed in MATLAB using a pairwise non-parametric test, Mann–Whitney-*U*-test, to determine the significant statistics. Multiple comparisons adjustments were excluded due to the exploratory aspect of the study. We performed different statistical comparisons to address our main questions. First, we compared patients to CTRL. We consistently report the patient values followed by CTRL unless stated otherwise. We then compared across patient groups to first determine if the prodromal αSYN group RBD started to already present abnormalities which were identified in PD and MSA, and then to identify which abnormalities reliably differentiated PSP from the αSYN groups.

Results

Low-level saccade statistics

Gaze distribution maps

We first analysed the distributions of all fixations for the 10 min of FV from all participants, which produced gaze distribution maps. Patient groups (Fig. 1A, top row) and their corresponding CTRL groups (Fig. 1A, bottom row) had a strong centre bias (indicated in yellow), spending most of their time fixating on locations around screen's centre [7]. We subtracted the gaze distribution maps of CTRL groups from the patient groups to reveal the differences in the centre bias (Fig. 1B). PD, MSA and PSP groups had a significantly greater centre bias than CTRL (Fig. 1C; PD: 0.0043 average gaze/visual degree versus 0.0039, $P < 0.05$, MSA: 0.0042 versus 0.0039, $P < 0.01$, PSP: 0.0047 versus 0.0039, $P < 0.0001$). That means patient groups spent less time exploring the peripheral

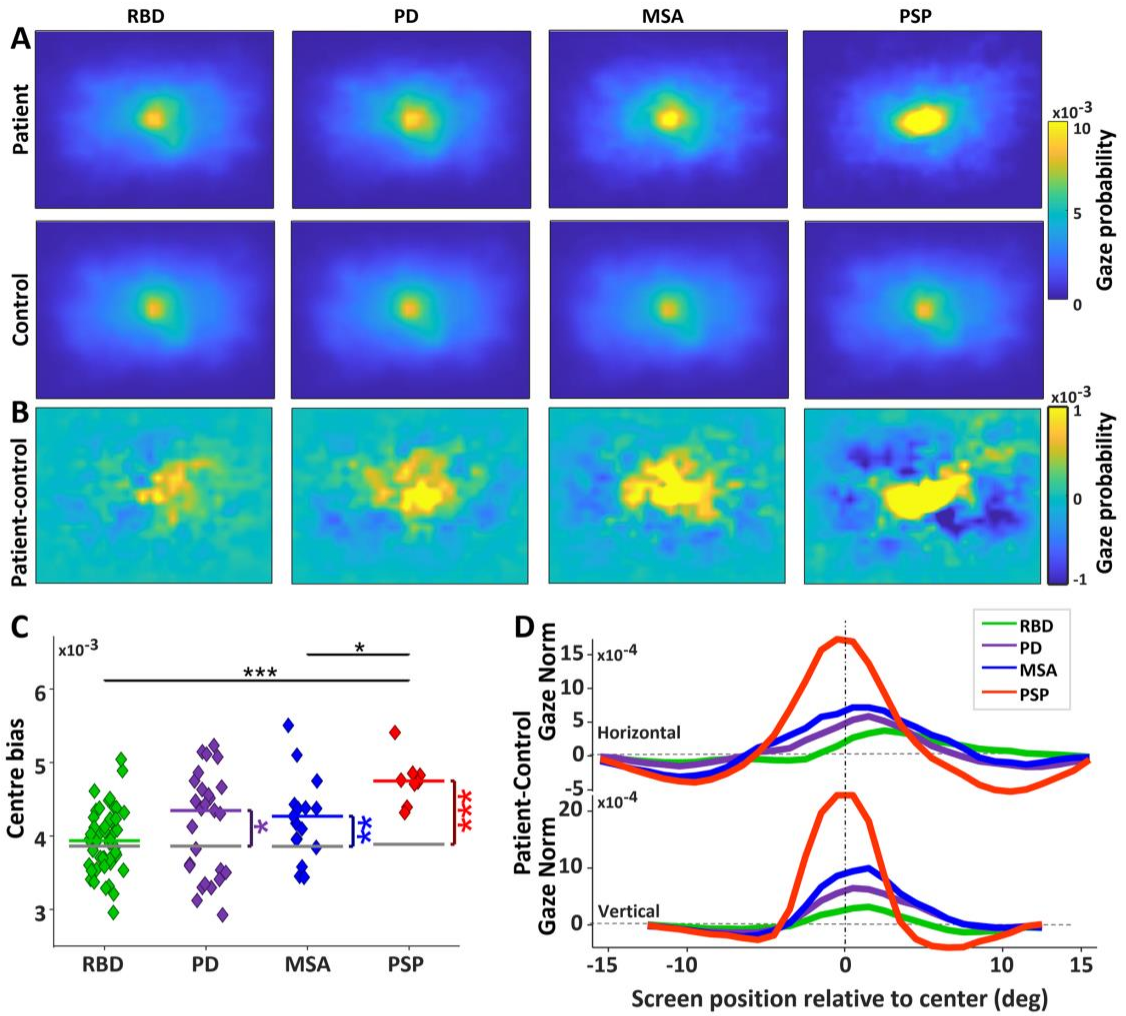

Fig. 1 Characteristics of gaze distribution. **A** Gaze distribution for each group. The screen spanned 32 deg horizontally and 26 deg vertically. Higher gaze probability is represented by yellow. **B** Difference gaze probability maps of the patients minus controls, with yellow (positive values) indicating higher gaze probability for patients than controls. **C** Individual values of centre bias, which was defined as the value at the centre of the gaze probability map in A for each participant. The gray horizontal lines indicate the CTRL group's median, and the colorful horizontal lines indicate the patient groups' median. Comparisons between the patients and CTRL were shown with vertical lines with asterisks if significant. Horizontal bares with asterisks indicate comparison between the disease groups. **D** Difference in gaze probability between each patient group and their respective control group, extracted from a slice through the horizontal and vertical meridian of the difference gaze probability maps in C (positive values indicate higher gaze probability for patients relative to controls). Asterisks show a significance level of $*P < .05$ and $**P < .01$ and $***P < .001$(same in all further figures). *RBD* REM sleep behaviour disorder, *PD* Parkinson's disease, *MSA* Multiple system atrophy, *PSP* Progressive supranuclear palsy

parts of the video clips than CTRL. We then compared the patient groups to one another. RBD and MSA had a significantly smaller centre bias than PSP (RBD versus PSP: $P < 0.001$, MSA versus PSP: $P < 0.05$). We also looked at the difference in gaze distributions between patients and controls along the horizontal and vertical meridians (Fig. 1D; patient-CTRL). The PSP group had a greater centre bias along horizontal and vertical meridians compared to all other groups.

Saccade and fixation duration distributions

We computed low-level statistics of saccade frequency, direction, and amplitude, as well as fixation durations.

For these analyses, we separated macro-saccades from micro-saccades. All patient groups made fewer macro-saccades than CTRL (Fig. 2A; RBD: 1.74 saccades/s versus 1.89, $P<0.05$; PD: 1.51 versus 1.87, $P<0.0001$; MSA: 1.49 versus 1.92, $P<0.0001$; PSP: 1.13 versus 1.94, $P<0.0001$). Among patient groups, RBD had a higher macro-saccade frequency, not only relative to PD ($P<0.05$) and MSA ($P<0.05$) but also relative to PSP ($P<0.001$). Both PD and MSA had a higher macro-saccades rate relative to PSP (both $P<0.05$). The overall micro-saccade rate (Fig. 2B) was not significantly different across groups. As a direct result of fewer macro-saccades, PSP and PD had longer fixation durations than CTRL (Fig. 2C; PD: 384 ms versus 357, $P<0.05$; PSP: 416 versus 348, $P<0.001$). PSP also had significantly longer fixation durations than RBD ($P<0.01$) and MSA ($P<0.05$).

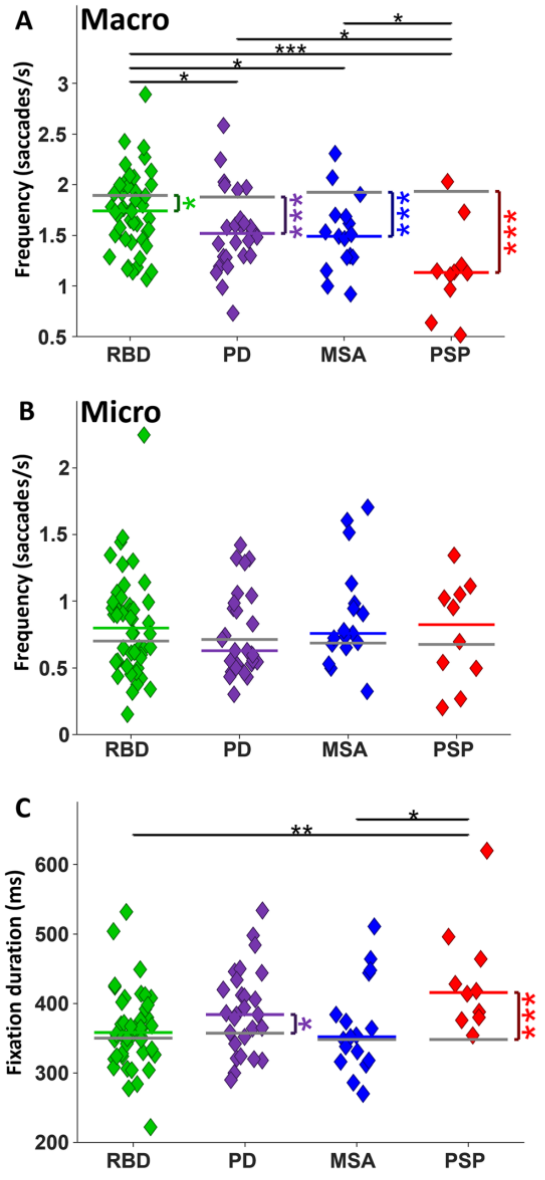

Fig. 2 Saccade frequency and median fixation duration. **A** Macro-saccade rate per second for each group. The most important finding is the difference between RBD and PD. **B** Micro-saccade rate per second. **C** Median Fixation duration of each group

Distribution of macro- and micro-saccade directions

PSP patients develop vertical gaze palsy during disease progression [46]. To determine if there were directional biases in the distribution of saccade directions, we computed the frequency of macro-and micro-saccades in 60 different directions (Fig. 3 A–B). PD, MSA, and PSP had reduced horizontal macro-saccade frequency compared to CTRL, but RBD did not differ from CTRL (Fig. 3C; RBD: 1.21 saccades/s versus 1.28, $P=0.08$; PD: 1.05 versus 1.25, $P<0.01$; MSA: 0.97 versus 1.28, $P<0.01$; PSP: 1.05 versus 1.31, $P<0.05$). Overall micro-saccade frequency in the horizontal direction did not differ between patient groups and CTRL (Fig. 3D). Vertical macro-saccades were reduced in all patient groups relative to CTRL (Fig. 3E, RBD: 0.51 saccades/s versus 0.63, $P<0.001$, PD: 0.44 versus 0.60, $P<0.0001$, MSA: 0.42 versus 0.64, $P<0.0001$, and PSP: 0.07 versus 0.62, $P<0.0001$). Comparisons among αSYN groups revealed a significant difference between RBD and MSA ($P<0.05$), while all αSYN groups had more vertical macro-saccades than PSP (all $P<0.001$). PSP displayed lower vertical micro-saccade frequency than CTRL (Fig. 3F, RBD: 0.28 saccades/s versus 0.31, $P=0.39$; PD: 0.29 versus 0.32, $P=0.28$; MSA: 0.32 versus 0.31, $P=0.77$; PSP:0.15 versus 0.26, $P<0.05$) and lower than all patient groups (all $P<0.05$).

Saccade amplitude

We determined the average saccade amplitude for each of the 60 directions (Fig. 4A–B). PSP participants made the smallest macro-saccades amplitude in all directions, followed by MSA, then PD, and finally RBD, while CTRL made the largest macro-saccades (Fig. 4A).

Horizontal macro-saccade amplitude was reduced in all patient groups compared to CTRL (Fig. 4C; RBD: 7.04 saccades/s versus 7.49, $P<0.05$, PD: 6.76 versus 7.46, $P<0.01$, MSA: 6.46 versus 7.60, $P<0.0001$, and PSP: 4.30 versus 7.52, $P<0.0001$). RBD made larger macro-saccades than MSA ($P<0.01$) and PSP ($P<0.0001$). All αSYN groups made larger horizontal macro-saccades than PSP

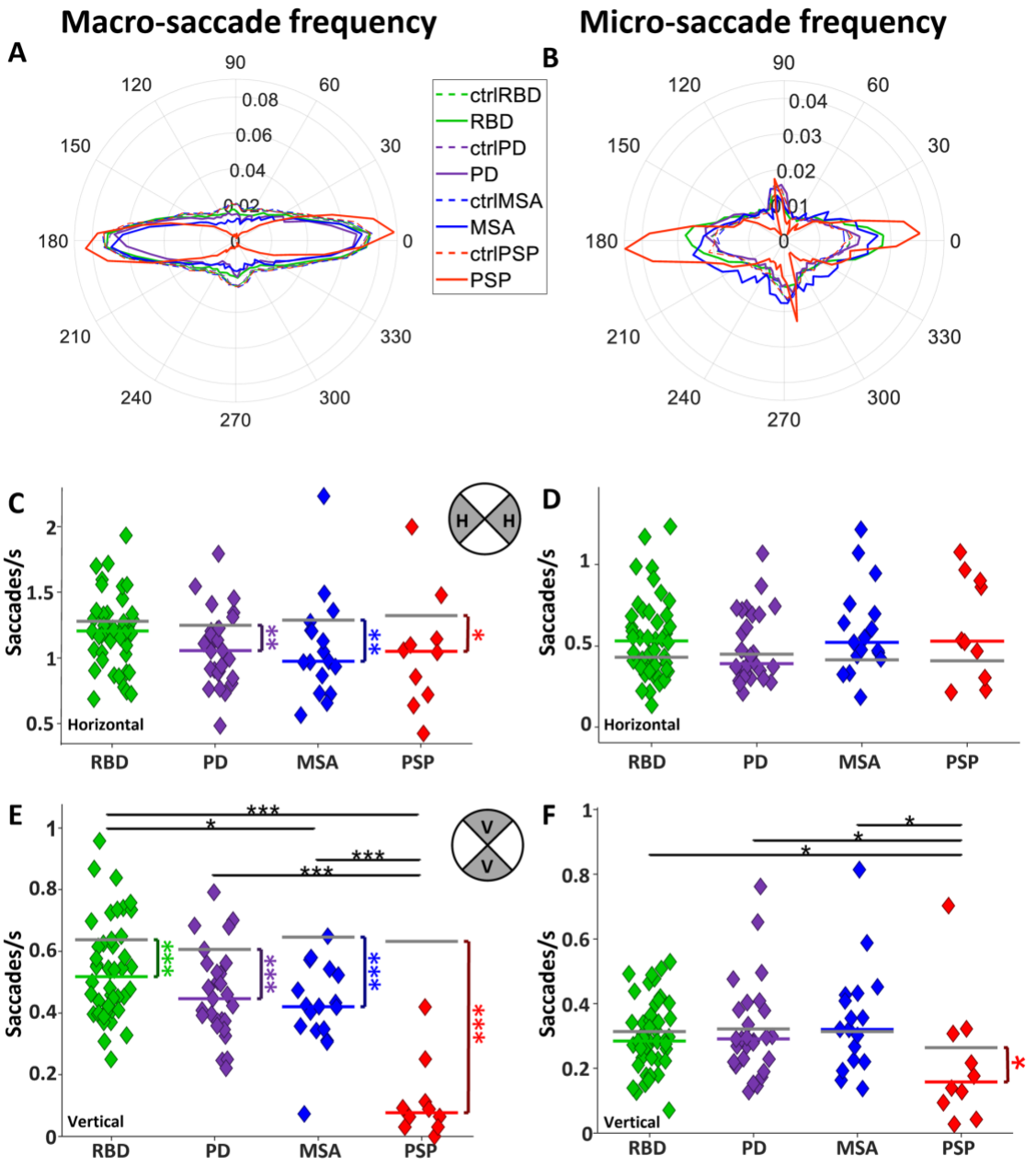

Fig. 3 Saccade rate in different directions. **A** Polar histogram of macro-saccades frequency and **B** polar histogram of micro-saccades frequency for every group. Polar coordinates are saccade directions, and each circle represents the average macro/micro-saccade frequency within each group. **C** and **D** Horizontal macro and micro-saccade frequency, respectively. **E** and **F** vertical macro and micro-saccade frequency of each individual, respectively

($P < 0.001$). Horizontal micro-saccade amplitude was significantly larger in PSP versus CTRL (Fig. 4D; 1.33 degree versus 1.2, $P < 0.01$, all other comparisons of patients to CTRL were not significant (all $P > 0.05$)). PSP had a horizontal larger micro-saccade amplitude than RBD ($P < 0.05$) and PD ($P < 0.01$).

