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STUDIES ON EYE MOVEMENTS IN PARKINSON'S DISEASE

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Studies on eye movements in Parkinson's Disease

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Panagiota Tsitsi

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To my families,

the one I was born into,

the one I created,

and the one I earned.

POPULAR SCIENCE SUMMARY OF THE THESIS

Parkinson's Disease is a common disorder of the brain that affects, however, the whole body. Patients are mainly identified by their tremors and walking difficulties, but Parkinson's Disease is more than that. Apart from motor symptoms, patients suffer also memory difficulties, stress and depression as well as problems that are related to functions that are not under their voluntary control, like constipation, urinary urgency, and sleep problems. Vision changes and reading difficulties are among the most common complaints.

During my PhD studies, I tried to describe these changes in vision with the help of technology. We used modern devices called eye trackers that measure eye movements. Together with my coauthors, we calculated how fast and accurate these movements are, and how long it takes to initiate them. We also calculated the speed by which Parkinson's patients read a simple text and compared it to that of healthy volunteers. Last, we asked the patients to look steadily at a target and tried to identify if they get distracted more easily than healthy participants, by computing the number of eye movements they do during the task.

Overall, we found that patients make shorter, and slower eye movements, make more directional errors when asked to look in the opposite direction of a target, and that medication affects some of these characteristics. We also noticed that medication is not effective against reading difficulties and that patients read slower than normal, probably because they need to spend more time on each word. Last, we noticed that Parkinson's patients get easily distracted when trying to focus on a visual target.

In addition to our eye-movement studies, we examined a small but very interesting population of ten people who live in the area of Norrbotten, in Northern Sweden, and are diagnosed with a rare variant of a well-described metabolic disease with eye movement difficulties, namely the Norrbottnian type of Gaucher Disease 3. According to our findings, these patients suffer memory and attention difficulties but seem to have good language and visuospatial skills.

We concluded that eye tracking can assist our clinical evaluation by providing objective calculations of eye movements. This could help physicians when giving a diagnosis but also while following up patients. Additionally, we pointed out the need for a better understanding of the mental functions of Norrbottnian Gaucher Disease type 3 patients, in order to provide better care and a better quality of life.

ΠΕΡΙΛΗΨΗ

Η νόσος του Πάρκινσον είναι μια διαταραχή του εγκεφάλου που επηρεάζει, ωστόσο, ολόκληρο το σώμα. Οι ασθενείς εμφανίζουν τρόμο και δυσκολίες βάδισης, αλλά η νόσος του Πάρκινσον δε χαρακτηρίζεται μόνο από κινητικά συμπτώματα. Οι διαταραχές μνήμης, το άγχος και η κατάθλιψη καθώς και προβλήματα που σχετίζονται με ακούσιες λειτουργίες όπως η δυσκοιλιότητα, η έπειξη για ούρηση και τα προβλήματα ύπνου, είναι μερικά από τα προβήματα των ασθενών με νόσο Παρκινσον. Οι αλλαγές στην όραση και οι δυσκολίες στην ανάγνωση είναι ανάμεσα στα πιο κοινά συμπτώματα που ταλαιπωρούν τους ασθενείς.

Κατά τη διάρκεια των διδακτορικών μου σπουδών, προσπάθησα να περιγράψω αυτές τις αλλαγές στην όραση με τη βοήθεια της τεχνολογίας. Χρησιμοποιήσαμε σύγχρονες συσκευές που ονομάζονται eye trackers και οι οποίες μετρούν τις κινήσεις των ματιών. Μαζί με τους συνεργάτες μου, υπολογίσαμε πόσο γρήγορες και ακριβείς είναι αυτές οι κινήσεις. Υπολογίσαμε επίσης την ταχύτητα με την οποία οι ασθενείς με Πάρκινσον διαβάζουν ένα απλό κείμενο και κάναμε συγκρίσεις με υγιείς εθελοντές. Τέλος, ζητήσαμε από τους ασθενείς να κοιτάζουν σταθερά έναν στόχο και προσπαθήσαμε να προσδιορίσουμε αν αποσπώνται πιο εύκολα σε σχέση με τους υγιείς εθελοντές που πήραν μέρος στη μελέτη, υπολογίζοντας τον αριθμό των κινήσεων που κάνουν τα μάτια κατά τη διάρκεια του τεστ.

Συνολικά, διαπιστώσαμε ότι οι ασθενείς κινούν τα μάτια τους πιο αργά και με μικρότερο εύρος σε σχέση με τους υγιείς, κάνουν περισσότερα σφάλματα κατεύθυνσης όταν τους ζητείται να κοιτάξουν προς την αντίθετη κατεύθυνση ενός στόχου, και ότι η φαρμακευτική αγωγή επηρεάζει ορισμένα, μόνο, από αυτά τα χαρακτηριστικά. Παρατηρήσαμε επίσης ότι η φαρμακευτική αγωγή δεν είναι αποτελεσματική έναντι στις δυσκολίες ανάγνωσης και ότι οι ασθενείς διαβάζουν πιο αργά από το κανονικό, πιθανώς επειδή πρέπει να συγκεντρώνονται περισσότερο χρόνο σε κάθε λέξη. Τέλος, παρατηρήσαμε ότι οι ασθενείς με Πάρκινσον αποσπώνται εύκολα όταν προσπαθούν να εστιάσουν σε έναν οπτικό στόχο.