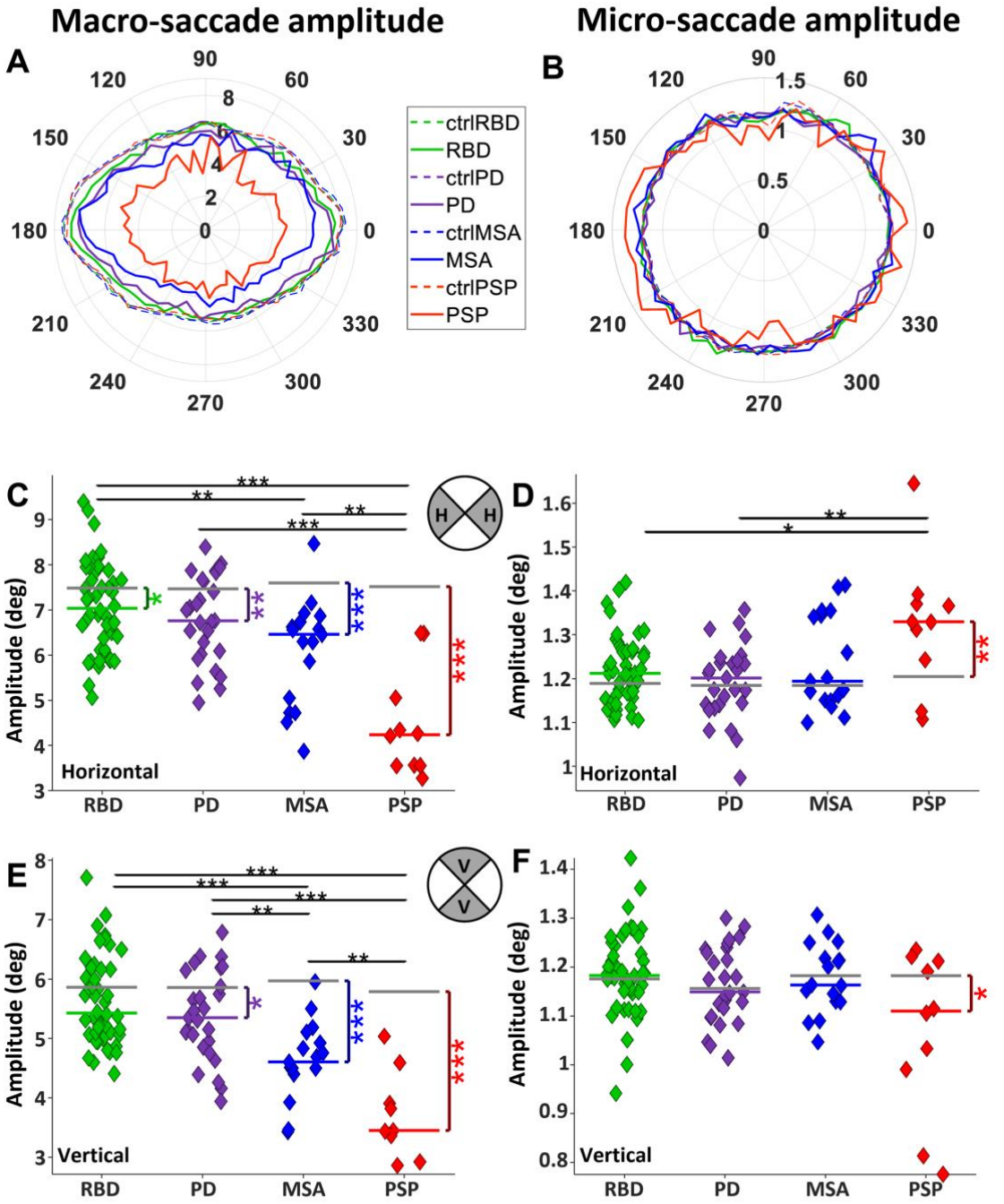

Fig. 4 Characteristic of saccade amplitude in different directions. **A** Polar histogram of macro-saccade amplitude, **B** polar histogram of micro-saccade amplitude for each group. Polar coordinates are saccade directions, and each circle represents the average saccade amplitude within each group. The bin angle was 10 degrees. **C** and **D** Horizontal macro- and micro-saccade amplitude, respectively. **E** and **F** Vertical macro- and micro-saccade amplitude, respectively

Vertical macro-saccades had reduced amplitude in PD, MSA, and PSP relative to CTRL (Fig. 4E; PD: 5.35 degree versus 5.86, $P<0.05$, MSA: 4.60 versus 5.97, $P<0.0001$, and PSP: 3.44 versus 5.82, $P<0.0001$). Comparisons of αSYN groups showed that both RBD and PD had larger vertical macro-saccade amplitude than MSA ($P<0.001$), while PSP had smaller vertical amplitude compared to all groups ($P<0.001$). PSP had a smaller vertical micro-saccade amplitude than CTRL (1.11 versus 1.18, $P<0.05$).

Saccade amplitude-velocity relationship

The average main sequence (saccade amplitude vs. velocity [43, 47]; see Supplementary Fig. 3 for single subject fit) of all groups showed that PSP patients had significantly slower saccades than CTRL and all other patient groups (Fig. 5A). The slopes of the individual participants' main sequence linear fits are shown in Fig. 5B. PSP had significantly slower saccades compared to CTRL (PSP: 118.37 degree/s versus 154.18, $P<0.01$, all other comparisons of patients to CTRL were not significant (all $P>0.05$)). PSP also had significantly slower saccades compared to RBD ($P<0.001$), PD ($P<0.001$), and MSA ($P<0.01$).

Analyses aligned on clip changes

Clip-aligned changes in saccade rate

The clip transition represents a large perturbation in visual input to the brain. We examined the results of saccade and pupil responses that were influenced by these clip changes. About 65 ms after clip change, there was a momentary suppression in macro-saccade rate, followed by a rebound that started ~ 120 ms and peaked at approximately 200–250 ms (Fig. 6A). Finally, the saccade rate returned to a steady state rate about 400–500 ms after clip change. The baseline saccade rate prior to clip change was reduced in all patient groups (Supplementary Fig. 4A), but the depth of the suppression was not different across groups (Supplementary Fig. 4B). Most importantly, although the start of the saccade rebound (120–170 ms after clip change) was similar in patients and controls, the peak of the rebound was significantly reduced in all patient groups relative to controls (Fig. 6B; RBD: 4.70 saccades/s versus 5.23, $P<0.01$, PD: 4.20 versus 5.08, $P<0.001$, MSA: 4.05 versus 5.2, $P<0.001$, and PSP: 3.70 versus 5.16, $P<0.0001$). RBD had a higher saccade peak than PD ($P<0.05$) and PSP ($P<0.001$). Because the start of the rebound was relatively normal, we interpret that all subjects were motivated and attending to the task. The average saccade rate in the epoch 1000–3000 ms (steady state) after the clip changes was reduced in all patient groups relative to CTRL (Fig. 6C; RBD: 1.57 saccades/s versus 1.71, $P<0.01$, PD: 1.36 versus 1.65, $P<0.0001$, MSA: 1.29 versus 1.73, $P<0.0001$, and PSP: 0.92 versus 1.79, $P<0.001$). RBD had a higher steady state saccade rate compared to PD ($P<0.05$), MSA ($P<0.05$), and PSP ($P<0.001$). PD also had a higher saccade rate compared to PSP ($P<0.05$). When we separated the clips for high and low luminance, we did not observe differences in saccade rate based upon luminance levels of the clips.

Micro-saccade rate was also affected by the clip change (Fig. 6D). In CTRL, the micro-saccade rate

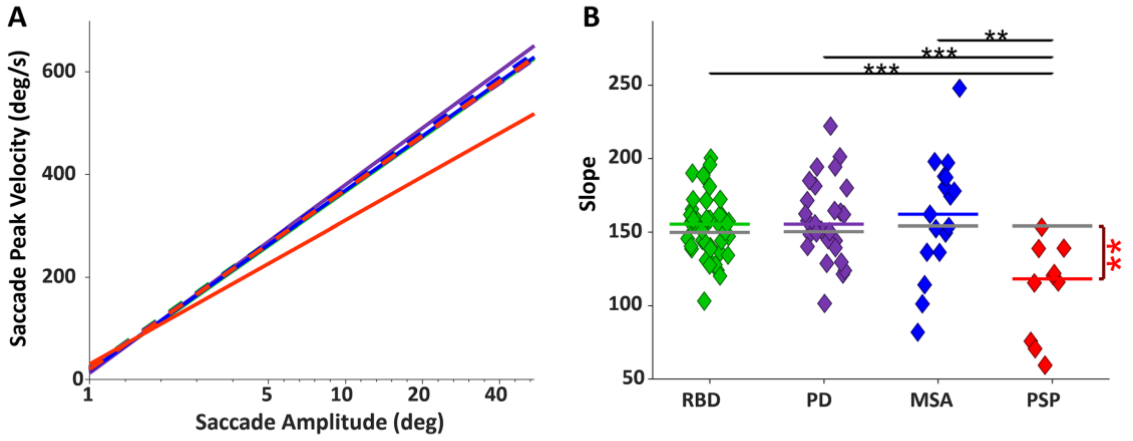

Fig. 5 Main sequence. **A** Main sequence of all patient groups along with their matched CTRL. The X-axis is amplitude on a logarithmic scale. The linear fitting line is applied over all data points of the subjects in 10 different movies in all directions. **B** Slope of the fit line for the main sequence of each individual

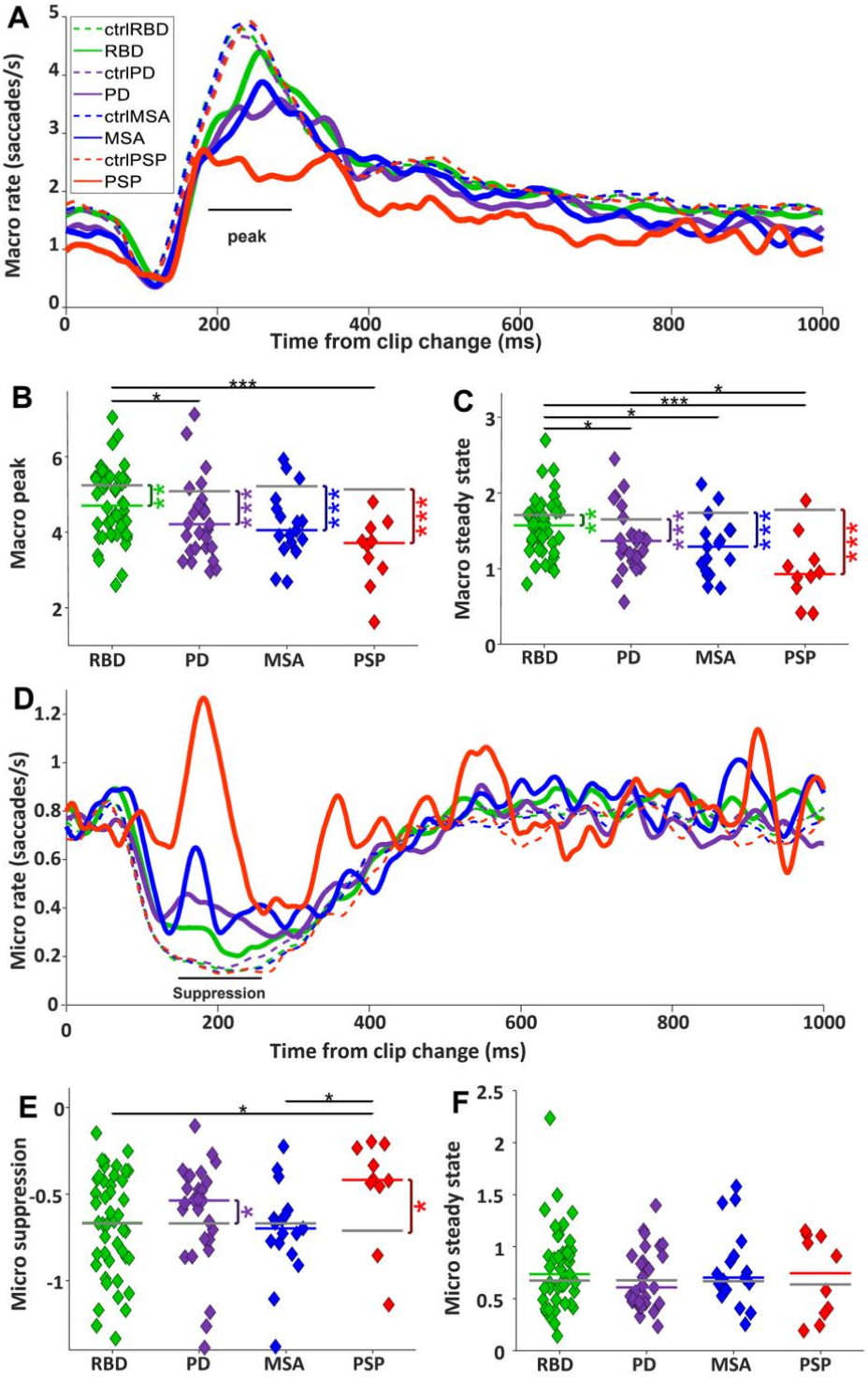
A
ctrlRBD
RBD
ctrlPD
PD
ctrlMSA
MSA
ctrlPSP
PSP
peak
Macro rate (saccades/s)
Time from clip change (ms)
B
Macro peak
C
Macro steady state
RBD
PD
MSA
PSP
D
Micro rate (saccades/s)
Suppression
Time from clip change (ms)
E
Micro suppression
F
Micro steady state

Fig. 6 Saccade rate after clip change. **A** Macro-saccade rate after clip change. The black horizontal line shows the epoch in which the average macro-saccade peak was measured. Every trace represents the mean macro-saccades of all participants in all trials. **B** Median macro-saccade peak for each participant. **C** Median macro-saccade rate in steady state (1000–3000 ms after clip change). Notably, the most critical finding in panels B and C is the distinction between RBD and PD. **D** Micro-saccade rate after clip change. Every trace represents the mean micro-saccades of all participants in all trials. The black horizontal line shows the epoch in which the micro-saccade rate suppression has been measured. **E** Median of micro-saccade suppression magnitude. **F** Median micro-saccade rate in steady state

dropped ~ 70 ms after clip change, and this suppression persisted until ~ 500 ms before returning to a steady state. The magnitude of suppression of micro-saccade rate was reduced in PD and PSP relative to CTRL (Fig. 6E; RBD: -0.67 saccades/s versus -0.67, $P = 0.54$, PD: -0.53 versus -0.67, $P < 0.05$, MSA: -0.70 versus -0.67, $P = 0.90$, and PSP: -0.42 versus -0.72, $P < 0.05$). RBD and MSA had larger suppressions than PSP (both $P < 0.05$). Steady state micro-saccade rate (1000–3000 ms after clip change) did not differ between the groups.

Clip-aligned changes in pupil size

Changes in global luminance evoke transient pupil responses [48], and the clip changes included significant luminance changes on the screen that drive changes in pupil size. For the clip changes with the 20% most significant luminance increase (Fig. 7A), a robust constriction of the pupil was initiated ~ 300 ms after clip change and peaked at ~ 800 ms, followed by a gradual increase in pupil size over the next 2 s. The absolute pupil constriction change was smaller in PSP than CTRL but failed to reach a significance level (Fig. 7B; PSP: −169.21 pixels versus −217.73, $P = 0.19$). MSA and PD had a bigger pupil constriction delta than CTRL but failed to reach a significance level (PD: −262.9 pixels versus −235.26, $P = 0.25$, MSA: −273.96 versus −212.53, $P = 0.06$). RBD was very similar to CTRL in the size of pupil constriction delta (RBD: −236.53 pixels versus −245.25, $P = 0.72$). MSA had a significantly greater pupil constriction delta than PSP ($P < 0.05$). Relative pupil size in steady state following luminance increase (Fig. 7C) was more constricted in MSA relative to CTRL (RBD: −154,16 pixels versus −168.11, $P = 0.80$, PD: −179.92 versus −153.62, $P = 0.53$, MSA: −231,52 versus −148.86, $P < 0.01$, and PSP: −116.27 versus −148.86, $P = 0.11$). In the steady state epoch, MSA had more constriction than RBD ($P < 0.05$) and PSP ($P < 0.05$).

For the clip changes with the 20% greatest decrease in global luminance, there was a robust dilation of the pupil that began ~ 400 ms after clip change, followed by an increase in pupil size until a steady state was reached at approximately 1000 ms (Fig. 7D). However, there were significant differences in the magnitude of this dilation response across groups. MSA had larger pupil dilation compared to CTRL, while PSP elicited smaller dilation than CTRL, but this was not significant (Fig. 7E, RBD: 84.59 pixels versus 88.72, $P = 0.51$, PD: 84.85 versus 88.31, $P = 0.80$, MSA: 110.36 versus 79.18, $P < 0.05$, and PSP: 62.44 versus 78.87, $P = 0.25$). Pupil dilation was larger in MSA than PSP ($P < 0.05$). Relative to CTRL, median pupil size after dilation in steady state was bigger in MSA (Fig. 7F, 129.09 pixels versus 89.23, $P < 0.05$), while it was smaller (not significant) in PSP (68.46 pixels versus 86.85, $P = 0.10$). RBD and PD displayed a similar pupil dilation with CTRL (RBD: 95.75 pixels versus 101.38, $P = 0.91$, PD: 97.75 versus 92.95, $P = 0.64$). MSA had larger pupil dilation in steady state than PSP ($P < 0.05$).

Some of these changes in the dynamics of pupil responses following luminance changes could be the result of different baseline pupil sizes in the different disorders. It is intriguing that pupil baseline size was elevated in MSA, but slightly reduced in PD and RBD (Supplementary Fig. 5). Baseline pupil size was greatly reduced in PSP, compared to CTRL and the αSYN groups.

Correlations between oculomotor and clinical assessment

A correlation analysis with the UPDRS-III scores of all patients from all groups and their saccade (Fig. 8; Supplementary Fig. 7) and pupil (Supplementary Fig. 8) parameters was performed. We also repeated the analysis without including the PSP patients to isolate the correlations for the αSYN groups. Spearman correlation revealed that macro-saccade frequency was negatively associated with the severity of motor symptoms in the combined patient group (Fig. 8A; with PSP: $\rho = -0.38$, $P < 0.001$; without PSP: $\rho = -0.31$, $P < 0.005$). Saccade amplitude was also negatively correlated with UPDRS-III (Fig. 8B, with PSP: $\rho = -0.39$, $P = 0.0002$, without PSP: $\rho = -0.33$, $P = 0.003$). The rebound in saccade rate following the clip changes was negatively correlated to UPDRS-III score (Fig. 8C, with PSP: $\rho = -0.41$, $P < 0.0001$, without PSP: $\rho = -0.36$, $P < 0.001$), as well as the steady state saccade rate 1000–3000 ms after clip change (Fig. 8D, with PSP: $\rho = -0.44$, $P < 0.001$, without PSP: $\rho = -0.37$, $P < 0.001$). Neither micro-saccade rate (Supplementary Fig. 7A) nor micro-saccade suppression magnitude (Supplementary Fig. 7B) was correlated with UPDRS-III score, either with or without PSP included. We did not identify any significant correlations between pupil parameters and UPDRS-III scores (Supplementary Fig. 8A–D).