Εκτός από τις μελέτες μας για την κίνηση των ματιών, εξετάσαμε επίσης έναν μικρό αλλά πολύ ενδιαφέροντα πληθυσμό δέκα ατόμων που ζουν στην περιοχή του Norrbotten, στη Βόρεια Σουηδία, και έχουν διαγνωστεί με μια σπάνια παραλλαγή μιας μεταβολικής νόσου, που ονομάζεται Norrbottnian μορφή της νόσου Gaucher τύπου 3. Σύμφωνα με τα ευρήματά μας, αυτοί οι ασθενείς αντιμετωπίζουν δυσκολίες μνήμης και προσοχής, αλλά φαίνεται να έχουν καλές γλωσσικές και οπτικοχωρικές δεξιότητες.

Καταλήξαμε στο συμπέρασμα ότι η χρήση της τεχνολογίας μπορεί να βελτιώσει την αξιολόγησή μας, παρέχοντας αντικειμενικούς υπολογισμούς των οφθαλμικών κινήσεων. Αυτό θα μπορούσε να βοηθήσει στην κλινική πράξη, τόσο στη διαδικάσία της μια διάγνωσης αλλά και κατά την παρακολούθηση ασθενών. Επιπρόσθετα, επισημάναμε την ανάγκη για καλύτερη κατανόηση των νοητικών λειτουργιών των ασθενών με τη Norrbottnian μορφή της νόσου Gaucher τύπου 3, προκειμένου να παρέχουμε καλύτερη φροντίδα και καλύτερη ποιότητα ζωής.

ABSTRACT

Heterogeneity in Parkinson's Disease (PD) phenotype and genotype is probably the main reason why, despite the abundance of biomarkers, we still lack a robust method for diagnosis and prognosis, besides clinical evaluation. Subjective changes in vision and objective measures in eye movements have been extensively studied, but the results are mainly used to better understand the pathophysiology of PD and are not integrated into the clinical praxis.

The aim of this doctoral project was to examine if eye movements could serve as useful biomarkers for PD diagnosis and prognosis, and investigate their association with motor function, cognition, and medication effect. In addition, we aimed to examine cognition in a group of patients with a rare metabolic disorder and prominent eye-movement difficulties, the Norrbottnian Gaucher Disease 3 (GD3).

Saccades, reading, and sustained fixation were examined in PD patients and healthy controls (HC) in the first three studies. Recruitment took place at Karolinska University Hospital Huddinge for the first two studies, and for the third study at Academic Specialist Center in Stockholm. Three different eye trackers were used, a head-mounted and two screen based, and the assessments were performed in a clinical setting. In the first two studies patients were examined in ON and OFF medication status, in order to evaluate the role of levodopa. In study 1, we examined saccadic parameters in 20 HC and 40 PD patients; study 2 involved reading assessments for 13 HC and 19 PD patients; in study 3 we examined sustained fixation in 43 HC and 50 PD patients. Recruitment for study 4 took place at Sunderby Regional Hospital, in Luleå, and we examined 10 patients with the Norrbottnian type of GD3. Cognitive evaluation was done with the Repeatable Battery for Assessment of Neuropsychological Status (RBANS).

PD participants had worse saccadic performance, a slower reading speed, and deficient fixation control. Saccadic gain was associated with motor performance, while latency was related to cognition. Levodopa had no effect on saccadic gain, it worsened latency for the horizontal visually guided saccades and ameliorated the latency of antisaccades, but not the error rate or reading performance. We assumed that reading difficulties were attributed to cognitive, rather than oculomotor deficits. Fixation was more easily interrupted in PD compared to HC, and PD participants' pupils did not dilate to the same extent as HC, in response to the cognitive effort put during sustained fixation. In study 4 we found that patients with the Norrbottnian type of GD3 have an overall worse cognitive performance compared to that of healthy population, scoring worse in memory and attention tests, present however with preserved language and visuospatial skills.

The eye-tracking studies led to the conclusion that this method could be integrated into the clinical praxis as part of the clinical evaluation. It is easy to perform and provides reliable results that enable the understanding of motor, cognitive, and behavioral changes in PD. In order to do so, we would need a common protocol of assessment, so that the results would be comparable between different populations. The last study identified RBANS as a useful and easy-to-use tool for the cognitive examination of Norrbottnian GD3 patients.

LIST OF SCIENTIFIC PAPERS

I. Waldthaler J, **Tsitsi P**, Svenningsson P. Vertical saccades and antisaccades: complementary markers for motor and cognitive impairment in Parkinson's disease. NPJ Parkinsons Dis. 2019 Jun 24;5:11.

II. Waldthaler J, **Tsitsi P**, Seimyr GÖ, Benfatto MN, Svenningsson P. Eye movements during reading in Parkinson's disease: A pilot study. Mov Disord. 2018 Oct;33(10):1661-1662.

III. **Tsitsi P**, Benfatto MN, Seimyr GÖ, Larsson O, Svenningsson P, Markaki I. Fixation Duration and Pupil Size as Diagnostic Tools in Parkinson's Disease. J Parkinsons Dis. 2021;11(2):865-875.

IV. **Tsitsi P**, Markaki I, Waldthaler J, Machaczka M, Svenningsson P. Neurocognitive profile of adults with the Norrbottnian type of Gaucher disease. JIMD Rep. 2021 Nov 21;63(1):93-100.

SCIENTIFIC PAPERS NOT INCLUDED IN THE THESIS

I. Bereczki E, Bogstedt A, Höglund K, **Tsitsi P**, Brodin L, Ballard C, Svenningsson P, Aarsland D. Synaptic proteins in CSF relate to Parkinson's disease stage markers. NPJ Parkinsons Dis. 2017 Feb 8;3:7.