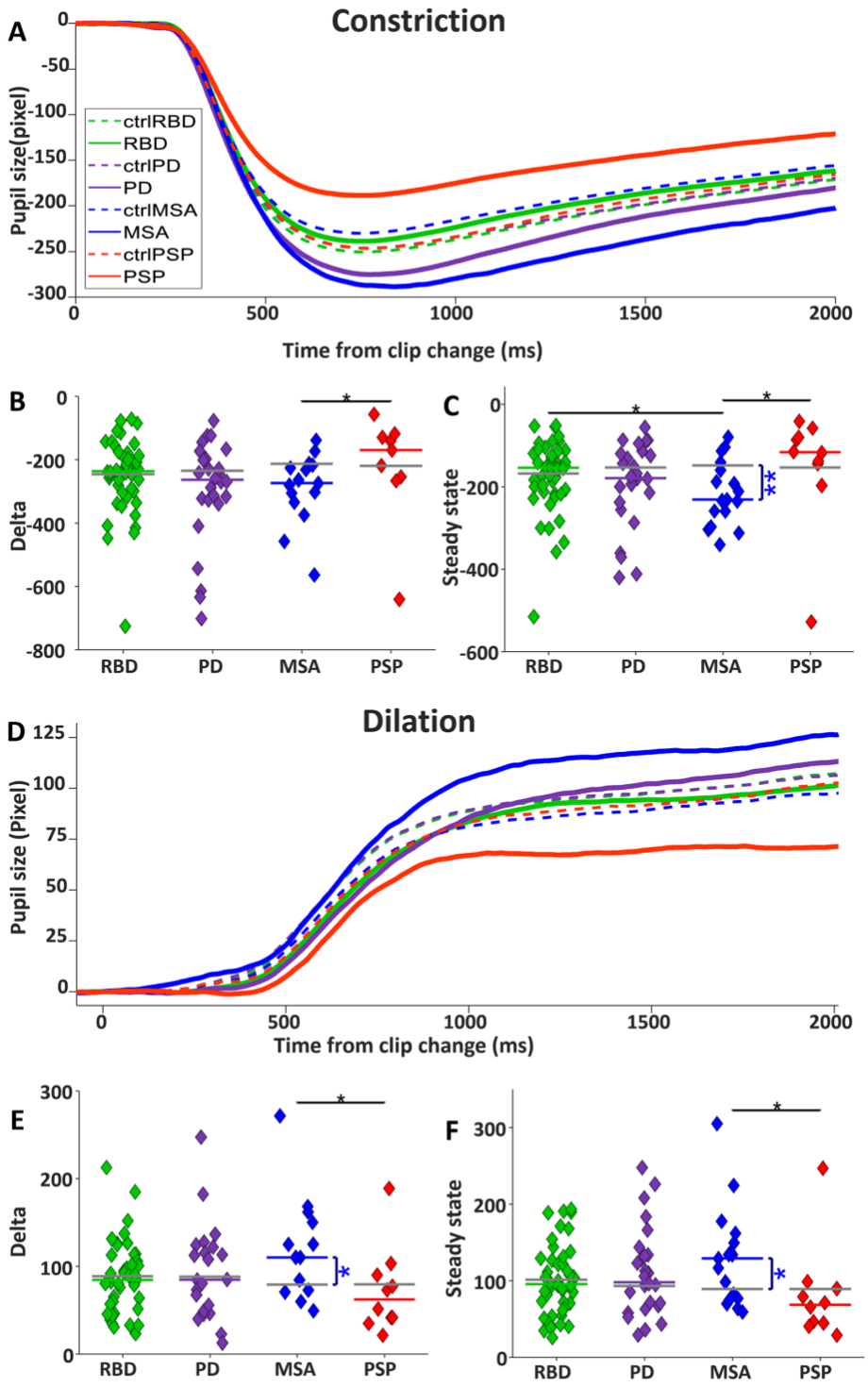
A
Constriction
Pupil size(pixel)
ctrlRBD
RBD
ctrlPD
PD
ctrlMSA
MSA
ctrlPSP
PSP
Time from clip change (ms)
B
Delta
C
Steady state
RBD
PD
MSA
PSP
D
Dilation
Pupil size (Pixel)
Time from clip change (ms)
E
Delta
F
Steady state

Fig. 7 Pupil response. **A** Pupil constriction after clip change with positive luminance change. Time zero shows the onset of the clip change. **B** Median pupil constriction Delta and **C** median pupil size in steady state for each participant. **D** Pupil dilation after clip change with negative luminance change. **E** Median pupil dilation magnitude and **F** median pupil size in steady state

Discussion

In this perspective exploratory study, we investigated parameters of oculo- and pupillomotor function in the manifest αSYN PD, MSA, and the prodromal αSYN RBD in comparison to the tauopathy PSP. We employed a Free Viewing paradigm (FV)—in combination with novel analysis methods of saccade and pupil behaviours- to study the above mentioned movement disorders. Previous studies have used visually guided saccade tasks to quantify horizontal and vertical gaze abnormalities [21, 22]. When uninstructed participants watched short video clips for only 10 min, this FV paradigm allowed us to answer the three questions lined out in the introduction as follows: (1) FV revealed qualitatively similar vertical gaze abnormalities as reported for the visually guided saccade task, but in addition, we describe several novel findings related to saccade and pupil behaviour as detailed below; (2) the behavioural results from FV differentiated between patients with αSYN and PSP –in principle in line with the results obtained with the visually guided saccade task; and (3) in the αSYN prodrome RBD, the FV paradigm allowed us to identify already discrete, but distinct saccadic abnormalities, which however are less pronounced than in PD and MSA patients.

Saccade abnormalities in neurodegenerative movement disorders

All patient groups had altered saccade behaviour during the FV task, including increased centre bias (Fig. 1) and reduced saccade amplitude and frequency (Figs. 2, 3, 4). Thus, all patients with αSYNs or PSP – to varying degrees—harvested less visual information from the peripheral visual display, and instead focused their limited resources on the centre of the screen, which would greatly reduce their ability to process the whole gist of any clip.

The clip transitions had a profound impact on saccade production (Fig. 6). Within ~ 70 ms of clip transition, the macro-saccade rate plunged to a nadir ~ 120 ms before rebounding. This initial suppression in saccade rate was the result of large changes in the visual display at clip change [45, 49] and was likely produced by visual input passing through the superior colliculus (SC) to the brainstem omnipause neurons (OPNs) [50] which gate all saccades via direct inhibition of premotor excitatory and inhibitory burst neurons [51–53] in the paramedian pontine reticular formation (PPRF) and the rostral interstitial nucleus of the medial longitudinal fasciculus (riMLF). OPNs have transient visual responses [54–56] and so the visual perturbation produced by the clip change, which is known to activate neurons in the SC [34], likely led to an increase in OPN discharge which would immediately inhibit saccade burst neurons in the riMLF and PPRF and lead to saccade suppression.

In structured oculomotor tasks, visually triggered saccades are typically initiated more than 90 ms after target appearance and can be further characterized as express saccades or regular latency saccades [32, 57]. Saccades with reaction times < 90 ms are not visually triggered [33, 58]. Analogous to the structured pro-saccade task, in FV, saccade triggered < 90 ms after clip change preceded the transient epoch of saccade suppression, and the ensuing rebound in saccade rate represents the shortest latency visually triggered saccades, which could include both express (90–140 ms) and regular (> 140 ms) latency saccades. Express saccades, the shortest latency visually triggered saccades that human can make [57], are produced when transient visual signals travelling through the SC become the saccade command [59, 60].

Following the clip transitions in the FV task, the depth of the saccade suppression and initial part of rebound was intact in all patient groups. However, the peak of the saccade rebound was significantly blunted in all patient groups (Fig. 6B), which is analogous to the time of regular latency saccades in the pro and anti-saccade tasks (SRT > 140 ms) [32]. Because the initial part of the saccade rebound was intact, we interpret this to mean that all participants were motivated and attended to the task. The reduced frequency of saccades at this time was likely the result of cognitive impairments due to neurodegeneration in cortical/basal ganglia circuits affecting or delaying key inputs to the SC [21, 23, 30, 61]. This observation which is analogous to increased latency of correct saccades among PD patients performing the anti-saccade task. In contrast, the generation of automatic visually triggered pro-saccades remained relatively unimpaired in PD [23, 30], likely because these automatic saccades are driven by visual inputs from occipital and parietal cortex to the SC, regions of the brain that are less impacted in the diseases studied here.

The FV task provided an assessment of many saccade parameters. However, we were not able to determine subtle saccade abnormalities related to dysmetria because we did not define visual targets in the video clips. The visually guided saccade task is ideal to investigate saccade dysmetria and the difference between vertical and horizontal saccades. The FV task is better for measuring ongoing and continuous saccade and micro-saccade behaviour, and pupil behaviour without having to introduce any complex instructions or task parameters.

The SC represents a competition map for the generation of saccades in a winner take all manner [62] in which only

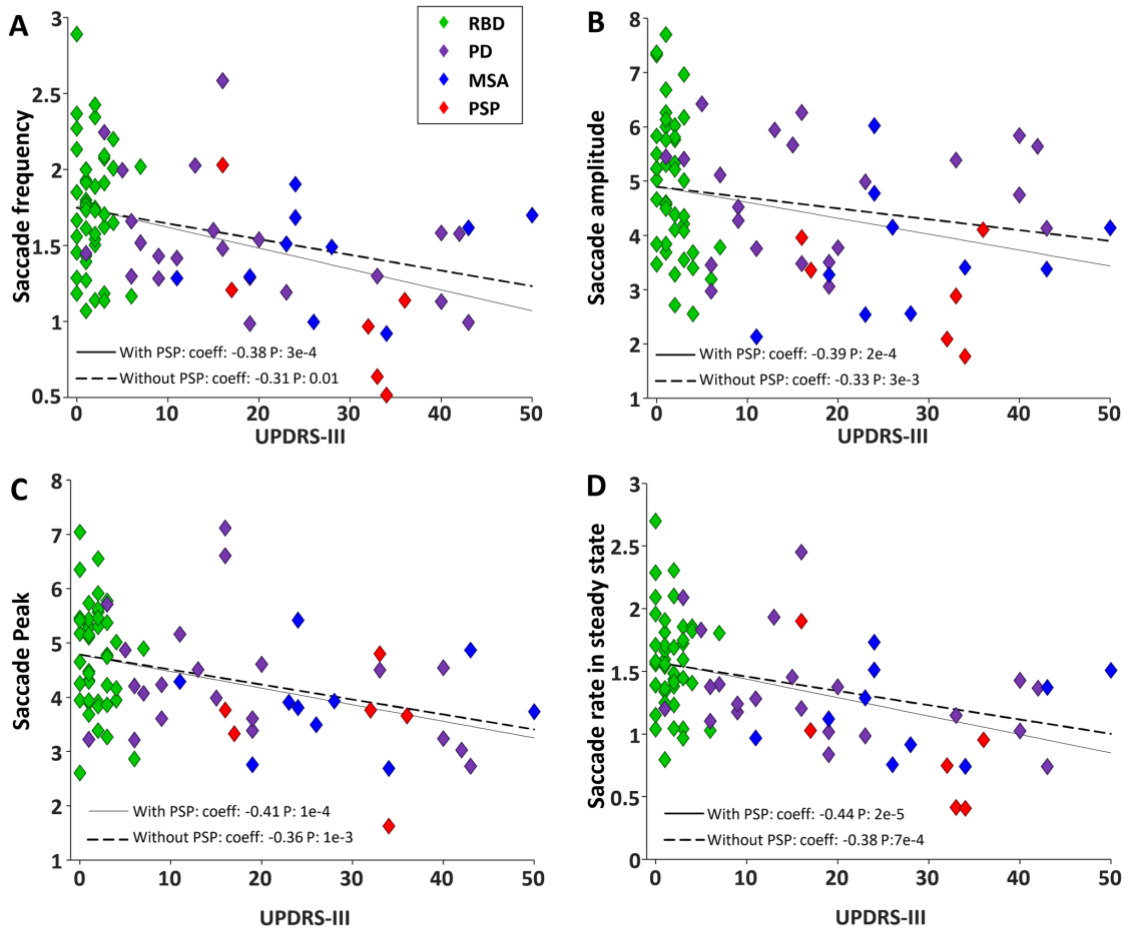

Fig. 8 Relation between UPDRS-III and saccade. **A** Negative correlation between saccade frequency and UPDRS-III score. **B** Negative correlation between saccade amplitude and UPDRS-III score. **C** Negative correlation between saccade peak and UPDRS-III score. **D** Negative correlation between saccade rate in steady state and UPDRS-III. The solid and dashed black lines show the linear fit over data including PSP and without PSP, respectively

one spatial location can issue a saccade burst at any one time. Likely due to the reduced macro-saccade rate following the clip change (Fig. 6A, B), the micro-saccade rate was less suppressed following clip change in PD and PSP (Fig. 6D, E). However, the micro-saccade steady state was not increased in the patient groups (Fig. 6F), despite the significant reductions in macro-saccade steady state in all patient groups (Fig. 2C). So this inverse relation between macro- and micro-saccade rates was not consistent across the entire clip but was most evident immediately following clip transition (< 500 ms).

Other brain disorders, such as the psychiatric disorder schizophrenia, have also been studied in terms of eye movement dysfunctions. According to a recent study, patients with schizophrenia showed fewer fixations with longer duration and smaller and lower saccades during a free visual exploration compared to CTRL [63]. Silberg et al. also showed that when patients with schizophrenia explore movies of real-life scenes, they had a strong centre bias behaviour and their gaze was independent of saliency based features of the movie [64, 65]. Schizophrenic individuals explored a smaller area of the visual scene compared to CTRL [65]. This pattern of results is similar to what we observed in all of our patient groups and may be indicative of general frontal cortex dysfunction. Whether this is a genuine feature of schizophrenia or due to antidopaminergic therapy needs to be clarified.

Vertical saccade deficits in neurodegeneration

All patients had a significant reduction in vertical saccade rate which was greatest in PSP (Fig. 3E). PD patients make hypometric saccades in vertical and horizontal directions [66, 67], but do not exhibit downward vertical gaze paresis, which is typical in PSP [18, 20, 39]. This dramatic vertical gaze palsy in PSP is likely the result of degeneration in the midbrain that impacted the riMLF. This structure houses the vertical saccade burst neurons that project directly to the pools of vertical extraocular muscle motoneurons in the oculomotor and trochlear nuclei [68]. Reduction in signals from these burst neurons in the riMLF will make it harder to initiate the vertical component of saccades, and those saccades will have a reduced amplitude and velocity. This is the pattern we observed in PSP, where it appears that these neurons were selectively damaged, leading to vertical gaze palsy. This hypothesis is supported by structural abnormalities in PSP that are known to often impact the midbrain and hence riMLF [69], which may appear small and pathologic [13].

Pupil characteristics in neurodegeneration—opposite effects in PSP versus MSA

Pupil responses were abnormal in the different patient groups, but in dramatically different ways for the PSP versus the MSA group (Fig. 7) which suggest very different actions of pathophysiology. All participants showed a very robust centre bias (Fig. 1), and pupil size is determined by global luminance. Therefore, the pupil differences we described cannot be attributed to local luminance differences based upon the location of fixation. Across the duration of the free viewing of video, pupil size for the PSP group was significantly smaller than for the MSA group (Supplementary Fig. 5). Following clip transition to darker or brighter clips, pupil dilation and constriction responses were attenuated in PSP but exaggerated in MSA (Fig. 7). Despite these large differences in the magnitude of the pupil responses between PSP and MSA, there were no differences in the onset latency of the constriction or dilation responses (Supplementary Fig. 6), suggesting that the deficits likely arise from central (i.e., brainstem) rather than peripheral (i.e., retinal), origin.

A number of factors influence pupil size in addition to luminance, such as cognitive and emotional factors, sensory saliency, and arousal [70]. The dominant luminance pathway consists of retinal input to the pretectal olivary nuclei via intrinsically photosensitive retinal ganglion cells [71]. Neurons in the pretectal olivary nucleus project directly to the Edinger Westphal nucleus (EW) [72, 73]. Many different brainstem nuclei and pathways are responsible for the non-luminance modulations of pupil size [74]. The locus coeruleus (LC) in the pons is a key structure in pupil control [75]. The discharge of LC neurons is correlated to the slow changes in pupil size that are related to arousal [75]. More recently, another non-luminance pathway has been identified through the SC [74]. The same SC neurons that project to riMLF and PPRF also collateralize into regions of the central mesencephalic reticular formation (cMRF) [76, 77], which then projects to EW [78] to influence pupil size. As a result, cognitive control signals from cortex that flow through the SC have a route to influence pupil size.

Pathophysiology of the LC has been implicated in the early stages of PD, typically at the prodromal stage II of Braak and coworkers [79]. Thus, alterations in LC activity, which likely occur in αSYN, would lead to altered pupil control. Consistent with our findings, previous studies have also identified exaggerated pupil responses in αSYN, including larger pupil diameter after light adaptation in PD [80], larger pupil size after both light and dark adaptation in MSA [81]. However, other studies have identified conflicting results regarding pupil dysregulation in αSYN, including finding similarities in pupil baseline between PD and CTRL [82], reduced constriction amplitudes in PD, and longer latency of the light reflex [80, 82]. However, we observed no differences in constriction or dilation latency (Supplementary Fig. 6). PD patients have an autonomic imbalance and are more sensitive to light [27, 83–85]. Previous studies have also identified additional abnormal pupil behaviour in MSA; for instance, they lack a bigger pupil response to stress [86, 87], the average constriction and dilation velocities were considerably slower than controls [11], and larger pupil size after both light and dark adaptation in MSA [81]. The above conflicting findings are likely the result of different stimulus manipulations on the retina. The pupil responses that we observed in the FV task involved stimulation of much of the retina. Additional research will be necessary to determine what is the optimal visual stimulus required to reveal consistent pupil deficits in these patient groups.

Part of the hypothesis of the spread of pathophysiology in αSYN includes early involvement of the LC [79], which plays a critical role in regulating pupil size concerning arousal [75]. It has been shown in monkeys that LC discharge is tightly correlated to pupil size; greater discharge leads to increases in pupil size, and microstimulation of LC also increases pupil size [88]. It is hard to reconcile how the loss of neurons in LC leads to increased pupil size in αSYN.

PSP is known to have pathophysiology in the midbrain that may impact EW and cMRF, which are near riMLF [13, 14]. Therefore, midbrain pathophysiology may impact either neurons within EW or afferents to this nucleus in the midbrain. EW receives both excitatory and inhibitory connections from the cMRF and could conceivably produce the opposite pupil effects we observed in PSP versus MSA (Fig. 7).

Discrete saccadic abnormalities in RBD are pronounced in PD and MSA

We specifically included the isolated RBD patient group in our study to determine whether this prodromal αSYN group started to reveal patterns of abnormality identified in PD and MSA. Although centre bias was exaggerated in PD and MSA, RBD was similar to CTRL (Fig. 1). RBD made less macro-saccades than CTRL, but more than PD and MSA (Fig. 2A, 6A–C). All patient groups made smaller macro-saccades than CTRL, but this effect was very modest in RBD and much stronger in PD and MSA (Fig. 3A, C, E). Pupil responses in RBD were not predictive of changes in PD and MSA. These results reveal that RBD patients already display some saccade control deficits (macro-saccade frequency and amplitude) which are intensified in PD and MSA. Our results suggest that saccade parameters were already changing in RBD, but pupil responses were not. These altered saccade responses in RBD might represent early markers of αSYN. However, long-term studies, particularly including subjects who phenoconvert from RBD to PD or MSA during the study, are needed to confirm these findings.

Other studies have tried to identify early abnormalities in oculo-pupillo-motor function in the prodromal RBD condition [6, 21, 22, 89, 90] that could be used as indicators for early diagnosis of αSYN. Perkins et al. [21] identified attenuated pupil responses for RBD and PD patients performing an interleaved pro and anti-saccade response following the appearance of a central fixation spot, but this visual stimulus was a tiny spot confined to the fovea. In our study, the clip change was a substantial visual stimulus, covering the entire screen in front of the participant that presumably activated most of the retina. In this situation, RBD and PD pupil responses were not different from CTRL, however MSA had exaggerated responses that were significant for dilation (Fig. 7E). Additional research is required to identify whether retinal disturbances contribute to the pupil abnormalities we have reported in one, but not in the other αSYN disorders and whether these disturbances are uniform across the retina or are confined to specific regions of the retina (e.g., fovea vs. extrafoveal).

Linking eye tracking to UPDRS-III

The UPDRS is part of the standard for diagnosis of PD [91]. We found that saccade frequency, average saccade amplitude, and the magnitude of the rebound burst of saccades after the clip change were all negatively correlated to motor function, assessed with the UPDRS-III (Fig. 8). Other studies have also identified saccade parameters that correlated with clinical scores [92–94]. None of our pupil measures were correlated to UPDRS-III (Supplementary Fig. 8). Pupil assessment is not part of UPDRS-III [95] but may provide some unique measures that may be altered in αSYN, at least for MSA. Pupil measures may also be sensitive for distinguishing PSP from PD and MSA. Our results suggest that pupillometry may tap into additional brainstem circuits and provide additional measures of dysfunction.

Conclusions

We used a simple FV paradigm to identify oculo-pupillo-motor abnormalities in various neurodegenerative movement disorders. We identified potential prodromal biomarkers in RBD and differences between αSYN and the tauopathy PSP, suggesting that the FV task may be a tool to identify prodromal αSYN and help to distinguish early manifest αSYN from early PSP. Future intra-individual follow-up studies are required in RBD patients to determine whether the so far observed subtle changes in oculo-pupillo-motor measures will progressively increase over time and allow the prediction of the phenoconversion of RBD into manifest αSYN. These longitudinal studies will show whether oculo-pupillo-motor parameters can reliably classify neurodegenerative movement disorders in the manifest stage, and even more challenging, during their prodromal progression towards phenoconversion.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00415-022-11136-5.

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Data availability The original data will be available upon request.

Declarations

Conflicts of interest The authors declare that they have no conflicts of interest.

Ethical approval The study protocol was approved by the human research ethics board of the Faculty of Medicine, Philipps-University Marburg.

Informed consent Voluntary informed consent was obtained from each participant after a verbal and written explanation of the study, according to the Declaration of Helsinki.

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Saccadic eye movements and pupil behavior under free-viewing condition in prodromal (RBD) and manifest PD - search for a prodromal biomarker

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Idiopathic REM sleep behavior disorder (iRBD) is considered a specific prodromal stage of Parkinson's disease (PD)[1]. Within 15 years, patients with RBD converge in up to 85% of cases to a neurodegenerative disease of the α-synucleinopathy type, the best known disease example of which is PD. Thus RBD is a suitable disease stage to search for prodromal biomarkers of PD.

PD patients suffer from impaired cognitive control based on dysfunction within the prefrontal cortex, pre-motor cortex, and basal ganglia. Less well known are the low-level visuomotor circuits of the brainstem in PD patients. It is possible that low-level saccadic and pupillary responses could be affected. We performed video-based eye tracking in a free viewing task in order to assess both high and low-level visuomotor impairment in PD patients. A saliency-based model [2] that uses visual scenes with some pre-attentive features like color, motion, luminance, and flicker to have a good estimation of the human bottom-up, involuntary saliency maps. We added feature-based maps to the model to find the correlation between CTRL, RBD and PD in observer maps. We also calculate the effects of luminance on pupillary response and saccadic behavior.

Methods:

All subjects were required to sit in a chair in front of a stabilized chin-rest. Pupil was recorded noninvasively with a video-based monocular eye tracker (Eyelink-1000 Plus, SR Research). A monitor mounted camera with a 500 Hz sampling rate was used to measure eye movements and pupil size. Clippets of short movies (3-5s duration) were presented to subjects and they were free to look anywhere on the screen. Each ~60s movie consisted of 10-12 clippets of non-relevant scenes to avoid predictability and vary low level visually features. The eye-tracker calibrated data were segmented into saccade, fixation, pupil size, and position. The scan path of every subject was gained on each clippet. Pupil constriction and dilation were analyzed separately based on the screen luminance change.

Results:

Using observer maps of CTRLs we found CTRL and RBD subjects had similar correlation in scan path but PD patients had lower correlation with CTRL. RBD and PD showed more pupil constriction after clippets changed from dark to bright condition. These findings suggests that pupil circuitry may be affected in PD early on. The observed pupillary changes may serve as biomarkers to detect the disease before motor symptoms occur.

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Do the saliency features of a scene fade over time?

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Specific components may draw attention simply by standing out from their surroundings. Numerous research has been conducted to construct a model that considers all of the variables influencing visuomotor behavior. While most of these models work well on images, they can not predict all salient things in a video. The models, in particular, fail when a predicted but unseen incoming item directs a person's attention to an area that may be vacant. We employ video-based eye tracking with instruction-free viewing of video clips to assess the gaze pattern of the control (CTRL) subjects.

We recruited 280 CTRLs from different ages (>20 years) while they sat in front of a video-based monocular eye tracker (Eyelink-1000 Plus) in a light-controlled, quiet room. A monitor-mounted camera with a 500 Hz sampling rate was used to measure eye-movements. All participants viewed 10 short videos (~1 minute), consisting of 16-17 clippets of 3-5s duration without further instructions.

We then focused on one specific clippet that included multiple faces on screen and while the camera shifted to the left side, more faces entered the scene. We assessed each subject's scan paths on the screen. Simultaneously, we used a deep-gaze model to evaluate the salient locations of each frame and compared it to the participants' actual gaze location.

The findings indicated that not only are faces the most prominent locations in a scene, but also that newly arrived faces aroused more attention than the recently presented faces. The deep-gaze model predicted all faces on the screen as salient objects but could not identify which face had priority. We indicated that time is critical for developing a gaze prediction model and that the arrival time of each feature would affect the attention. Additionally, the scene's scanned history and the positions of previously represented objects affect observation preferences.

IX.2. Tables of statistics

Using the G*power2 test, the powers of the statistics for IPAST have been provided in the table below. Power analyses have only been calculated for comparisons that showed significant differences in IPAST results. The majority of the comparisons had very high power.

POWER		RBD ↔ CTRL	PD ↔ CTRL	MSA ↔ CTRL	PSP ↔ CTRL	RBD ↔ PD	RBD ↔ MSA	RBD ↔ PSP	PD ↔ MSA	PD ↔ PSP	MSA ↔ PSP
Correct median SRT	**Pro**			0.73							
	Anti		0.72	0.76	0.80			0.68		0.45	
Express latency saccades	**Pro**		0.55	0.56							
Regular latency saccades	**Pro**		0.93	0.98			0.60				
	Anti		0.77	0.98	0.41		0.84		0.66		
ITI blink rate	**Pro**			0.81	0.98			0.91		0.94	0.73
	Anti	0.47		0.76	0.98			0.90		0.90	0.64
Fixation blink rate	**Pro**	0.27		0.68	0.94			0.75	0.56	0.88	
	Anti			0.21	0.80			0.64		0.82	0.36
Direction error viable	**Anti**		0.99	0.92	0.99	0.92	0.82	0.99		0.86	
Direction error express	**Anti**		0.76	0.76		0.47	0.58				
Direction error regular	**Anti**		0.97		0.99	0.76		0.99		0.94	0.42
Correct amplitude viable	**Pro**		0.99	0.97	0.98	0.98	0.94	0.96		0.80	
Fixation onset time	**Pro+ anti**		0.99	0.99	0.99	0.94	0.99	0.99	0.75	0.94	
Pupil peak constriction time	**Pro**		0.47	0.97			0.90				
	Anti			0.96			0.96				
Pupil dilation size at target onset	**Pro**	0.90	0.86	0.97	0.99		0.67	0.97		0.65	
	Anti	0.70	0.82	0.99	0.99		0.91	0.97	0.63	0.76	
Pupil peak dilation velocity	**Pro**	0.91	0.13	0.89	0.99			0.94		0.69	
	Anti	0.91	0.42	0.91	0.99			0.91		0.67	0.99

[2] Faul, F., Erdfelder, E., Lang, AG. et al. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior Research Methods 39, 175–191 (2007). https://doi.org/10.3758/BF03193146

Free viewing statistics have been provided in the tables below:

CENTER BIAS		U	Z	P
CTRLRBD	**RBD**	2156.000	-1.375	0.169
CTRLPD	**PD**	1041.000	-2.402	0.016
CTRLMSA	**MSA**	329.000	-3.103	0.002
CTRLPSP	**PSP**	62.000	-3.436	0.001
RBD	**PD**	500.000	-1.383	0.167
RBD	**MSA**	267.000	-1.920	0.055
RBD	**PSP**	70.000	-3.423	0.001
PD	**MSA**	227.000	-0.060	0.952
PD	**PSP**	80.000	-1.881	0.060
MSA	**PSP**	41.000	-2.209	0.027

SACCADE RATE		U	Z	P
CTRLRBD	**RBD**	1854.500	-2.556	0.011
CTRLPD	**PD**	768.000	-3.880	0.000
CTRLMSA	**MSA**	223.000	-4.170	0.000
CTRLPSP	**PSP**	46.000	-3.808	0.000
RBD	**PD**	437.000	-2.102	0.036
RBD	**MSA**	251.000	-2.168	0.030
RBD	**PSP**	72.000	-3.380	0.001
PD	**MSA**	217.000	-0.301	0.763
PD	**PSP**	62.000	-2.497	0.013
MSA	**PSP**	41.000	-2.209	0.027

MICRO SACCADE RATE		U	Z	P
CTRLRBD	**RBD**	2413.000	-0.368	0.713
CTRLPD	**PD**	1247.500	-1.285	0.199
CTRLMSA	**MSA**	596.000	-0.417	0.676
CTRLPSP	**PSP**	205.000	-0.116	0.908
RBD	**PD**	512.500	-1.240	0.215
RBD	**MSA**	324.000	-1.038	0.299
RBD	**PSP**	223.000	-0.150	0.881
PD	**MSA**	148.500	-1.952	0.051
PD	**PSP**	122.000	-0.445	0.657
MSA	**PSP**	80.000	-0.251	0.802

FIXATION DURATION		U	Z	P
CTRLRBD	**RBD**	2379.500	-0.500	0.617
CTRLPD	**PD**	1072.500	-2.232	0.026
CTRLMSA	**MSA**	636.000	-0.015	0.988
CTRLPSP	**PSP**	56.500	-3.565	0.000
RBD	**PD**	459.500	-1.846	0.065
RBD	**MSA**	378.500	-0.194	0.846
RBD	**PSP**	79.500	-3.220	0.001
PD	**MSA**	169.000	-1.459	0.145
PD	**PSP**	90.000	-1.539	0.124
MSA	**PSP**	36.000	-2.461	0.014

HORIZONTAL SACCADE RATE		U	Z	P
CTRLRBD	**RBD**	2071.500	-1.706	0.088
CTRLPD	**PD**	965.500	-2.811	0.005
CTRLMSA	**MSA**	320.500	-3.189	0.001
CTRLPSP	**PSP**	114.500	-2.217	0.027
RBD	**PD**	462.000	-1.817	0.069
RBD	**MSA**	271.000	-1.858	0.063
RBD	**PSP**	143.000	-1.861	0.063
PD	**MSA**	213.000	-0.398	0.691
PD	**PSP**	117.000	-0.616	0.538
MSA	**PSP**	78.000	-0.352	0.725

VERTICAL SACCADE RATE		U	Z	P
CTRLRBD	**RBD**	1541.000	-3.784	0.000
CTRLPD	**PD**	671.000	-4.405	0.000
CTRLMSA	**MSA**	137.500	-5.030	0.000
CTRLPSP	**PSP**	3.000	-4.806	0.000
RBD	**PD**	456.500	-1.880	0.060
RBD	**MSA**	240.500	-2.331	0.020
RBD	**PSP**	14.000	-4.621	0.000
PD	**MSA**	200.500	-0.699	0.485
PD	**PSP**	15.000	-4.104	0.000
MSA	**PSP**	12.000	-3.666	0.000

HORIZONTAL MICRO SACCADE RATE		U	Z	P
CTRLRBD	**RBD**	2111.500	-1.549	0.121
CTRLPD	**PD**	1432.500	-0.284	0.776
CTRLMSA	**MSA**	447.500	-1.911	0.056
CTRLPSP	**PSP**	170.000	-0.929	0.353
RBD	**PD**	528.500	-1.057	0.291
RBD	**MSA**	355.500	-0.550	0.583
RBD	**PSP**	217.500	-0.267	0.789
PD	**MSA**	169.000	-1.458	0.145
PD	**PSP**	108.000	-0.923	0.356
MSA	**PSP**	84.000	-0.050	0.960

VERTICAL MICRO SACCADE RATE		U	Z	P
CTRLRBD	**RBD**	2290.500	-0.848	0.396
CTRLPD	**PD**	1288.500	-1.063	0.288
CTRLMSA	**MSA**	609.000	-0.287	0.774
CTRLPSP	**PSP**	96.500	-2.636	0.008
RBD	**PD**	612.500	-0.097	0.923
RBD	**MSA**	335.000	-0.867	0.386
RBD	**PSP**	120.500	-2.343	0.019
PD	**MSA**	191.500	-0.916	0.360
PD	**PSP**	73.500	0.035	0.034
MSA	**PSP**	38.000	-2.360	0.018

HORIZONTAL SACCADE AMPLITUDE		U	Z	P
CTRLRBD	**RBD**	1877.000	-2.468	0.014
CTRLPD	**PD**	965.000	-2.814	0.005
CTRLMSA	**MSA**	186.000	-4.542	0.000
CTRLPSP	**PSP**	12.000	-4.597	0.000
RBD	**PD**	535.000	-0.983	0.326
RBD	**MSA**	207.000	-2.849	0.004
RBD	**PSP**	26.000	-4.364	0.000
PD	**MSA**	150.000	-1.916	0.055
PD	**PSP**	19.000	-3.967	0.000
MSA	**PSP**	26.000	-2.962	0.003

VERTICAL SACCADE AMPLITUDE		U	Z	P
CTRLRBD	**RBD**	2053.000	-1.778	0.075
CTRLPD	**PD**	1021.000	-2.511	0.012
CTRLMSA	**MSA**	111.000	-5.297	0.000
CTRLPSP	**PSP**	9.000	-4.447	0.000
RBD	**PD**	529.000	-1.051	0.293
RBD	**MSA**	109.000	-4.367	0.000
RBD	**PSP**	11.000	-4.459	0.000
PD	**MSA**	105.000	-3.001	0.003
PD	**PSP**	12.000	-4.000	0.000
MSA	**PSP**	27.000	-2.668	0.008

HORIZONTAL MICRO SACCADE AMPLITUDE		U	Z	P
CTRLRBD	**RBD**	2112.000	-1.547	0.122
CTRLPD	**PD**	1425.000	-0.325	0.745
CTRLMSA	**MSA**	508.000	-1.303	0.193
CTRLPSP	**PSP**	93.000	-2.717	0.007
RBD	**PD**	532.000	-1.017	0.309
RBD	**MSA**	378.000	-0.201	0.840
RBD	**PSP**	123.000	-2.289	0.022
PD	**MSA**	187.000	-1.024	0.306
PD	**PSP**	57.000	-2.668	0.008
MSA	**PSP**	62.000	-1.155	0.248

VERTICAL MICRO SACCADE AMPLITUDE		U	Z	P
CTRLRBD	**RBD**	2259.000	-0.971	0.331
CTRLPD	**PD**	1444.000	-0.222	0.824
CTRLMSA	**MSA**	591.000	-0.468	0.640
CTRLPSP	**PSP**	114.000	-2.229	0.026
RBD	**PD**	510.000	-1.268	0.205
RBD	**MSA**	360.000	-0.480	0.631
RBD	**PSP**	141.000	-1.904	0.057
PD	**MSA**	203.000	-0.639	0.523
PD	**PSP**	89.000	-1.573	0.116
MSA	**PSP**	52.000	-1.657	0.098