II. Svenningsson P, Johansson A, Nyholm D, **Tsitsi P**, Hansson F, Sonesson C, Tedroff J. Safety and tolerability of IRL790 in Parkinson's disease with levodopa-induced dyskinesia-a phase 1b trial. NPJ Parkinsons Dis. 2018 Dec 6;4:35.

III. Vinding MC, **Tsitsi P**, Piitulainen H, Waldthaler J, Jousmäki V, Ingvar M, Svenningsson P, Lundqvist D. Attenuated beta rebound to proprioceptive afferent feedback in Parkinson's disease. Sci Rep. 2019 Feb 22;9(1):2604.

IV. Green H, **Tsitsi P**, Markaki I, Aarsland D, Svenningsson P. Novel Treatment Opportunities Against Cognitive Impairment in Parkinson's Disease with an Emphasis on Diabetes-Related Pathways. CNS Drugs. 2019 Feb;33(2):143-160.

V. Vinding MC, **Tsitsi P**, Waldthaler J, Oostenveld R, Ingvar M, Svenningsson P, Lundqvist D. Reduction of spontaneous cortical beta bursts in Parkinson's disease is linked to symptom severity. Brain Commun. 2020 Apr 28;2(1):fcaa052.

VI. Svenningsson P, Odin P, Dizdar N, Johansson A, Grigoriou S, **Tsitsi P**, Wictorin K, Bergquist F, Nyholm D, Rinne J, Hansson F, Sonesson C, Tedroff J; IRL752 Collaborators. A Phase 2a Trial Investigating the Safety and Tolerability of the Novel Cortical Enhancer IRL752 in Parkinson's Disease Dementia. Mov Disord. 2020 Jun;35(6):1046-1054.

VII. Markaki I, Bergström S, **Tsitsi P**, Remnestål J, Månberg A, Hertz E, Paslawski W, Sorjonen K, Uhlén M, Mangone G, Carvalho S, Rascol O, Meissner WG, Magnin E, Wüllner U, Corvol JC, Nilsson P, Svenningsson P. Cerebrospinal Fluid Levels of Kininogen-1 Indicate Early Cognitive Impairment in Parkinson's Disease. Mov Disord. 2020 Nov;35(11):2101-2106.

VIII. Eriksson A, **Tsitsi P**, Vinding MC, Ingvar M, Svenningsson P, Lundqvist D. Changes in emotion processing in early Parkinson's disease reflect disease progression. Neuropsychology. 2022 Mar;36(3):206-215.

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LIST OF ABBREVIATIONS

ANS	Autonomous Nervous System
AS	Antisaccades
AUC	Area Under the Curve
BG	Basal Ganglia
CI	Confidence Interval
CNS	Central Nervous System
COMT	Catechol-O-methyltransferase
CSF	Cerebrospinal Fluid
DLPFC	Dorsolateral Prefrontal Cortex
ERT	Enzyme Replacement Therapy
EWN	Edinger-Westphal Nucleus
F	Female
FAB	Frontal Assessment Battery
GBA	Glucocerebrosidase gene
GCase	Glucocerebrosidase enzyme
GD	Gaucher Disease
GPe	Globus Pallidus External
GPi	Globus Pallidus Internal
HC	Healthy Controls
HSCT	Hematopoietic Stem Cell Transplantation
IQR	Interquartile Range
LC	Locus Coeruleus
LEDD	Levodopa Equivalent Daily Dose
M	Male
MAO-B	Monoaminoxidase-B
MCI	Mild Cognitive Impairment
MDS	Movement Disorders Society
MDS-UPDRS	Movement Disorders Society revised version of the Unified Parkinsons Disease Rating Scale

MMSE	Mini Mental State Examination
MRI	Magnetic Ressonance Imaging
mSST	Modified Severity Scoring Tool
N	No
NA	Non Applicable
NS	Non signifficant
OR	Odds Ratio
PD	Parkinson’s Disease
PDD	Parkinson’s Disease Dementia
PEF	Parietal Eye Fields
PLR	Pupil Light Reflex
PNR	Pupil Near Response
PPN	Pendiculopontine Nucleus
PPR	Psychosensory Pupil Response
PPRF	Parapontine Reticular Formation
RBANS	Repeatable Battery for Assessment of Neuropsychological Status
RBD	Rapid-Eye-Movement Behavior Disorder
ROC	Receiver Operator Characteristic
SC	Superior Colliculus
SCT	Splenectomy
SEF	Supplementary Eye Fields
SNc	Substantia Nigra pars compacta
SNr	Substantia Nigra pars reticulata
SRT	Substrate Replacement Therapy
STN	Subthalamic Nucleus
SWJ	Square Wave Jerks
UPDRS	Unified Parkinson’s Disease Rating Scale
VGS	Visually Guided Saccades
Y	Yes

1 INTRODUCTION

Parkinson's Disease (PD) is considered a neurological disorder, but it is more than that. Alterations in the brain with loss of dopaminergic cells in the substantia nigra and accumulation of Lewy bodies in the neurons are the main pathological findings (1). These changes alone, however, cannot explain the signs and symptoms of PD, nor the phenotypical heterogeneity between patients. Diagnosis can therefore be challenging, and it is mainly based on clinical examination and medical history.

Oculomotor alterations in neurodegenerative disorders are a commonly neglected sign during everyday clinical evaluation. Eye movements are usually hard to examine in the clinical setting, both because of inexperience and lack of knowledge on behalf of the examiner, but also due to difficulties in cooperation with patients, especially those that are cognitively affected. Eye trackers, on the other hand, can measure eye movements in a quick and simple manner, making the evaluation of the oculomotor system easy. They have, extensively, been used to understand the pathophysiology of the oculomotor system in primates and humans. Whether this technique could easily be integrated into the outpatient clinic, though, is a matter of question. Are the results reliable? What are the practical difficulties of such assessments in a clinical setting? From a clinician's perspective, it would be interesting to have an objective method for assessing patients and obtain information regarding their diagnosis and prognosis, and eye-movement assessments with eye trackers could serve as such.