MAIN SEQUENCE SLOPE		U	Z	P
CTRLRBD	**RBD**	2390.000	-0.458	0.647
CTRLPD	**PD**	1261.000	-1.212	0.225
CTRLMSA	**MSA**	548.000	-0.900	0.368
CTRLPSP	**PSP**	70.000	-3.251	0.001
RBD	**PD**	557.000	-0.731	0.465
RBD	**MSA**	326.000	-1.006	0.314
RBD	**PSP**	48.000	-3.894	0.000
PD	**MSA**	212.000	-0.422	0.673
PD	**PSP**	28.000	-3.659	0.000
MSA	**PSP**	29.000	-2.812	0.005

SACCADE RATE PEAK		U	Z	P
ctrlRBD	**RBD**	1750.00	-2.965	0.003
ctrlPD	**PD**	796.000	-3.728	0.000
ctrlMSA	**MSA**	239.000	-4.009	0.000
ctrlPSP	**PSP**	44.000	-3.854	0.000
RBD	**PD**	443.000	-2.034	0.042
RBD	**MSA**	268.000	-1.905	0.057
RBD	**PSP**	73.000	-3.359	0.001
PD	**MSA**	221.000	-0.205	0.838
PD	**PSP**	83.000	-1.778	0.075
MSA	**PSP**	50.000	-1.757	0.079

SACCADE RATE IN STEADY STATE		U	Z	P
TRLRBD	**RBD**	1842.00	-2.605	0.009
CTRLPD	**PD**	794.000	-3.739	0.000
CTRLMSA	**MSA**	219.000	-4.210	0.000
CTRLPSP	**PSP**	45.000	-3.831	0.000
RBD	**PD**	419.000	-2.308	0.021
RBD	**MSA**	233.000	-2.447	0.014
RBD	**PSP**	71.000	-3.402	0.001
PD	**MSA**	202.000	-0.663	0.507
PD	**PSP**	64.000	-2.428	0.015
MSA	**PSP**	48.000	-1.858	0.063

MICRO SACCADE RATE SUPPRESSION MAGNITUDE		U	Z	P
ctrlRBD	**RBD**	2350.000	-0.615	0.539
ctrlPD	**PD**	1114.000	-2.007	0.045
ctrlMSA	**MSA**	625.000	-0.126	0.900
ctrlPSP	**PSP**	101.000	-2.531	0.011
RBD	**PD**	540.000	-0.926	0.355
RBD	**MSA**	366.000	-0.387	0.699
RBD	**PSP**	131.000	-2.118	0.034
PD	**MSA**	168.000	-1.482	0.138
PD	**PSP**	82.000	-1.813	0.070
MSA	**PSP**	44.000	-2.059	0.040

MICRO SACCADE RATE IN STEADY STATE		U	Z	P
ctrlRBD	**RBD**	2345.000	-0.635	0.526
ctrlPD	**PD**	1345.000	-0.758	0.449
ctrlMSA	**MSA**	570.000	-0.679	0.497
ctrlPSP	**PSP**	207.000	-0.070	0.944
RBD	**PD**	540.000	-0.926	0.355
RBD	**MSA**	390.000	-0.015	0.988
RBD	**PSP**	211.000	-0.406	0.684
PD	**MSA**	193.000	-0.880	0.379
PD	**PSP**	134.000	-0.034	0.973
MSA	**PSP**	75.000	-0.502	0.616

SACCADE RATE BASELINE		U	Z	P
CTRLRBD	**RBD**	1952.000	-2.174	0.030
CTRLPD	**PD**	887.000	-3.236	0.001
CTRLMSA	**MSA**	276.000	-3.637	0.000
CTRLPSP	**PSP**	54.000	-3.622	0.000
RBD	**PD**	478.000	-1.634	0.102
RBD	**MSA**	308.000	-1.285	0.199
RBD	**PSP**	77.000	-3.273	0.001
PD	**MSA**	218.000	-0.277	0.782
PD	**PSP**	63.000	-2.462	0.014
MSA	**PSP**	34.000	-2.561	0.010

SACCADE RATE SUPPRESSION MAGNITUDE		U	Z	P
ctrlRBD	RBD	2264.000	-0.952	0.341
ctrlPD	PD	1181.000	-1.645	0.100
ctrlMSA	MSA	453.000	-1.856	0.063
ctrlPSP	PSP	157.000	-1.231	0.218
RBD	PD	531.000	-1.028	0.304
RBD	MSA	356.000	-0.542	0.588
RBD	PSP	207.000	-0.492	0.623
PD	MSA	213.000	-0.398	0.691
PD	PSP	130.000	-0.171	0.864
MSA	PSP	81.000	-0.201	0.841

PUPIL CONSTRICTION DELTA		U	Z	P
ctrlRBD	RBD	2415.000	-0.360	0.719
ctrlPD	PD	1213.000	-1.138	0.255
ctrlMSA	MSA	386.000	-1.911	0.056
ctrlPSP	PSP	131.000	-1.433	0.152
RBD	PD	509.000	-1.043	0.297
RBD	MSA	269.000	-1.273	0.203
RBD	PSP	147.000	-1.365	0.172
PD	MSA	187.000	-0.217	0.829
PD	PSP	70.000	-1.774	0.076
MSA	PSP	34.000	-1.998	0.046

PUPIL CONSTRICTION IN STEADY STATE		U	Z	P
ctrlRBD	RBD	2443.000	-0.251	0.802
ctrlPD	PD	1368.000	-0.633	0.527
ctrlMSA	MSA	369.000	-2.701	0.007
ctrlPSP	PSP	135.000	-1.741	0.082
RBD	PD	563.000	-0.663	0.507
RBD	MSA	255.000	-2.106	0.035
RBD	PSP	144.000	-1.840	0.066
PD	MSA	181.000	-1.169	0.242
PD	PSP	79.000	-1.915	0.055
MSA	PSP	39.000	-2.310	0.021

PUPIL DILATION DELTA		U	Z	P
ctrlRBD	**RBD**	1858.000	-0.662	0.508
ctrlPD	**PD**	965.000	-0.261	0.794
ctrlMSA	**MSA**	263.000	-2.139	0.032
ctrlPSP	**PSP**	126.000	-1.232	0.218
RBD	**PD**	443.000	-0.416	0.677
RBD	**MSA**	179.000	-1.950	0.051
RBD	**PSP**	157.000	-1.319	0.187
PD	**MSA**	103.000	-1.366	0.172
PD	**PSP**	78.000	-1.301	0.193
MSA	**PSP**	31.000	-2.109	0.035

PUPIL DILATION IN STEADY STATE		U	Z	P
ctrlRBD	**RBD**	2477.000	-0.118	0.906
ctrlPD	**PD**	1399.000	-0.465	0.642
ctrlMSA	**MSA**	440.000	-1.987	0.047
ctrlPSP	**PSP**	128.000	-1.904	0.057
RBD	**PD**	577.000	-0.503	0.615
RBD	**MSA**	295.000	-1.487	0.137
RBD	**PSP**	143.000	-1.861	0.063
PD	**MSA**	182.000	-1.145	0.252
PD	**PSP**	81.000	-1.847	0.065
MSA	**PSP**	39.000	-2.310	0.021

IX.3. Information for participants

Philipps-Universität Marburg

Fachbereich Medizin

Klinik für Neurologie

Prof. Dr. Dr. h.c. W. H. Oertel
Hertie Senior-Forschungsprofessor

Informationen für die Studienteilnehmer

Untersuchung der Okulomotorik bei Patienten mit REM-Schlafverhaltensstörung (RBD), Parkinson-Krankheit (PK), Multisystematrophie (MSA), Progressive supranukleäre Blickparese (PSP), und gesunden Kontrollpersonen.

Verantwortliche Versuchsleiter

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Durchführender Versuchsleiter: _____

Allgemeine Informationen:
Wir bitten Sie um Teilnahme an einer Studie. Dieses Formular dient dem Zweck, Ihnen alle Informationen zu geben, die erforderlich sind, damit Sie entscheiden können, ob Sie an der Studie teilnehmen möchten oder nicht. Bitte lesen Sie sich das Formular aufmerksam durch. Sollten für Sie, nachdem Sie alles gelesen haben, nicht alle Fragen beantwortet sein, dann zögern Sie bitte nicht mit dem/der Versuchsleiter/Versuchsleiterin die restlichen Unklarheiten auszuräumen. Wenn alle Ihre Fragen beantwortet sind, können Sie sich entscheiden, ob Sie bei der Studie mitwirken möchten oder nicht. **Die Teilnahme an der Studie ist freiwillig. Sie können jederzeit und ohne Angaben von Gründen Ihre Einwilligung zur Studienteilnahme widerrufen, ohne dass Ihnen daraus Nachteile entstehen.**

Zweck des Versuchs:
Ziel der Untersuchung ist es, die Augenbewegungen bei gezielter Änderung der Blickrichtung während einer visuellen Wahrnehmungsaufgabe zu untersuchen. Die Muster der Augenbewegungen sollen bei verschiedenen Personengruppen miteinander verglichen werden. Vorausgegangene Studien deuten darauf hin, dass es bei einigen neurodegenerativen Erkrankungen (wie z.B. der Parkinson Erkrankung) zu Veränderungen der Augenbewegungen kommt. Ziel unserer Studie ist zu untersuchen, ob sich die Bewegungsmuster der Augen bei einer Änderung der Blickrichtung bei Gesunden im Vergleich zu Patienten mit einer Parkinson-Krankheit (oder einer verwandten Erkrankung) unterscheiden. Sofern die Ergebnisse systematischeUnterschiede aufzeigen, könnte man mit dieser Messmethode eine mögliche diagnostische Früherkennung für neurodegenerative Erkrankungen sowie eine schnelle, unkomplizierte und zuverlässige Methode zur Symptombeobachtung etablieren.

Philipps-Universität Marburg
Fachbereich Medizin
Klinik für Neurologie
Prof. Dr. Dr. h.c. W. H. Oertel
Hertie Senior-Forschungsprofessor

Ablauf des Versuchs:
Wir möchten Sie bitten zwei unterschiedliche Aufgaben am PC durchzuführen während wir Ihre Augenbewegungen aufzeichnen möchten. Beide Aufgaben sind weiter unten ausführlich beschrieben. Darüber hinaus möchten wir vorher gerne in einem 10-minütigen Test Ihre kognitive Leistungsfähigkeit (d.h. z.B. Konzentration und Aufmerksamkeit) untersuchen und Ihnen Fragen zu motorischen (z.B. ob und wie stark Sie zittern) und nicht-motorischen Symptomen wie z.B. Ihrer Stimmung stellen (max. 30 Minuten).

1) In der ersten Aufgabe werden wir Ihnen Punkte auf dem PC Bildschirm präsentieren, die Sie nach Errscheinen entweder direkt anschauen sollen oder in die genau entgegengesetzte Richtung blicken.
2) In der zweiten Aufgabe werden wir Ihnen unterschiedliche Videoclips zeigen (Tier-/Naturaufnahmen, Trickfilmausschnitte und kurze Sequenzen von Orten in einer kanadischen Stadt – Anmerkung: diese Methode ist in Kanada entwickelt worden.)

Ausführliche Beschreibung:
1) Während der **ersten Aufgabe** werden Ihnen verschiedene Punkte (s. Abb.1) auf dem Bildschirm gezeigt. Bei jeder einzelnen Wahrnehmungsaufgabe sehen Sie zuerst jeweils einen roten oder grünen Punkt in der Mitte des Bildschirms. Danach wird Ihnen ein grauer Punkt entweder rechts oder links am Bildschirmrand präsentiert. In Abhängigkeit von der Farbe des vorangeganenen Punktes, werden Sie gebeten, entweder den zweiten, grauen Punkt, rechts oder links mit den Augen zu folgen oder ganz bewusst in die entgegengesetzte Richtung zu blicken (siehe Abb 1). Wir werden Sie bitten, diese Wahrnehmungsaufgaben mehrfach hintereinander wiederholen.

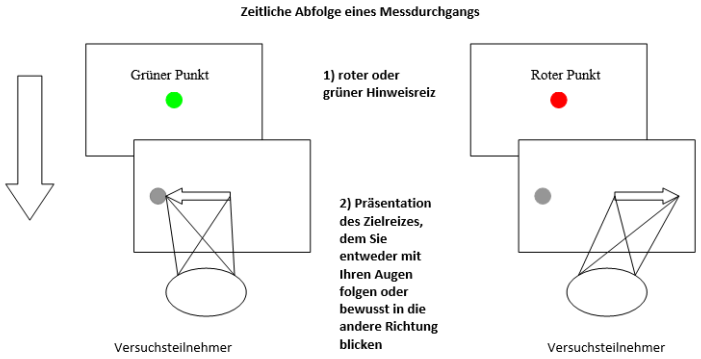

Abb. 1: grafische Darstellung der Änderung der Blickrichtungen während der visuellen Wahrnehmungsaufgabe

Vor und gelegentlich während des Versuches werden Sie gebeten, einzelne Punkte mit den Augen anzuschauen (zu "fixieren"). Diese Fixationen dienen der genauen Anpassung des Augenbewegungsmessgerätes (Kalibrierung).
Für diese Untersuchung ist es notwendig, die Farben „grün“ und „rot“ gut unterscheiden zu können. Manche Menschen leiden unter einer Farbfehlsichtigkeit wie z.B. der Rot-Grün-Schwäche. Diese Menschen haben Schwierigkeiten Unterschiede zwischen diesen Farben wahrzunehmen. Nicht immer wissen Personen, ob Sie unter einer solchen Farbfehlsichtigkeit leiden. Daher würden wir bei Ihnen vor Beginn der experimentellen Untersuchung einen kurzen Test zur Prüfung des Farbensinns durchführen (ca. 5 Minuten), um sicherzugehen, dass Sie die Aufgabe gut durchführen können. Auf

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den verwendeten Farbtafeln befinden sich runde Farbflecken, die in unterschiedlichen Farbnuancen und Größen angeordnet sind. Personen ohne Farbfehlsichtigkeit können darauf Zahlen oder Buchstaben lesen, während Personen mit einer Farbfehlsichtigkeit (z.B. Rot-Grün-Schwäche) große Mühe damit haben oder dies überhaupt nicht gelingt. Wir möchten Sie darauf aufmerksam machen, dass dieser Farbsehtest keinesfalls eine Diagnose durch einen Facharzt für Augenheilkunde ersetzt.

2) Bei der **zweiten Aufgabe** werden wir Sie bitten kurze Videoclips anzuschauen. Sie werden kurze Ausschnitte aus Tier-/Naturaufnahmen, Trickfilmen und unterschiedlichen Orten in einer kanadischen Stadt (Beispiele entnehmen Sie bitte Abb. 2a, b und c) sehen. Hier möchten wir Sie bitten diese einfach anzuschauen, ohne weiteren Anweisungen folgen zu müssen.

a)  b)  c) 

Abb. 2: Beispiele von Videoclips mit **a)** Tier-/Naturaufnahmen, b) Trickfilmsequenzen und c) unterschiedlichen Orten in und um einer kanadischen Stadt

Versuchsdauer:
Ein einzelner Messabschnitt mit beiden Aufgaben dauert in der Regel insgesamt 35 Minuten, keinesfalls länger als 45 Minuten. Die eigentliche Messzeit beträgt zwei Mal sieben Minuten (für die erste Aufgabe) und einmal 10 Minuten (für die zweite Aufgabe).

Erfasste Daten:
Zunächst werden einige Daten zu Ihrer Person mit Hilfe des beigefügten Fragebogens erfasst. Während des Versuchs werden Ihre Augenbewegungen mittels eines dafür zugelassenen Gerätes gemessen, sowie deren Zeitpunkte erfasst. Weiterhin werden Ihre Augenposition und der Durchmesser Ihrer Pupille digital aufgezeichnet. Dieses Gerät (s.g. Eye-Tracking-System) beleuchtet Ihr Auge mit unsichtbarem, infrarotem Licht und erfasst auf diese Weise die Position Ihrer Pupille, Ihrer Augen sowie die Größe Ihrer Pupille (siehe Abb. 3). Die Untersuchung ist nicht schmerzhaft und wird in der Regel auch nicht als unangenehm empfunden.

 

Abb. 3: Messgerät zur Aufzeichung von Augenbewegungen

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Datenspeicherung und Datenschutz:
Alle Daten werden unter Einhaltung des Datenschutzgesetzes erhoben, gespeichert und verarbeitet. Zugriff auf die Daten haben nur unmittelbar mit der Untersuchung befasste Personen. Die Speicherung und Auswertung der Messdaten erfolgt in elektronischer Form. Hierbei wird Ihren Messdaten eine Nummer zugeordnet („Pseudonymisierung"). Die Zuordnung zwischen dieser Nummer („Pseudonym") und personenbezogenen Daten erfolgt ausschließlich in Papierform in einfacher Ausfertigung; die zugehörigen Dokumente werden in verschlossenen Schränken, zu denen nur die Versuchsleiter Zugang haben, verwahrt. Daten auf elektronischen Speichermedien werden durch Passwortschutz vor unerlaubtem Zugriff geschützt; die Speichermedien selbst werden in verschlossenen, nur dem oben genannten Personenkreis zugänglichen, Räumen aufbewahrt. Gemäß gesetzlichen Regelungen werden alle Daten zehn Jahre aufbewahrt und anschließend vernichtet. Alle Untersucher/innen sowie die anderen genannten mit der Verarbeitung der Daten betrauten Personen unterliegen der Verschwiegenheitsverpflichtung nach §40 Bundesdatenschutzgesetz.

Mögliche Risiken, Stress oder Unannehmlichkeiten:
Mit diesem Versuch sind keine uns bekannten Risiken verbunden. Sie könnten es eventuell als unangenehm empfinden, über den Untersuchungszeitraum relativ still im Stuhl zu sitzen und auf den Bildschirm zu schauen. Sie haben aber jederzeit die Möglichkeit, den/die Versuchsleiter/Versuchsleiterin deswegen anzusprechen und eine Pause einzulegen oder den Versuch abzubrechen. Hierdurch entstehen Ihnen keinerlei Nachteile.

Nutzen des Versuches:
Für Sie wird aus der Versuchsdurchführung kein weiterer Nutzen resultieren. Wir erhoffen uns aber aus den Ergebnissen Rückschlüsse auf die Funktionsweise des menschlichen Gehirnes.

Deklaration von Helsinki:
Der Versuch steht in Einklang mit den in der Deklaration von Helsinki niedergelegten ethischen Standards für die Forschung an Menschen.