The main idea for this PhD project was to examine whether eye tracking is a feasible and reliable method in the outpatient clinic.

In Northern Sweden, there is a subpopulation of the rare metabolic disorder Gaucher Disease (GD) type 3 that shares some similarities with a genetic subtype of PD. This subpopulation presents with gaze palsy. We added a cognitive examination of these patients to the project.

2 LITERATURE REVIEW

2.1 PARKINSON'S DISEASE

PD is one of the most common neurodegenerative diseases with a higher prevalence in older populations. It comprises motor and non-motor symptoms, while subclinical signs and symptoms occur long before the diagnosis is established (2). PD has multifactorial pathogenesis; age, genetic alterations, environmental factors, concomitant disease, and medication account for some of the risk factors for developing the disease (3). Nevertheless, we are still far from identifying why not all individuals with similar risk factors develop the disease. The variability in motor and non-motor symptoms combined with variable disease progression makes each patient unique.

According to the United Kingdom Brain Bank Criteria (4), PD diagnosis is based on key features of the motor system: bradykinesia combined with rest tremor, rigidity, and postural instability are the basic clinical characteristics along with a number of exclusion and supportive criteria. However, PD is far from a pure movement disorder, and this is the reason why the Movement Disorders Society (MDS) has established a new set of diagnostic criteria that also involve the non-motor component of the disease (5).

Non-motor symptoms may present far in advance, and most often they are not related to any neurological diagnosis, not until they are recognized as prodromal symptoms of PD when motor symptoms occur. Anosmia, sleep disorders such as insomnia and rapid-eye-movement behavior disorder (RBD), depression, constipation, autonomic dysfunction, cognitive impairment, and speech problems are some of the non-motor symptoms that add to the list of motor symptoms (Table 1) (2). Clinical diagnosis is supported by imaging, wet biomarkers in cerebrospinal fluid (CSF) and blood, as well as genetic testing (6). However, post-mortem pathological examination is the only way to confirm the clinical diagnosis, and this highlights the need for diagnostic biomarkers as early as possible during disease progression.

2.3 COGNITIVE CHANGES IN PD

Alterations in cognition are recognized early during PD, even at early stages in non-medicated patients (7). Diagnostic criteria have been established for PD dementia (PDD) and mild cognitive impairment (MCI) (8, 9). The risk for dementia increases with disease progression, which also affects quality of life (10). Several parameters have been identified as risk factors for cognitive impairment in PD such as the akinetic-rigid type of the disease, older age, presence of hyposmia, and RBD (11). Additionally, genetic variations play an important role in the patients' cognitive profile: mutations in catechol-O-methyltransferase (*COMT*), microtubule-associated protein tau, apolipoprotein E, glucocerebrosidase (*GBA*), and α-synuclein genes have been associated with a higher risk for PDD (12).

Table 1. Motor and non-motor symptoms of Parkinson's Disease.

Motor symptoms	**Non-motor Symptoms**
Bradykinesia	Cardiovascular symptoms: orthostatism, decreased heart rate variability
Tremor (mainly rest tremor 4-6 Hz)	Psychiatric symptoms: hallucinations, depression, apathy, anxiety, impulse control disorders
Rigidity	Cognitive impairment
Postural instability	Sleep disorders, insomnia
Gait disturbances/Freezing	Fatigue, daytime somnolence
Camptocormia	Gastrointestinal tract problems: dysphagia, sialorrhea, delayed gastric emptying, constipation
Dystonia	Genito-urinary tract problems: impotence, reduced libido, overactive bladder
Hypomimia	Anosmia, hyposmia
	Dysarthria, hypophonia
	Visual and ocular abnormalities: diplopia, reading difficulties, eye-movement abnormalities, reduced blinking
	Sensory complaints, paresthesias, pain, peripheral neuropathy, restless legs syndrome
	Weight loss
	Hyperhidrosis, hypohidrosis

Two distinct clinical types of cognitive decline in PD have been suggested: one that is mostly frontal/executive, related to dopaminergic deficits, and a second that involves more posterior cortical areas, leading to a deterioration of attentional and visuospatial memory functions, mainly attributed to cholinergic and other neurotransmitter deficits (13). Studies on functional brain connectivity have revealed patterns that relate the striatum to the prefrontal cortex with connections that are affected in PD (14). Degeneration of the raphe nucleus and locus coeruleus (LC) alters the connections of these nuclei with the cortex and results in the depletion of serotonin and noradrenaline in respective cortical areas. Similarly, acetylcholine reduction in the nucleus basalis and pedunculopontine nucleus (PPN) has been described in demented but also in non-demented PD patients (14).

MCI in early PD patients might implicate progression to PDD, but many patients remain stable, while others return to normal, especially after treatment optimization (14). The clinical phenotype of cognitive impairment that presents with global memory deficits is related to a more severe prognosis (15). Typical frontal-executive dysfunction can include difficulties in planning, impaired inhibition, and working memory (16, 17). Visuospatial deficits, memory and language impairment, functions that are controlled by more posterior regions of the cortex, indicate a higher possibility for progression to PDD (18).

Attention is a complex function and as such, there are multiple areas of the brain that control it, and several neurotransmitters that are involved (18, 19). It can be seen as a mechanism for maintaining focus on a task, but also for switching between different tasks. It can be selective, towards a specific goal, or non-selective, targeting a high-performance level across various tasks. It can be driven by specific characteristics of the task or from factors that are internal such as previous experiences, while it is also highly dependent on the level of arousal (19). The main characteristic of attention is flexibility, which allows for shifting, prioritizing, filtering, and selecting information and in close relation to working memory. It requires the cooperation of subcortical and cortical areas that are controlled by neuromodulators. An intact executive function involves recruiting both attention and working memory resources in order to effectively achieve a goal (20). In PD, executive function deficits are attributed to the disruption of the dopaminergic neural connections between the striatum and frontal and prefrontal areas (14, 20).

Visual attention is necessary to filter the incoming information from the eyes and only select data that are important and relevant during a task (21). In PD, visual attention is subject to a strong top-down inhibition due to planning dysfunction that affects eye movements. On the other hand, dysfunction of the oculomotor system is hypothesized to lead to excessive bottom-up facilitation of visual attentional processes. This disequilibrium is reflected on altered eye movements that are controlled both by fast bottom-up functions as well as top-down cognitive processes during focusing and decision making (22, 23).

2.4 VISUAL COMPLAINTS

Obtaining visual information from the environment requires combined cooperation of various functions of the eyes and brain. Vision and gaze control, motion perception, working memory and attention, memory processes that decide which information is further analyzed and which is, temporarily or permanently, discarded, are some of the neural activities that are required to reach optimal results (24). Visual problems are commonly reported by patients (25). Double vision, alterations in visual perception, impairment in color vision and contrast, as well as visual hallucinations, are usual complaints (24, 26). Clinical examination often reveals saccadic hypometria, increased latency of voluntary saccades, hyperreflexivity, and saccadic intrusions during smooth pursuit (27).

Visual dysfunction seems to affect overall perception and motor function in PD, and reports of visuospatial impairment in PD are not new (28, 29). These symptoms are not specific for PD although they may appear early in the disease course. Visual changes and lower-level visual dysfunction seem to explain some of the visuospatial deficits in PD (30), while some of the functional visual disturbances could be attributed to impaired saccade or smooth pursuit performance (24). Some of the deficits are predictive of cognitive decline in the disease course (31).

2.5 EYE MOVEMENTS

Apart from an intact visual pathway, we need eye movements to acquire visual information. The aim of the oculomotor system is to stabilize the gaze and shift it fast and accurately when needed, so that images can be focused on the fovea (32). Various involuntary and voluntary movements are involved for this purpose. Saccades, smooth pursuit, fixation, vergence, and vestibulo-ocular movements, namely vestibular and optokinetic nystagmus, share many similarities and differ in various ways, but most importantly, the eyes shift between these movements in a continuous manner (32).

2.5.1 Saccades

Saccades refer to the eye movements that shift the gaze between two different targets, or fixation points (33). Normally they are fast and accurate. They can be elicited voluntarily or in a reflexive manner. Saccadic velocity and amplitude are similar for reflexive and volitional saccades, and these parameters are mainly controlled by the superior colliculus (SC), the cerebellum, and the saccade generator in the brainstem, the parapontine reticular formation (PPRF) for horizontal saccades and the rostral interstitial nucleus of medial longitudinal fasciculus for vertical saccades. Latency, however, defined as the time required for the onset of a saccade, is determined by the complexity of the task; during this delay, the brain has to compute the rest of the saccadic characteristics, such as amplitude and velocity, and it depends on various external parameters i.e. characteristics of the stimulus and possible distractors, as well as internal ones, i.e. cognitive and arousal state (34). Internal processes for the initiation of a saccade include analysing the stimulus in the cortex and the transmission of the signal via more complex pathways (32, 33). Voluntary saccades require internal planning, and they don’t

necessarily need an external stimulus. They can be purely intentional, memory-guided, or in response to a command: look towards a target or opposite from a target, called antisaccades (AS) in that case. Contrary to that, reflexive saccades, looking towards a sound source, for example, require less planning, and their initiation is subject to less control (33). Reflexive saccades are most often visually guided (VGS) in the clinical setting of examining eye movements.

2.5.2 Fixation

The role of fixation is to maintain the image of the object of interest in the fovea which is the area of the retina where the visual acuity is best (35). The decision to move the eyes, generate a saccade, and pause fixation, requires an evaluation of the need to maintain fixation but also of the significance of the stimulus towards which the eyes shall move. Therefore, fixation is closely related to attention and other cognitive functions. It requires the involvement of multiple brain structures; cortical, frontal and parietal, and subcortical, such as the nucleus raphe interpositus and SC. A fine balance between excitatory signals that stabilize the gaze, and inhibitory ones that prevent the generation of unwanted eye movements, is necessary for successful fixation (35). Small regulatory movements, ocular tremor and drift, microsaccades, and small saccades, are, nevertheless, required to avoid blurring of the image as well as to compensate for small head movements (36, 37).

2.5.3 Pupil responsivity during fixation

Pupil-size changes depend on different kinds of stimuli: constriction to light, namely the pupillary light reflex (PLR), constriction to accommodation, focusing that is to a near target, namely the pupil near response (PNR), and dilation due to cognitive load, namely the psychosensory pupil response (PPR) (38). The role of the autonomous nervous system (ANS) in pupil function is cardinal, but pupil size, is subject to adjustments controlled by multiple brain areas, and modulations by cognition, emotion, and arousal (38).