Im Falle von Rückfragen, können Sie jederzeit mit uns Kontakt aufnehmen. Sie erreichen uns unter o.g. Adresse, unter Telefon 06421-5863798 oder via Email an Habibi@staff.uni-marburg.de

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Fragebogen für StudienteilnehmerInnen

Sehr geehrte Studienteilnehmerin, sehr geehrter Studienteilnehmer,

wir freuen uns über Ihr Interesse, an unserer Untersuchung zur Augenbewegungen und Pupillenerweiterung teilzunehmen. Um sicherzustellen, dass Sie als Teilnehmer für die Studie geeignet sind, bitten wir Sie, auch in Ihrem Interesse, die folgenden Fragen wahrheitsgemäß zu beantworten. Sollten Sie sich bei der Beantwortung unsicher sein, zögern Sie nicht, die durchführende Person zu kontaktieren.
Ihre Ansprechperson ist _____

Vor- und Nachname: _____

Geburtsdatum: _____

Geschlecht: □ männlich □ weiblich

Leiden oder litten Sie an einer der folgenden Erkrankungen oder Fehlsichtigkeiten (Zutreffendes bitte ankreuzen)

□ Katarakt („Grauer Star“)
□ Glaukom („Grüner Star“)
□ Strabismus („Schielen“)
□ Myopie („Kurzsichtigkeit“)
□ Hyperopie („Weitsichtigkeit“)
□ Presbyopie („Altersweitsichtigkeit“)

Falls Sie an einer dieser Fehlsichtigkeiten leiden, wie stark ist diese ausgeprägt („Dioptrien-Wert“)? _____

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Leiden Sie an einer Farb-Fehlsichtigkeit (rot/grün-Schwäche)? ☐ ja ☐ nein

Wurden Sie bereits an den Augen / an einem Auge operiert: ☐ ja ☐ nein

Haben Sie eine andere Augenerkrankung? ☐ ja ☐ nein

Wenn ja, welche? _____

Nehmen Sie regelmäßig Medikamente ein? Wenn ja, welche?

_____

Benutzen Sie regelmäßig Augentropfen? Wenn ja, welche?

_____

Haben Sie sonstige Erkrankungen? Wenn ja, welche?

_____

Bitte noch auf der nächsten Seite die Einverständniserklärung unterschreiben!

→

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Hertie Senior-Forschungsprofessor

Einverständniserklärung

Untersuchung von Augenbewegungen bei Patienten mit REM-Schlafverhaltensstörung (RBD), Parkinson-Krankheit (PK), Multisystematrophie (MSA) Progressive supranukleäre Blickparese (PSP), und gesunden Kontrollpersonen.

Ich habe die Probandeninformation über Ziel und Ablauf der Untersuchung sowie studienbedingte Erfordernisse und mögliche Nebenwirkungen erhalten, gründlich durchgelesen und verstanden. Ich hatte ausreichend Gelegenheit, mich bei dem/der Versuchsleiter/Versuchsleiterin über den Untersuchungshergang zu informieren, sowie auftretende Fragen zu stellen. Diese wurden mir von dem/der Versuchsleiter/Versuchsleiterin verständlich beantwortet. Eine Kopie der Probandeninformation habe ich erhalten. Ich hatte ausreichend Zeit, mich für oder gegen eine Teilnahme an dieser Studie zu entscheiden. Mit meiner Unterschrift erkläre ich, dass ich das Vorhaben und die Information verstanden habe und freiwillig an der Studie teilnehme. Ich habe verstanden, dass ich jederzeit ohne Angabe von Gründen aus der Studie ausscheiden kann, ohne dass mir persönliche Nachteile entstehen. Auch der Versuchsleiter kann die Studie jederzeit beenden. Mir ist bekannt, dass diese Studie in erster Linie der Wissenserweiterung dient und gegebenenfalls keinen persönlichen Vorteil für mich bringen kann. Ich erkläre mich damit einverstanden, dass meine Daten unter Einhaltung des Datenschutzgesetzes erhoben, gespeichert und verarbeitet werden. Ich bin darüber informiert, dass alle Untersucher/innen der Verschwiegenheitsverpflichtung nach §40 Bundesdatenschutzgesetz unterliegen und die Speicherung und Auswertung meiner studienbezogenen Daten nach gesetzlichen Bestimmungen in anonymisierter oder pseudonymisierter Form erfolgt.

Studienteilnehmer:

_____

Datum Name in Blockschrift Unterschrift

Versuchsleiter/Versuchsleiterin:

_____

Datum Name in Blockschrift Unterschrift

IX.4. Consent forms

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Fachbereich Medizin

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Prof. Dr. Dr. h.c. W. H. Oertel
Hertie Senior-Forschungsprofessor

Einverständniserklärung

Untersuchung von Blicksakkaden bei Patienten mit REM-Schlafverhaltensstörung (RBD), Parkinson-Krankheit (PK), Multisystematrophie (MSA), und progressive supranukleäre Blickparese (PSP).

Ich habe die Probandeninformation über Ziel und Ablauf der Untersuchung sowie studienbedingte Erfordernisse und mögliche Nebenwirkungen erhalten, gründlich durchgelesen und verstanden. Ich hatte ausreichend Gelegenheit, mich bei dem/der Versuchsleiter/Versuchsleiterin über den Untersuchungshergang zu informieren, sowie auftretende Fragen zu stellen. Diese wurden mir von dem/der Versuchsleiter/Versuchsleiterin verständlich beantwortet. Eine Kopie der Probandeninformation habe ich erhalten. Ich hatte ausreichend Zeit, mich für oder gegen eine Teilnahme an dieser Studie zu entscheiden. Mit meiner Unterschrift erkläre ich, dass ich das Vorhaben und die Information verstanden habe und freiwillig an der Studie teilnehme. Ich habe verstanden, dass ich jederzeit ohne Angabe von Gründen aus der Studie ausscheiden kann, ohne dass mir persönliche Nachteile entstehen. Auch der Versuchsleiter kann die Studie jederzeit beenden. Mir ist bekannt, dass diese Studie in erster Linie der Wissenserweiterung dient und gegebenenfalls keinen persönlichen Vorteil für mich bringen kann. Ich erkläre mich damit einverstanden, dass meine Daten unter Einhaltung des Datenschutzgesetzes erhoben, gespeichert und verarbeitet werden. Ich bin darüber informiert, dass alle Untersucher/innen der Verschwiegenheitsverpflichtung nach §40 Bundesdatenschutzgesetz unterliegen und die Speicherung und Auswertung meiner studienbezogenen Daten nach gesetzlichen Bestimmungen in anonymisierter oder pseudonymisierter Form erfolgt.

Studienteilnehmer:

_____	_____	_____
Datum	Name in Blockschrift	Unterschrift

Versuchsleiter/Versuchsleiterin:

_____	_____	_____
Datum	Name in Blockschrift	Unterschrift

centre for neuroscience studies AT QUEEN'S UNIVERSITY

http://neuroscience.queensu.ca

Queen's UNIVERSITY

EYE MOVEMENTS IN HUMANS WITH FRONTAL LOBE/BASAL GANGLIA DYSFUNCTION
CONSENT FORM

You are being invited to participate in a research project directed by Dr. Douglas Munoz of the Centre for Neuroscience Studies at Queen's University. The aim of this work is to determine how people move their eyes to explore the visual environment and how these eye movements may be altered in people with a variety of neurological or psychiatric diagnoses. Each session takes up to three and a half hours to complete. You may be invited to participate in additional experiments on separate days. You will receive compensation at the rate of $20/hour or gift card equivalent for participating. You can refuse to participate in any part of the experiments. You may also terminate your involvement in these experiments at any time.

Eye movements will be measured using a video-based eye-tracker, a simple camera that tracks eye movements. You will be placed in front of a computer screen and look at dots or video clips that are displayed on the screen. This is a non-invasive technique for recording eye movements and produces no discomfort. You may be asked to complete a short computer task about social cognition (the way we feel, think and deal with social situations). You may be invited to have your heart rate measured using a chest band with an embedded heart rate sensor. Gel will be applied to the sensors before placement to enhance the signal. You may also be invited to measure galvanic skin response using sticky tab electrodes paired with a galvanic skin response sensor. You may be invited to complete an interview, personal history form, questionnaires and/or a short cognitive test to assess things like your current medications, your medical history, mental health, memory, verbal skills, and attention. These assessments have no diagnostic purposes, but will allow researchers to determine how behaviour, cognition or medication relates to eye movement measures.
You may also be invited to provide two buccal swabs (Q-tip swab inside cheek) or saliva (2ml) for genetic analysis.

The purpose of collecting genetic material is to allow researchers to investigate how genetic factors (i.e., variations in genes underlying neurotransmission) may relate to various measurements (metrics) of eye movements. *The data only describe a limited set of gene variation. The data does not have any diagnostic purposes or personal identification (we can't/won't identify family history), and therefore will not be reported back to you, the participant or any other entity.* Genetic material will be collected in a tube, marked with your participant ID number and held indefinitely at a Queen's University affiliated genetics laboratory for long-term storage. Genetics materials may be linked to other portions of this study and will be used to gain insight into the contribution of genetics to behaviour in the eye movement tasks. If you are a First Nations or an indigenous person who has contact with spiritual 'Elders', you may want to talk to them before you make a decision about this research study. Elders may have concerns about some research procedures including genetic testing. You have the right to request withdrawal of your genetic material from this study.

All information obtained during the course of this study is strictly confidential and your anonymity will be protected. Paper files associated with a participant's name will be locked and access will be restricted to the study team. Electronic data will be anonymized and stored in a secure database system indefinitely. Access to the database linking identifiable information to participant ID numbers will be restricted to the study team. In any publication of this work, participants will only be referred to by an arbitrary number. There is the risk of loss of confidentiality for you only if during any assessment you (a) disclose involvement in the abuse of children or elderly individuals, (b) disclose being the victim of abuse (if under age 16) or (c) disclose threat to seriously harm yourself or others. If current abuse or severe neglect is disclosed, the research team member will inform Child and Family Services and your treatment provider. The Research Ethics Board may review the study files to ensure the ethical conduct of the research.

Your participation in this study is voluntary. You may decline to participate in any aspect of this study without penalty (impact on health care access or academics) or loss of compensation. You may withdraw from this study at any time. If you wish to withdraw any data from the study, please email Dr. Douglas Munoz at doug.munoz@queensu.ca. The researcher may terminate the study without your consent if they feel you are unfit to continue.

CENTRE FOR NEUROSCIENCE STUDIES
AT QUEEN'S UNIVERSITY

Botterell Hall • 18 Stuart Street
Queen's University • Kingston, ON • K7L 3N6

PHONE 613-533-6360 FAX 613-533-6840
WEB http://neuroscience.queensu.ca

There is a remote possibility that during your research activities you could come into contact with someone with COVID-19. If this highly unlikely event were to occur, we are required by the Public Health Unit to retain on file your email address or phone number to share with them for contact tracing purposes. There are no risks involved in participating in this study. There are no benefits to the subject involved but future patients may be helped through the results derived from this project. It is hoped that this study will lead to increased understanding of how the brain controls eye movements and how this information may be used to help identify and treat different disorders.

You may retain a copy of the consent form. Please feel free to consult Dr. Douglas Munoz (doug.munoz@queensu.ca, 613-533-2111), the principal investigator in this study, or Dr. Albert Clark, Chair of the Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board (HSREB@queensu.ca, toll free number: 1-844-535-2988) at Queen's University at any time to discuss these procedures. This study has been reviewed for ethical compliance by the Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board. The researchers declare no conflict of interest or personal benefits related to this research.

- ☐ I wish to be contacted for future studies
- ☐ I authorize the inspection of my medical records that are related to this research by designated investigators
- ☐ I agree to participate in genetic sample collection
- ☐ I agree to complete an interview, personal history form, questionnaires and/or short cognitive test
- ☐ I agree to complete a computer task about social cognition
- ☐ I agree to participate in heart rate monitoring using a chest band
- ☐ I agree to participate in skin response monitoring using a sticky tab electrodes and sensor

PARTICIPANT'S NAME: _____

I have read and understand the fact sheet describing this study. I have had the purposes, procedures, and technical language of this study explained to me. I have been given sufficient time to consider the above information and to seek advice if I choose to do so. I have had the opportunity to ask questions which have been answered to my satisfaction. I am voluntarily signing this form. I understand that I may withdraw my consent to participate in this research project at any time. By signing this consent form, I am indicating that I agree to participate in this study.

Signature of Participant (if >18 years of age) Date

Signature of Legal Guardian if Participant is a minor Date

By signing this consent form, I (the parent/guardian) am indicating that my child, or my ward, may participate in this study. I am also confirming that all relevant parents/guardians have agreed to allow the child to participate in the study.

INVESTIGATOR:

I, or one of my assistants, have carefully explained to the participant the nature of the above research study. I certify that, to the best of my knowledge, the participant understands the nature of the study.

Signature of investigator Date

Version Date: 2021/OCT BKN PHGY-007-97

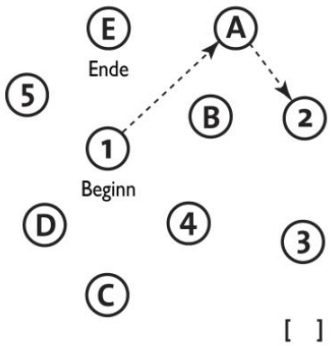
IX.5. Questionnaires

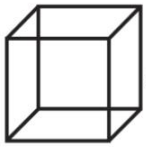
IX.5.1. Montreal cognitive assessment (MoCA)

MONTREAL COGNITIVE ASSESSMENT (MOCA)

NAME :
Ausbildung : Geburtsdatum :
Geschlecht : DATUM :

VISUOSPATIAL / EXEKUTIV





Würfel nachzeichnen

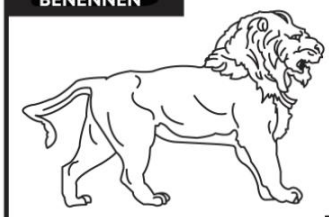
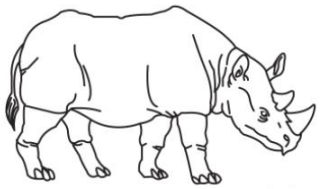
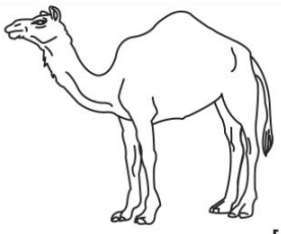
[ ] [ ]

Eine Uhr zeichnen (Zehn nach elf)
(3 Punkte)

[ ] Kontur [ ] Zahlen [ ] Zeiger

PUNKTE __/5

BENENNEN

[ ] [ ] [ ] __/3

GEDÄCHTNIS

Wortliste vorlesen, wiederholen lassen.
2 Durchgänge. Nach 5 Minuten überprüfen (s.u.)

	GESICHT	SAMT	KIRCHE	TULPE	ROT
1.Versuch					
2.Versuch					

Keine Punkte

AUFMERKSAMKEIT Zahlenliste vorlesen (1 Zahl/ Sek.)

In der vorgegebenen Reihenfolge wiederholen [ ] 2 1 8 5 4
Rückwärts wiederholen [ ] 7 4 2 __/2

Buchstabenliste vorlesen (1 Buchst./Sek.). Patient soll bei jedem Buchstaben „A" mit der Hand klopfen. Keine Punkte bei 2 oder mehr Fehlern
[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B __/1

Fortlaufendes Abziehen von 7 , mit 100 anfangen [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65
4 oder 5 korrekte Ergebnisse: **3 P.**, 2 oder 3 korrekt: **2 P.**, 1 korrekt: **1 P.**, 0 korrekt: **0 P.** __/3

SPRACHE **Wiederholen:** „Ich weiß lediglich, dass Hans heute an der Reihe ist zu helfen." [ ]
„Die Katze versteckte sich immer unter der Couch, wenn die Hunde im Zimmer waren." [ ] __/2

Möglichst viele Wörter in einer Minute benennen, die mit dem Buchstaben F beginnen [ ] _____ (N ≥ 11 Wörter) __/1

ABSTRAKTION Gemeinsamkeit von z.B. Banane und Apfelsine = Frucht [ ] Eisenbahn - Fahrrad [ ] Uhr - Lineal __/2

ERINNERUNG

	Worte erinnern OHNE HINWEIS	GESICHT [ ]	SAMT [ ]	KIRCHE [ ]	TULPE [ ]	ROT [ ]	Punkte nur bei richtigem Nennen OHNE Hinweis
Optional	Hinweis zu Kategorie						
	Mehrfachauswahl						

__/5

ORIENTIERUNG [ ] Datum [ ] Monat [ ] Jahr [ ] Wochentag [ ] Ort [ ] Stadt __/6

© **Z Nasreddine MD** Version 7. Nov. 2004 deutsche Übersetzung: **SM Bartusch, SG Zipper**

Normal ≥ 26 / 30

TOTAL __/30
+ 1 Punkt wenn ≤ 12 Jahre Ausbildung

www.mocatest.org Untersucher: _____

IX.5.2. Non-motor Symptoms Questionnaire and Scale for Parkinson's disease (PD NMS)

Fragebogen zu nicht-motorischen Symptomen beim Morbus Parkinson
(PD NMS Questionnaire)

Name: ..... Alter: ..... Datum: .....

Klinik/Zentrum:..... Mann ☐ Frau ☐

Nicht die Bewegung betreffende Probleme bei der Parkinson-Erkrankung

Die Bewegungsstörungen bei der Parkinson-Erkrankung sind gut bekannt. Es können aber manchmal auch andere Probleme auftreten, als Teil der Erkrankung oder deren Behandlung. Es ist wichtig, dass der Arzt über diese Probleme Bescheid weiß, v.a. wenn sie von Ihnen als störend empfunden werden.

Eine Reihe von Problemen ist unten angeführt. Bitte kreuzen Sie das Feld „Ja" an, wenn Sie das beschriebene Symptom während des letzten Monats erlebt haben. Der Arzt oder die Krankenschwester kann Ihnen Fragen stellen, um Ihnen bei der Entscheidung zu helfen. Wenn bei Ihnen das jeweilige Problem im Laufe des letzten Monats nicht aufgetreten ist, kreuzen Sie bitte das Feld „Nein" an. Sie sollten auch dann „Nein" antworten, wenn Sie die Symptome in der Vergangenheit, aber nicht während des letzten Monats hatten.