2.5.4 Reading

Reading is a complex process that involves not only oculomotor planning but also cognitive and linguistic processes. From an oculomotor function perspective, reading comprises alternating saccades and fixations. The goal of saccadic eye movements, forward (progressive) or backward (regressive), is to bring the words of interest to the fovea, where visual acuity is optimal, although information from the periphery influences the reading process as well. Words are then fixated in order to be interpreted, and fixation duration is determined by various factors, such as linguistic properties of the text as well as cognitive characteristics of the reader (39, 40). Hence, fixation duration represents a temporal measure and indicates processing load, while fixation position and saccade amplitude are measures of space that describe the direction and sequence of processing (41). Brain structures that are involved in natural reading and correlate to fixation duration have been identified in functional magnetic resonance imaging (fMRI) studies and they include cortical areas that are associated with attention, language processing, and oculomotor control, as well as striate and peristriate regions (40). Variations in

eye-movement patterns during reading are to some extent attributed to individual differences in working memory (42).

2.6 NEUROANATOMY OF THE EYE MOVEMENTS

In order for the eye to initiate a movement, towards or in the opposite direction of a stimulus, the signal must be first perceived by the visual cortex in the occipital area of the brain. This is done via the afferent visual pathway that leads from the retina to the thalamus, the geniculate body more specifically, and to the primary visual cortex.

2.6.1 Cortical areas of interest

From the visual cortex, the signal is redirected towards the frontal and parietal areas, the frontal eye fields (FEF) and parietal eye fields (PEF). Both FEF and PEF are involved in saccade generation and define its latency in variable grade, depending on the type of the saccade. The FEF are responsible for decision making and target selection, and therefore generate a motor response in the form of a saccade based on an internal or external command. Hence their contribution to the highly volitional saccade (longer latency) generation is crucial (33). The PEF´s role lies mostly in the visuospatial regulation of the saccadic movement, and therefore their role is more important during reflexive saccades (shorter latency). Processes that involve attention take place in the generation of eye movements at this level. The dorsolateral prefrontal cortex (DLPFC), together with the anterior cingulate cortex, both considered important in executive control (43), act as filters that cancel unwanted eye movements by delaying the latency of a saccade while protecting against distractors. Control of a higher level is further achieved by the supplementary eye field (SEF), in the frontal lobe (32, 33).

2.6.2 Subcortical areas of interest – The role of the basal ganglia

Although there are direct projections from the FEF and SEF towards the brainstem generator, saccade generation requires the activation of the SC (44). The SC in the brainstem plays an important role in the oculomotor pathway as it connects the brainstem saccade generator with the cortex. It is directly connected with the PEF. Connections with the FEF are both direct and indirect via the basal ganglia (BG), more specifically the substantia nigra pars reticulata (SNr) (44, 45). For the generation of saccades, the cortex (FEF, SEF, DLPFC) stimulates the caudate to inhibit the SNr (which normally exerts tonic inhibition on the SC) in a phasic way, thus resulting in disinhibition, and initiation of a saccade (45). There are, however, additional connections with the globus pallidus external (GPe), as part of the indirect pathway (45). Therefore, the BG participate in the modulation of volitional saccades while more reflexive saccades are thought to bypass the BG. Their generation requires the activation of parietal cortical areas that project directly to the SC (33, 44). The BG do not generate saccades; their role lies mainly in filtering the appropriate movements through inhibition. It is important to note that the SC receives mainly excitatory input, with the exception of the BG which send inhibitory signals. This inhibition from SNr is further modulated by the caudate, which can remove the SNr effect, thus disinhibiting the SC, while the GPe and the subthalamic nucleus (STN) add to this modulation, depending on behavioral parameters, like attention and working

memory (45). Inhibitory input is also sent directly to the brainstem generator from the cortex (46). The SC is involved in various parts of the generation of eye movements, from processing of the visual signal to preparation of the motor command of saccade generation, fixation, and microsaccades. It projects to the brainstem generator, to the cerebellum, and sends ascending fibers to the FEF via the thalamus (47). A simple illustration of the aforementioned pathways is illustrated in Fig 1.