Ist bei Ihnen innerhalb des letzten Monats Folgendes aufgetreten?

	JA	NEIN
1. Herauslaufen von Speichel tagsüber	☐	☐
2. Verlust oder Veränderung in Ihrer Fähigkeit zu schmecken oder zu riechen	☐	☐
3. Schwierigkeit beim Schlucken von Nahrung oder Getränken oder Probleme mit Verschlucken	☐	☐
4. Erbrechen oder Gefühl von Übelkeit	☐	☐
5. Verstopfung (weniger als 3 Stuhlentleerungen pro Woche) oder Notwendigkeit beim Stuhlgang stark zu pressen	☐	☐
6. Stuhlinkontinenz	☐	☐
7. Gefühl der unvollständigen Darmentleerung nach dem Toilettengang	☐	☐
8. Plötzlicher starker Harndrang, so dass Sie sich beeilen müssen, zur Toilette zu gehen	☐	☐
9. Regelmäßiges nächtliches Aufstehen zum Wasserlassen	☐	☐
10. Unerklärliche Schmerzen (nicht als Folge bekannter Erkrankungen wie z.B. Arthritis)	☐	☐
11. Unerklärliche Gewichtsveränderungen (nicht als Folge geänderter Ernährung)	☐	☐
12. Probleme sich an Dinge zu erinnern, die kürzlich passiert sind, oder vergessen, Dinge zu erledigen	☐	☐
13. Interesseverlust an dem was um Sie herum geschieht, oder an Aktivitäten	☐	☐
14. Sehen oder Hören von Dingen, von denen Sie wissen oder Ihnen gesagt wird, dass sie nicht da sind	☐	☐
15. Schwierigkeiten, sich zu konzentrieren oder aufmerksam zu bleiben	☐	☐
16. Sich traurig fühlen, niedergeschlagen oder schwermütig	☐	☐
17. Gefühl der Angst, Furcht oder Panik	☐	☐
18. Reduziertes oder gesteigertes Interesse an Sex	☐	☐
19. Gefühl von Schwierigkeiten beim Versuch Geschlechtsverkehr zu praktizieren	☐	☐
20. Gefühl von Blutleere im Kopf, Schwindel oder Schwäche beim Aufstehen aus dem Sitzen oder Liegen	☐	☐
21. Stürze	☐	☐
22. Schwierigkeiten, während Aktivitäten wie Arbeit, Autofahren oder Essen wach zu bleiben	☐	☐
23. Schwierigkeiten abends einzuschlafen oder nachts durchzuschlafen	☐	☐
24. Intensive lebhafte Träume oder Träume, die Angst machen	☐	☐
25. Sprechen oder Bewegungen während des Schlafs, so als ob Sie einen Traum „ausleben"	☐	☐
26. Unangenehme Empfindungen in Ihren Beinen nachts oder beim Ausruhen und das Gefühl, sich bewegen zu müssen	☐	☐
27. Geschwollene Beine	☐	☐
28. Übermäßiges Schwitzen	☐	☐
29. Doppelbilder	☐	☐
30. Glauben, dass Ihnen Dinge passieren, von denen andere sagen, dass sie nicht wahr sind	☐	☐

Alle Informationen, die Sie in diesem Formular angeben, werden vertraulich behandelt und nur zu dem Zweck verwendet, für die sie erhoben wurden. Die angegebenen Informationen werden zur Verlaufskontrolle benutzt. Ihre persönlichen Daten werden in Übereinstimmung mit dem Datenschutzgesetz verwendet und aufbewahrt.

IX.5.3. REM sleep Behavior Disorder Screening Questionnaire (RBDSQ)

REM Sleep Behavior Disorder Screening Questionnaire (RBDSQ)

Bitte beantworten Sie folgende Fragen zu Ihrem Schlafverhalten:

Datum: _____

Name: _____ **Geboren am:** _____

		Ja	Nein
1.	Ich habe teilweise sehr lebhafte Träume.	☐	☐
2.	Meine Träume haben des öfteren aggressiven oder aktionsgeladenen Inhalt.	☐	☐
3.	Die Trauminhalte stimmen meist mit meinem nächtlichen Verhalten überein.	☐	☐
4.	Mir ist bekannt, dass ich meine Arme oder Beine im Schlaf bewege.	☐	☐
5.	Es ist dabei vorgekommen, dass ich meinen Partner oder mich selbst (beinahe) verletzt habe.	☐	☐
6.	Bei mir treten oder traten während des Träumens folgende Erscheinungen auf:		
	6.1 laut Sprechen, Schreien, Schimpfen, Lachen	☐	☐
	6.2 plötzliche Bewegungen der Gliedmaßen/"Kämpfen".	☐	☐
	6.3 Gesten, Bewegungsabläufe, die im Schlaf sinnlos sind, wie z.B. winken, salutieren, Mücken verscheuchen, Stürze aus dem Bett.	☐	☐
	6.4 um das Bett herum umgefallene Gegenstände, wie z.B. Nachttischlampe, Buch, Brille.	☐	☐
7.	Es kommt vor, dass ich durch meine eigenen Bewegungen wach werde.	☐	☐
8.	Nach dem Erwachen kann ich mich an den Inhalt meiner Träume meist gut erinnern.	☐	☐
9.	Mein Schlaf ist häufiger gestört.	☐	☐
10.	Bei mir liegt/lag eine Erkrankung des Nervensystems vor (z.B. Schlaganfall, Gehirnerschütterung, Parkinson, RLS, Narkolepsie, Depression, Epilepsie, entzündl. Erkrankung des Gehirns).	☐	☐

Falls ja, welche?

Bitte geben Sie noch Ihre derzeitige Medikation an...

Anzahl der Ja-Antworten: ☐

Stiasny-Kolster K, Mayer G, Schäfer S, Möller JC, Heinzel-Gutenbrunner M, Oertel WH. The REM Sleep Behavior Disorder Screening Questionnaire - A New Diagnostic Instrument, Movement Disorders, Vol. 22, No. 16, 2007, pp. 2386 –2393
© RBDSQ, K. Stiasny-Kolster (e-mail: stiasny@med.uni-marburg.de)

IX.5.4. Unified Parkinson's Disease Rating Scale (UPDRS)

Patienten-ID

Arzt-ID

Zentrums-ID

Unterschrift des Arztes

Datum

Kompetenznetz Parkinson

UPDRS
Seite 1 von 10

UPDRS

Formular zurücksetzen

Stadium
War der Patient zum Zeitpunkt der Untersuchung im ON- oder OFF-Stadium?

ON ◯ *OFF* ◯

I. *Kognitive Funktionen, Verhalten und Stimmung*

1. Intellektuelle Einschränkung

0 Keine. (0)
1 Leicht. Vergeßlichkeit mit teilweiser Erinnerung an Ereignisse und keine anderweitigen Schwierigkeiten. (1)
2 Mäßiger Gedächtnisverlust mit Desorientierung und mäßigen Schwierigkeiten beim Meistern komplexer Probleme. Leichte, aber definitive Einschränkung zu Hause mit der Notwendigkeit einer gelegentlichen Hilfe. (2)
3 Schwerer Gedächtnisverlust mit zeitlicher und häufig örtlicher Desorientierung. Schwere Einschränkung bei der Bewältigung von Problemen. (3)
4 Schwerer Gedächtnisverlust, Orientierung nur zur Person erhalten. Kann keine Urteile fällen und keine Probleme lösen. Benötigt bei der persönlichen Pflege viel Hilfe. Kann nicht mehr allein gelassen werden. (4)

2. Denkstörungen: (als Folge von Demenz oder Medikamentenintoxikationen)

0 Keine. (0)
1 Lebhafte Träume. (1)
2 »Gutartige« Halluzinationen mit erhaltener Einsicht. (2)
3 Gelegentliche bis häufige Halluzinationen und Wahnvorstellungen; keine Einsicht; könnte sich störend auf die täglichen Aktivitäten auswirken. (3)
4 Persistierende Halluzinationen, Wahnvorstellungen oder floride Psychose. Kann sich nicht selbst versorgen. (4)

3. Depression:

0 Nicht vorhanden. (0)
1 Zeitweise Traurigkeit oder Schuldgefühl stärker als normal, niemals Tage oder Wochen anhaltend. (1)
2 Anhaltende Depression (1 Woche oder länger). (2)
3 Anhaltende Depression mit vegetativen Symptomen (Schlaflosigkeit, Appetitlosigkeit, Gewichtsabnahme, Verlust des Interesses). (3)
4 Anhaltende Depression mit vegetativen Symptomen und Selbstmordgedanken oder -absichten. (4)

4. Motivation/Initiative

0 Normal. (0)
1 Weniger energisch als sonst; stärker passiv. (1)
2 Fehlende Initiative oder Desinteresse an nicht routinemäßigen Aktivitäten. (2)
3 Fehlende Initiative oder Desinteresse an täglichen (routinemäßigen) Aktivitäten. (3)
4 In sich gekehrt, völliges Fehlen von Motivation. (4)

Patienten-ID

Kompetenznetz Parkinson

UPDRS
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II. *Aktivitäten des täglichen Lebens*

5. Sprache

0 Normal. (0)
1 Leicht beeinträchtigt. Keine Verständigungsschwierigkeiten. (1)
2 Mäßig beeinträchtigt. Wird bisweilen gebeten, etwas zu wiederholen. (2)
3 Stark beeinträchtigt. Wird häufig gebeten, etwas zu wiederholen. (3)
4 Meistens unverständlich. (4)

6. Speichelsekretion

0 Normal. (0)
1 Gering, aber eindeutig vermehrter Speichel im Mund; nachts gelegentlich Speichelaustritt. (1)
2 Mäßig vermehrte Speichelsekretion; eventuell minimaler Speichelaustritt. (2)
3 Deutlich vermehrte Speichelsekretion mit leichtem Speichelaustritt. (3)
4 Ausgeprägter Speichelaustritt, muß ständig Papiertuch oder Taschentuch benutzen. (4)

7. Schlucken

0 Normal. (0)
1 Seltenes Würgen/Verschlucken. (1)
2 Gelegentliches Würgen/Verschlucken. (2)
3 Weiche Nahrung erforderlich. (3)
4 Ernährung über Magensonde oder Gastrostomie erforderlich. (4)

8. Handschrift

0 Normal. (0)
1 Etwas langsam oder klein. (1)
2 Mäßig langsam oder klein; sämtliche Wörter leserlich. (2)
3 Stark beeinträchtigt; nicht alle Wörter leserlich. (3)
4 Die Mehrzahl der Wörter ist unleserlich. (4)

9. Speisen schneiden und mit Utensilien umgehen:

0 Normal. (0)
1 Etwas langsam und unbeholfen, aber keine Hilfe erforderlich. (1)
2 Kann die meisten Speisen schneiden, jedoch unbeholfen und langsam; etwas Hilfe erforderlich. (2)
3 Speisen müssen von jemandem geschnitten werden, kann aber noch langsam essen. (3)
4 Muß gefüttert werden. (4)

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Kompetenznetz Parkinson

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II. *Aktivitäten des täglichen Lebens – Fortsetzung*

10. Anziehen:

0 Normal. (0)
1 Etwas langsam, aber keine Hilfe erforderlich. (1)
2 Gelegentliche Hilfe beim Knöpfen, beim Schlüpfen in die Ärmel. (2)
3 Beträchtliche Hilfe erforderlich, kann aber manches allein schaffen. (3)
4 Hilflos. (4)

11. Hygiene:

0 Normal. (0)
1 Etwas langsam, aber keine Hilfe erforderlich. (1)
2 Braucht beim Duschen und Baden Hilfe; oder bei Körperpflege sehr langsam. (2)
3 Braucht beim Waschen, Zähneputzen, Haarekämmen und beim Gang auf die Toilette Hilfe. (3)
4 Dauer-Blasen-Katheter oder andere mechanische Hilfsmittel. (4)

12. Umdrehen im Bett und Bettwäsche zurechtziehen:

0 Normal. (0)
1 Etwas langsam und unbeholfen, benötigt aber keine Hilfe. (1)
2 Kann sich allein, jedoch unter großen Schwierigkeiten, herumdrehen und die Bettwäsche zurechtziehen. (2)
3 Beginnt, kann sich aber nicht allein im Bett umdrehen oder die Bettwäsche zurechtziehen. (3)
4 Hilflos. (4)

13. Fallen (unabhängig von Starre):

0 Kein Fallen. (0)
1 Seltenes Fallen. (1)
2 Gelegentliches Fallen, weniger als einmal pro Tag. (2)
3 Fällt durchschnittlich einmal pro Tag. (3)
4 Fällt häufiger als einmal pro Tag. (4)

14. Erstarren beim Gehen:

0 Kein Erstarren. (0)
1 Seltenes Erstarren beim Gehen; eventuell verzögerter Start. (1)
2 Gelegentliches Erstarren beim Gehen. (2)
3 Regelmäßiges Erstarren. Gelegentliches Fallen nach Erstarren. (3)
4 Häufiges Fallen nach Erstarren. (4)

Patienten-ID

Kompetenznetz Parkinson

UPDRS
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II. *Aktivitäten des täglichen Lebens – Fortsetzung*

15. Laufen:

0 Normal. (0)
1 Leichte Schwierigkeiten. Eventuell fehlendes Mitschwingen der Arme, eventuell Neigung, das Bein nachzuziehen. (1)
2 Mäßige Schwierigkeiten, benötigt jedoch wenig oder keine Hilfe. (2)
3 Schwere Gehstörung, benötigt Hilfe. (3)
4 Kann selbst mit Hilfe nicht mehr gehen. (4)

16. Tremor:

0 Kein Tremor. (0)
1 Leicht und selten auftretend. (1)
2 Mäßig; für den Patienten lästig. (2)
3 Stark, bei zahlreichen Aktivitäten hinderlich. (3)
4 Ausgeprägt; bei den meisten Aktivitäten hinderlich. (4)

17. Sensorische Beschwerden infolge von Parkinsonismus:

0 Keine Beschwerden. (0)
1 Gelegentliches Taubheitsgefühl, Kribbeln oder leichte Schmerzen. (1)
2 Häufiges Taubheitsgefühl, Kribbeln oder Schmerzen, nicht störend. (2)
3 Häufig schmerzhafte Empfindungen. (3)
4 Unerträgliche Schmerzen. (4)

III. *Motorische Untersuchung*

18. Sprache:

0 Normal. (0)
1 Leichte Abnahme von Ausdruck, Diktion und/oder Volumen. (1)
2 Monoton, verwaschen, aber verständlich; mäßig behindert. (2)
3 Deutliche Beeinträchtigung, schwer zu verstehen. (3)
4 Unverständlich. (4)

19. Gesichtsausdruck:

0 Normal. (0)
1 Minimal veränderte Mimik, könnte ein normales »Pokergesicht« sein. (1)
2 Leichte, aber eindeutig abnorme Verminderung des Gesichtsausdrucks. (2)
3 Mäßig verminderte Mimik; Lippen zeitweise geöffnet. (3)
4 Maskenhaftes oder erstarrtes Gesicht mit stark oder völlig fehlendem Ausdruck; Lippen stehen um 7 mm auseinander. (4)

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III. *Motorische Untersuchung – Fortsetzung*

20. Ruhetremor:

G = Gesicht, RH = rechte Hand, LH = linke Hand, RF = rechter Fuß, LF = linker Fuß

		G	RH	LH	RF	LF
0	Keine.	0	0	0	0	0
1	Leicht und selten vorhanden.	1	1	1	1	1
2	Geringe Amplitude persistierend; oder mäßige Amplitude, aber nur intermittierend	2	2	2	2	2
3	Mäßige Amplitude, die meiste Zeit vorhanden.	3	3	3	3	3
4	Ausgeprägte Amplitude, die meiste Zeit vorhanden.	4	4	4	4	4

21. Aktions- oder Haltetremor der Hände:

R = rechts, L = links

		R	L
0	Fehlt.	0	0
1	Leicht; bei Bewegung vorhanden.	1	1
2	Mäßige Amplitude, bei Bewegung vorhanden.	2	2
3	Mäßige Amplitude sowohl bei Haltung als auch bei Bewegung.	3	3
4	Ausgeprägte Amplitude; beim Essen störend.	4	4

22. Rigidität:

Geprüft bei passiver Bewegung der großen Gelenke am sitzenden Patienten. Zahnradphänomen kann ignoriert werden.

N = Nacken, ROE = rechte obere Extremität, LOE = linke obere Extremität, RUE = rechte untere Extremität, LUE = linke unter Extremität

		N	ROE	LOE	RUE	LUE
0	Fehlt.	0	0	0	0	0
1	Leicht oder nur erkennbar bei Aktivierung durch spiegelbildliche oder andere Bewegungen.	1	1	1	1	1
2	Leicht bis mäßig.	2	2	2	2	2
3	Ausgeprägt, jedoch voller Bewegungsumfang bleibt erreicht.	3	3	3	3	3
4	Stark; Schwierigkeit beim Ausführen aller Bewegungen.	4	4	4	4	4

23. Fingerklopfen

Patient berührt in rascher Reihenfolge und bei größtmöglicher Amplitude und mit jeder Hand gesondert den Daumen mit dem Zeigefinger.

R = rechts, L = links

		R	L
0	Normal.	0	0
1	Leichte Verlangsamung und/oder Verringerung der Amplitude.	1	1
2	Mäßig eingeschränkt. Eindeutige und frühzeitige Ermüdung. Bewegung kann gelegentlich unterbrochen werden.	2	2
3	Stark eingeschränkt. Verzögerter Start der Bewegungen oder Unterbrechung fortlaufender Bewegungen.	3	3
4	Kann die Aufgabe kaum ausführen.	4	4

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III. *Motorische Untersuchung – Fortsetzung*

24. Handbewegungen:

Patient öffnet und schließt die Hände in rascher Reihenfolge bei größtmöglicher Amplitude und mit jeder Hand gesondert. R = rechts, L = links

		R	L
0	Normal.	0	0
1	Leichte Verlangsamung und/oder Verringerung der Amplitude.	1	1
2	Mäßig eingeschränkt. Eindeutige und frühzeitige Ermüdung. Bewegung kann gelegentlich unterbrochen werden.	2	2
3	Stark eingeschränkt. Verzögerter Start der Bewegungen oder Unterbrechung fortlaufender Bewegungen.	3	3
4	Kann die Aufgabe kaum ausführen.	4	4

25. Rasch wechselnde Bewegungen der Hände:

Pronations-Supinations-Bewegungen der Hände, vertikal oder horizontal, mit größtmöglicher Amplitude, beide Hände gleichzeitig.