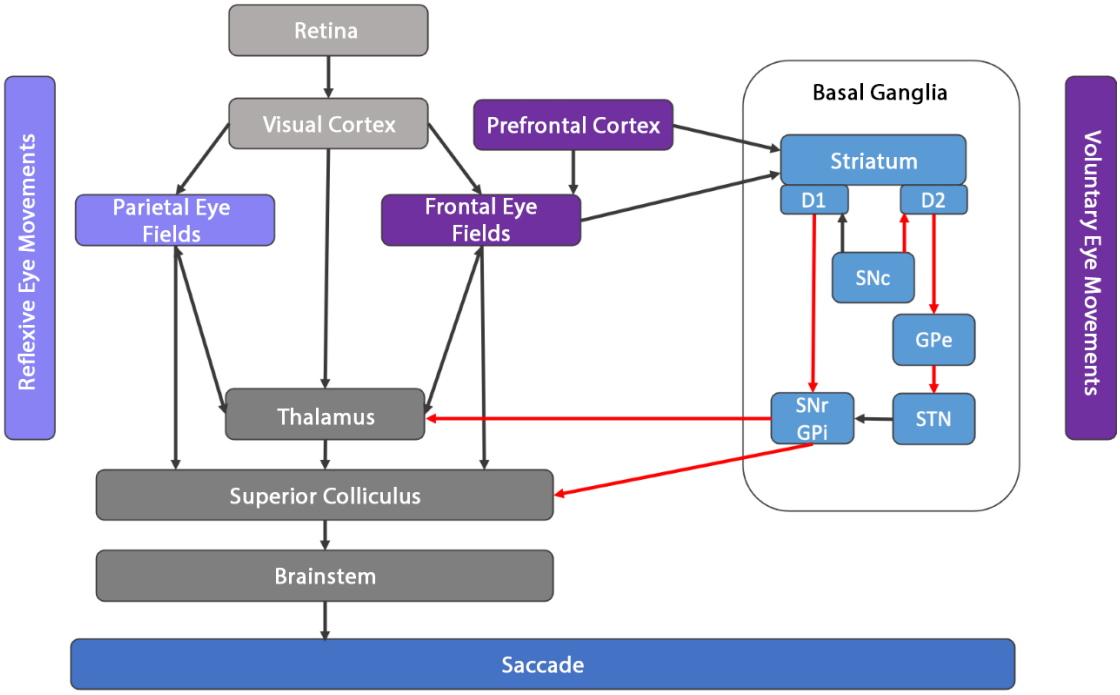

Figure 1. *Schematic illustration of the connections between various brain structures for the generation and control of saccades. Signaling from the retina travels to the visual cortices. From there, signals reach the parietal and frontal eye fields to initiate saccades. The BG are mainly involved in controlling voluntary saccades, together with the prefrontal cortices and the frontal eye fields. Reflexive saccades are mainly controlled by the parietal eye fields. Projections from the BG to the SC for saccadic control follow a similar pattern like the ones to the thalamus for movement control: Inhibition from the SNr and the GPi is finely adjusted by the direct and indirect pathways. Imbalance of the direct and indirect pathway in PD due to dopamine depletion leads to excessive inhibition of the SC and saccadic abnormalities. Inhibitory projections are demonstrated in red and excitatory ones are demonstrated in black. BG: Basal ganglia, SC: Superior colliculus, SNc: Substantia nigra pars compacta, SNr: Substantia nigra pars reticulata, GPe: Globus pallidus external, GPi: Globus pallidus internal, STN: Subthalamic nucleus*

Diagram created by Josefine Waldthaler (translated in English), published under permission of the creator.

2.6.3 Brainstem saccade generator and cerebellum

The brainstem saccade generator consists of two separate cell populations, one for the horizontal (pontine tegmentum) and one for the vertical saccades (pretectal tegmentum). The neurons that are involved in saccadic control are further divided into two groups. One group consists of omnipause cells, controlled by the SC. They are inactive during saccades but burst during fixation, and inhibit saccades towards any direction. The second population comprises excitatory burst neurons responsible for the initiation of the planned saccade, and inhibitory ones that inhibit the generation of unwanted saccades. The fine balance between these cell populations is necessary for the generation of fast and accurate saccadic movements (32, 33).

Regarding fixation, it is believed that omnipause neurons in the nucleus raphe interpositus of the PPRF, fire in a tonic manner during fixation in order to inhibit the firing of saccade generators of the mesencephalic and pontomedullary reticular formations, canceling unwanted vertical and horizontal saccades. They stop firing when a saccade starts and pass the signal to the motor neurons of the extraocular muscles (35). A similar mechanism of action has been described for neurons at the rostral end of the SC, where firing during fixation and pausing during saccades takes place, the mechanism though not being entirely similar to that of omnipause neurons (35). The SC neurons appear to stimulate fixation and saccades in a synergistic, rather than an antagonistic manner, so that the visual field is represented as a virtual map where all potential target locations are represented by active neurons (48).

The vermis, the caudal fastigial nucleus as well as lateral areas in the cerebellar hemispheres are involved in the fine adjustment of saccades, mostly their amplitude, and consequently their accuracy (33). The vermal lobules VIc–VII and the caudal fastigial nuclei have been indicated as areas of control of gaze accuracy during fixation (35).

2.6.4 Neuroanatomy of the pupil reflex

The iris sphincter muscle, innervated by the parasympathetic ANS, is responsible for constriction. Dilation is achieved by the iris dilator muscle, innervated by the sympathetic ANS. The constriction/parasympathetic pathway that uses acetylcholine as the main neurotransmitter is relatively short and mainly responsible for the PLR: fibers from the optic nerve and chiasma reach the Edinger-Westphal nucleus (EWN) via the pretectal nucleus; from there, signaling proceeds to the ciliary ganglion via the oculomotor nerve and innervates the sphincter muscle. Contrary to that, the dilation/sympathetic pathway uses catecholamines and is significantly longer and less understood. This complex pathway involves the frontal cortex along with subcortical domains such as the hypothalamus (projecting inhibitory signals to the EWN), and the LC. The ophthalmic branch of the sympathetic nerve descends to the spinal cord (C8-T2) and through the superior ciliary ganglions innervates the iris dilator muscles (38, 49). The role of LC in arousal and cognition has been extensively discussed and studies on how pupil dilation is affected by mental processes shed light on this aspect of the pupillary response (49, 50).

2.7 NEUROTRANSMITTERS

2.7.1 The role of dopamine

Dopamine is abundant in the nervous system. Its presence in the visual pathway and its role in the genesis of visual dysfunction in PD have been discussed (24). Dopamine can be found in the eye, in the amacrine cells of the retina, with projections towards the striatum. Dopaminergic pathways in the brain are numerous: the striatonigral pathway originating in the substantia nigra and terminating in the striatum (caudate nucleus and putamen), the mesolimbic and mesocortical pathway (from the ventral tegmentum to the nucleus accumbens and limbic system, and frontal cortex respectively), pathways within the hypothalamus (involving the pituitary gland, the amygdala, hippocampus, cingulate gyrus, and olfactory bulb), and within the cortex (mainly frontal and limbic areas, and less in the visual cortex) (24). Dopamine's role in the ANS has also been described. It can be found in the sympathetic and visceral ganglia as well as the artery walls. Dopamine depletion in the BG and the frontal cortex in PD may affect the function of the SC and, therefore, oculomotion, while its presence in the ANS could explain defects in pupil reactivity (24).