		R	L
0	Normal.	0	0
1	Leichte Verlangsamung und/oder Verringerung der Amplitude.	1	1
2	Mäßig eingeschränkt. Eindeutige und frühzeitige Ermüdung. Bewegung kann gelegentlich unterbrochen werden.	2	2
3	Stark eingeschränkt. Verzögerter Start der Bewegungen oder Unterbrechung fortlaufender Bewegungen.	3	3
4	Kann die Aufgabe kaum ausführen.	4	4

26. Agilität der Beine:

Der Patient klopft in rascher Reihenfolge mit der Ferse auf den Boden und hebt dabei das ganze Bein an. Die Amplitude soll mindestens 7,5 cm betragen.

		R	L
0	Normal.	0	0
1	Leichte Verlangsamung und/oder Verringerung der Amplitude.	1	1
2	Mäßig eingeschränkt. Eindeutige und frühzeitige Ermüdung. Bewegung kann gelegentlich unterbrochen werden.	2	2
3	Stark eingeschränkt. Verzögerter Start der Bewegungen oder Unterbrechung fortlaufender Bewegungen.	3	3
4	Kann die Aufgabe kaum ausführen.	4	4

27. Aufstehen vom Stuhl:

Patient versucht mit vor der Brust verschränkten Armen von einem geradelehnigen Holz- oder Metallstuhl aufzustehen.

0	Normal.	0
1	Langsam; kann mehr als einen Versuch benötigen.	1
2	Stößt sich an den Armlehnen hoch.	2
3	Neigt zum Zurückfallen und muß es eventuell mehrmals versuchen, kann jedoch ohne Hilfe aufstehen.	3
4	Kann ohne Hilfe nicht aufstehen.	4

Patienten-ID

Kompetenznetz Parkinson

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III. *Motorische Untersuchung – Fortsetzung*

28. Haltung:

0 Normal aufrecht. (0)
1 Nicht ganz aufrecht, leicht vorgebeugte Haltung; könnte bei einem älteren Menschen normal sein. (1)
2 Mäßig vorgebeugte Haltung; eindeutig abnorm, kann leicht zu einer Seite geneigt sein. (2)
3 Stark vorgebeugte Haltung mit Kyphose; kann mäßig zu einer Seite geneigt sein. (3)
4 Ausgeprägte Beugung mit extrem abnormer Haltung. (4)

29. Gang:

0 Normal. (0)
1 Geht langsam, kann einige kurze Schritte schlurfen, jedoch keine Festination oder Propulsion. (1)
2 Gehen schwierig, benötigt aber wenig oder keine Hilfe; eventuell leichtes Trippeln, kurze Schritte oder Propulsion. (2)
3 Starke Gehstörung, benötigt Hilfe. (3)
4 Kann überhaupt nicht gehen, auch nicht mit Hilfe. (4)

30. Haltungsstabilität:

Reaktion auf plötzliches Verlagern nach hinten durch Ziehen an den Schultern des Patienten, der mit geöffneten Augen und leicht auseinanderstehenden Füßen geradesteht. Der Patient ist darauf vorbereitet.

0 Normal. (0)
1 Retropulsion, gleicht aber ohne Hilfe aus. (1)
2 Fehlen einer Haltungsreaktion; würde fallen, wenn er nicht vom Untersucher aufgefangen würde. (2)
3 Sehr instabil; neigt dazu, spontan das Gleichgewicht zu verlieren. (3)
4 Kann nicht ohne Unterstützung stehen. (4)

31. Bradykinesie und Hypokinesie des Körpers:

Kombination aus Langsamkeit, Zögern, verminderten Mitbewegungen der Arme, geringer Bewegungsamplitude und allgemeiner Bewegungsarmut.

0 Keine. (0)
1 Minimale Verlangsamung, Bewegung wirkt beabsichtig; könnte bei manchen Menschen normal sein. Möglicherweise herabgesetzte Amplitude. (1)
2 Leichte Verlangsamung und Bewegungsarmut, die eindeutig abnorm sind. Alternativ auch herabgesetzte Amplitude. (2)
3 Mäßige Verlangsamung und Bewegungsarmut oder Herabsetzung der Amplitude. (3)
4 Ausgeprägte Verlangsamung und Bewegungsarmut oder Herabsetzung der Amplitude. (4)

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Kompetenznetz Parkinson

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IV. Komplikationen der Behandlung (in der vergangenen Woche)

A. Dyskinesien

32. Dauer: Zu welcher Tageszeit traten die Dyskinesien auf?

0 Keine. (0)
1 1 - 25% des Tages. (1)
2 26 - 50% des Tages. (2)
3 51 - 75% des Tages. (3)
4 76 - 100% des Tages. (4)

33. Behinderung: Wie hinderlich sind die Dyskinesien?

Anamnestische Angaben; können durch Untersuchung in der Sprechstunde modifiziert werden.

0 Keine Behinderung. (0)
1 Leichte Behinderung. (1)
2 Mäßige Behinderung. (2)
3 Starke Behinderung. (3)
4 Vollständige Behinderung. (4)

34. Schmerzhafte Dyskinesien: Wie schmerzhaft sind die Dyskinesien?

0 Keine schmerzhaften Dyskinesien. (0)
1 Leicht. (1)
2 Mäßig. (2)
3 Stark. (3)
4 Ausgeprägt. (4)

35. Auftreten von Dystonie am frühen Morgen: (Anamnestische Angaben)

0 Nein. (0)
1 Ja. (1)

B. Klinische Fluktuationen

36. Gibt es nach einer Medikamenteneinnahme zeitlich vorhersagbare »OFF«-Perioden?

0 Nein. (0)
1 Ja. (1)

37. Gibt es zeitlich nicht vorhersagbare »OFF«-Perioden?

0 Nein. (0)
1 Ja. (1)

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IV. Komplikationen der Behandlung – Fortsetzung

38. **Treten »OFF«-Perioden plötzlich auf, z.B. innerhalb von wenigen Sekunden?**

0 Nein. (0)
1 Ja. (1)

39. **Für welche Dauer befindet sich der Patient tagsüber durchschnittlich im »OFF«-Stadium?**

0 Überhaupt nicht. (0)
1 1 - 25% des Tages. (1)
2 26 - 50% des Tages. (2)
3 51 - 75% des Tages. (3)
4 76 - 100% des Tages. (4)

C. Anderweitige Komplikationen

40. **Leidet der Patient an Appetitlosigkeit, Übelkeit oder Erbrechen?**

0 Nein. (0)
1 Ja. (1)

41. **Leidet der Patient an Schlafstörungen, z.B. Schlaflosigkeit oder Schläfrigkeit?**

0 Nein. (0)
1 Ja. (1)

42. **Hat der Patient orthostatische Symptome?**

0 Nein. (0)
1 Ja. (1)

Blutdruck – RR ____ / ____ *mm Hg*

Pulsfrequenz ____ */min*

Körpergewicht ____ *kg*

Patienten-ID

Kompetenznetz Parkinson

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VI. *Modifizierte Schwab- und England-Skala*

		ON	OFF
Aktivitäten des täglichen Lebens			
100%	Völlig unabhängig. Kann sämtliche Verrichtungen ohne Verlangsamung, Schwierigkeiten oder Behinderung ausführen. Völlig gesund. Keine Schwierigkeiten wahrgenommen.	○	○
90%	Völlig unabhängig. Kann sämtliche Verrichtungen mit geringer Verlangsamung, Schwierigkeiten und Behinderung ausführen. Kann doppelt so lange dazu brauchen. Schwierigkeiten werden bewußt.	○	○
80%	Bei den meisten Verrichtungen völlig unabhängig. Braucht dafür doppelt so viel Zeit. Ist sich der Schwierigkeiten und Verlangsamung bewußt.	○	○
70%	Nicht völlig unabhängig. Bei manchen Verrichtungen größere Schwierigkeiten. Braucht für einige drei- bis viermal so lange. Muß einen großen Teil des Tages auf die Verrichtungen verwenden.	○	○
60%	Leichte Abhängigkeit. Kann die meisten Verrichtungen ausführen, jedoch äußerst langsam und unter viel Anstrenung; manche unmöglich; Fehler.	○	○
50%	Stärker abhängig. Hilfe bei der Hälfte der Verrichtungen, langsamer usw. Schwierigkeiten bei allem.	○	○
40%	Sehr abhängig. Kann bei sämtlichen Verrichtungen mithelfen, nur einige allein sehr langsam.	○	○
30%	Kann bei Anstrengungen hier und da einige Verrichtungen allein ausführen oder beginnen. Benötigt viel Hilfe.	○	○
20%	Kann nichts allein tun. Kann bei manchen Verrichtungen etwas mithelfen. Stark behindert.	○	○
10%	Völlig abhängig, hilflos. Völlig behindert.	○	○
0%	Vegetative Funktionen wie Schlucken, Blasen- und Stuhlentleerung sind ausgefallen. Bettlägerig.	○	○

IX.5.5. Beck-Depressions-Inventar (BDI-II)

BDI-2

Dieser Fragebogen enthält 21 Gruppen von Aussagen. Bitte lesen Sie jede dieser Gruppen von Aussagen sorgfältig durch und suchen Sie sich dann in jeder Gruppe eine Aussage heraus, die am besten beschreibt, wie Sie **sich in den letzten zwei Wochen, einschließlich heute, gefühlt haben.** Kreuzen Sie die Zahl neben der Aussage an, die Sie sich herausgesucht haben (0, 1, 2 oder 3). Falls in einer Gruppe mehrere Aussagen gleichermaßen auf Sie zutreffen, kreuzen Sie die Aussage mit der höheren Zahl an. Achten Sie bitte darauf, dass Sie in jeder Gruppe nicht mehr als eine Aussage ankreuzen, das gilt auch für Gruppe 16 (Veränderungen der Schlafgewohnheiten) oder Gruppe 18 (Veränderungen des Appetits).

1) Traurigkeit		Patientenetikett:	
Ich bin nicht traurig.	0		
Ich bin oft traurig.	1		
Ich bin ständig traurig.	2		
Ich bin so traurig oder unglücklich, dass ich es nicht aushalte.	3		
2) Pessimismus		**7) Selbstablehnung**	
Ich sehe nicht mutlos in die Zukunft.	0	Ich halte von mir genauso viel wie immer.	0
Ich sehe mutloser in die Zukunft als sonst.	1	Ich habe Vertrauen in mich verloren.	1
Ich bin mutlos und erwarte nicht, dass meine Situation besser wird.	2	Ich bin von mir enttäuscht.	2
Ich glaube, dass meine Zukunft hoffnungslos ist und nur noch schlechter wird.	3	Ich lehne mich völlig ab.	3
3) Versagensgefühle		**8) Selbstvorwürfe**	
Ich fühle mich nicht als Versager.	0	Ich kritisiere oder tadle mich nicht mehr als sonst.	0
Ich habe häufiger Versagensgefühle.	1	Ich bin mir gegenüber kritischer als sonst.	1
Wenn ich zurückblicke, sehe ich eine Menge Fehlschläge.	2	Ich kritisiere mich für all meine Mängel.	2
Ich habe das Gefühl, als Mensch ein völliger Versager zu sein.	3	Ich gebe mir die Schuld für alles Schlimme, was passiert.	3
4) Verlust von Freude		**9) Selbstmordgedanken**	
Ich kann die Dinge genauso gut genießen wie früher.	0	Ich denke nicht daran, mir etwas anzutun.	0
Ich kann die Dinge nicht mehr so genießen wie früher.	1	Ich denke manchmal an Selbstmord, aber ich würde es nicht tun.	1
Dinge, die mir früher Freude gemacht haben, kann ich kaum mehr genießen.	2	Ich möchte mich am liebsten umbringen.	2
Dinge, die mir früher Freude gemacht haben, kann ich überhaupt nicht mehr genießen.	3	Ich würde mich umbringen, wenn ich die Gelegenheit dazu hätte.	3
5) Schuldgefühle		**10) Weinen**	
Ich habe keine besonderen Schuldgefühle.	0	Ich weine nicht öfter als früher.	0
Ich habe oft Schuldgefühle wegen Dingen, die ich getan habe oder hätte tun sollen.	1	Ich weine jetzt mehr als früher.	1
Ich habe die meiste Zeit Schuldgefühle.	2	Ich weine beim geringsten Anlass.	2
Ich habe ständig Schuldgefühle.	3	Ich möchte gern weinen, aber ich kann nicht.	3
6) Bestrafungsgefühle		**11) Unruhe**	
Ich habe nicht das Gefühl, für etwas bestraft zu sein.	0	Ich bin nicht unruhiger als sonst.	0
Ich habe das Gefühl, vielleicht bestraft zu werden.	1	Ich bin unruhiger als sonst.	1
Ich erwarte, bestraft zu werden.	2	Ich bin so unruhig, dass es mir schwerfällt, still zu sitzen	2
Ich habe das Gefühl, bestraft zu sein.	3	Ich bin so unruhig, dass ich mich ständig bewegen oder etwas tun muss.	3

12) Interessenverlust		17) Reizbarkeit	
Ich habe das Interesse an anderen Menschen oder an Tätigkeiten nicht verloren	0	Ich bin nicht reizbarer als sonst.	0
Ich habe weniger Interesse an anderen Menschen oder an Dingen als sonst.	1	Ich bin reizbarer als sonst.	1
Ich habe das Interesse an anderen Menschen oder Dingen zum größten Teil verloren.	2	Ich bin viel reizbarer als sonst.	2
Es fällt mir schwer, mich überhaupt für irgendetwas zu interessieren.	3	Ich fühle mich dauernd gereizt.	3
13) Entschlussfähigkeit		**18) Veränderungen des Appetits**	
Ich bin so entschlussfreudig wie immer.	0	Mein Appetit hat sich nicht verändert.	0
Es fällt mir schwerer als sonst, Entscheidungen zu treffen.	1	Mein Appetit ist etwas schlechter als sonst.	1a
		Mein Appetit ist etwas größer als sonst.	1b
Es fällt mir sehr viel schwerer als sonst, Entscheidungen zu treffen.	2	Mein Appetit ist viel schlechter als sonst.	2a
		Mein Appetit ist viel größer als sonst.	2b
Ich habe Mühe, überhaupt Entscheidungen zu treffen.	3	Ich habe überhaupt keinen Appetit.	3a
		Ich habe ständig Heißhunger.	3b
14) Wertlosigkeit		**19) Konzentrationsschwierigkeiten**	
Ich fühle mich nicht wertlos.	0	Ich kann mich so gut konzentrieren wie immer.	0
Ich halte mich für weniger wertvoll und nützlich als sonst.	1	Ich kann mich nicht mehr so gut konzentrieren wie sonst.	1
Verglichen mit anderen Menschen fühle ich mich viel weniger wert.	2	Es fällt mir schwer, mich längere Zeit auf irgendetwas zu konzentrieren.	2
Ich fühle mich völlig wertlos.	3	Ich kann mich überhaupt nicht mehr konzentrieren.	3
15) Energieverlust		**20) Ermüdung oder Erschöpfung**	
Ich habe so viel Energie wie immer.	0	Ich fühle mich nicht müder oder erschöpfter als sonst.	0
Ich habe weniger Energie als sonst.	1	Ich werde schneller müde oder erschöpft als sonst.	1
Ich habe so wenig Energie, dass ich kaum noch etwas schaffe.	2	Für viele Dinge, die ich üblicherweise tue, bin ich zu müde oder erschöpft.	2
Ich habe keine Energie mehr, um überhaupt noch etwas zu tun.	3	Ich bin so müde oder erschöpft, dass ich fast nichts mehr tun kann.	3
16) Veränderungen der Schlafgewohnheiten		**21) Verlust an sexuellem Interesse**	
Meine Schlafgewohnheiten haben sich nicht verändert.	0	Mein Interesse an Sexualität hat sich in letzter Zeit nicht verändert.	0
Ich schlafe etwas mehr als sonst.	1a	Ich interessiere mich weniger für Sexualität als früher.	1
Ich schlafe etwas weniger als sonst.	1b		
Ich schlafe viel mehr als sonst.	2a	Ich interessiere mich jetzt viel weniger für Sexualität.	2
Ich schlafe viel weniger als sonst.	2b		
Ich schlafe fast den ganzen Tag.	3a	Ich habe das Interesse an Sexualität völlig verloren.	3
Ich wache 1-2 Stunden früher auf als gewöhnlich und kann dann nicht mehr einschlafen.	3b		

SUMME: _____

IX.6. Curriculum vitae

IX.7. Verzeichnis der akademischen Lehrer

Meine akademischen Lehrer, denen ich an dieser Stelle herzlich danken möchte, waren in Bojnord, Teheran, und Marburg, die Damen und Herren Professor*innen, Doktor*innen und Dozent*innen:

Bojnord:

Dr. Mehran	Garmehi
Dr. Elham	Hajian
Dr. Ahmad	Jamshidpour
Dr.Vahid	Kiani
Dr. Shadi	Langari
Hamid	Moghadam
Rabee	Ravanifard
Dr. Mehdi	Sargolzayi
Dr. Ghodrat	Sepidnam
Dr. Mohammad	Shabani

Tehran:

Dr. Negin	Daneshpour
Prof. Reza	Ebrahimpour
Dr. Mohammad	Kalantari
Dr. Zahra	Mirzamomen
Dr. Ali	Nourollah
Dr. Masoumeh	Safkhani
Dr. Hamidreza	Shayegh

Canada:

Prof. Douglas	Munoz

Marburg:

Dr. Annette	Janzen
Prof. Wolfgang	Oertel

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IX.9. Ehrenwörtliche Erklärung