2.7.2 The role of other neurotransmitters

Apart from dopamine denervation, signaling with other neurotransmitters is affected in PD. Studies on the LC focusing on the malfunction of noradrenergic neurotransmission (51), on the role of the dorsal raphe nuclei affecting the serotoninergic neurotransmission (52), and the cholinergic brainstem nuclei, especially the basal nucleus of Meynert that leads to dysfunctional cholinergic transmission (53), explain some of the symptom variability in PD. The role of acetylcholine in cognition, balance, and freezing of gait has been depicted in various studies (13, 53). On the other hand, the LC which is affected in PD, is mainly a noradrenalin-producing nucleus that plays an important role in cognition, attention, and learning, behavioral flexibility, pain modulation, arousal, and wakefulness, as well as pupil reactivity, among other functions (54).

2.8 OCULOMOTOR CHANGES IN PD

2.8.1 Saccades

Saccades can be used to differentiate between various neurological syndromes such as PD and atypical Parkinsonian syndromes (55-57). Hypometric saccades in multiple system atrophy, prolongation of saccadic latency in corticobasal syndrome, and slow saccades together with alterations in their latency in progressive supranuclear palsy, are usually described (58). The most common finding in PD is hypometria of both VGS and voluntary saccades (59) which results in multiple corrective saccades when trying to reach a visual target, described as gaze fragmentation (60, 61). Saccadic latency and amplitude worsen with disease progression (62). A higher error rate in AS is expected in PD (63). The AS task has been studied with functional and imaging methods and it seems to serve as a possible marker of executive function in different populations (64, 65) as well as in early-diagnosed PD patients (66). Study results on

the effect of levodopa administration on the latency of voluntary saccades, such as AS, saccadic amplitudes, VGS latency, and AS error rate, are inconsistent (66-68).

2.8.2 Fixation and pupil reflex

During fixation PD patients have more saccadic intrusions, most of them being square wave jerks (SWJ). The SC and the fastigial oculomotor region of the cerebellum appear to play a role in the control of these movements (69). The PLR has been suggested to be impaired in PD and this can be attributed to both pre-and postganglionic changes (70-72). However, pupil response during fixation in stable light conditions in PD has not been extensively studied.

2.8.3 Reading

Reading difficulties in PD have been studied to some extent, and the role of cognition seems to be important (73-75). Slower reading is a common finding in the existing studies, but whether it is a result of longer fixations, shorter progressive saccades, or multiple regressive saccades is not completely clear.

2.9 GAUCHER DISEASE, THE NORRBOTTNIAN GAUCHER DISEASE TYPE 3 AND THE LINK TO PD

Among the different genes and mutations that are related to PD, homozygous and heterozygous *GBA* gene mutations have been confirmed as risk factors (3, 76-78). The *GBA* gene that is located on chromosome 1q21, encodes the lysosomal enzyme, glucocerebrosidase (GCase), which metabolizes glycosylceramide into glucose and ceramide. The most common lysosomal storage disorder, GD, is caused by homozygous mutations in the *GBA* gene (3).

GD is characterized by decreased enzyme activity that leads to the broad accumulation of glucosylceramide in monocytes-macrophages (79, 80). Despite its heterogeneity, three clinical subtypes are featured: a non-neuronopathic form (GD1), an acute neuronopathic form (GD2), and a chronic neuronopathic form (GD3) (80). GD1 is characterized by hematological, visceral and bone manifestations, while clinical phenotypes GD2 and GD3 present with symptoms from the central nervous system (CNS), including oculomotor disturbance (80). In GD2, signs can be found very early, in utero and infancy but they usually come later in life in GD3 (79). The Norrbottnian subtype of GD3 is found with a high prevalence (1:17.500) in northern Sweden (81). The majority of these patients are homozygotes for the missense mutation L444P (c.1448T>C) in the *GBA* gene (82), receive enzyme replacement therapy (ERT) and they have an longer life expectancy compared to other neuronopathic forms of GD (83). Norrbottnian GD3 patients present with visceral, bone, and hematologic features as well as a saccadic supranuclear gaze palsy (84, 85). Ataxia, myoclonus, and epilepsy, as well as dystonia-like hyperkinetic symptoms have been described with great variability between individuals (83). To date, ERT and Substrate Replacement Therapy (SRT) are the most common treatments, with no effect against neurological symptoms, whatsoever (79, 80). Splenectomy and allogeneic hematopoietic stem cell transplantation (allo-HSCT) were previously among the therapeutic options but are no longer applied.

Epidemiological studies on GD3 have indicated a broad spectrum of neurological symptoms, including cognitive deficits. Little is known about the pathological processes that cause those signs and symptoms from the CNS but it is speculated that, although glucosylceramide accumulates rarely in neurons (80), perivascular accumulation of Gaucher cells (86), and mechanisms that involve glucosylsphingosine (80, 87), could be responsible. It is, therefore, interesting to examine to a greater extent the neurocognitive profile of adult patients with the Norrbottnian type of GD3 with respect to their therapeutic management, surgical and medical.

3 RESEARCH AIMS

During this PhD project, the aim was to study eye movements in PD and detect possible relations between cognitive and clinical aspects of the disease (Studies 1, 2, 3). Additionally, we investigated a special population of GD patients that shares some similarities with PD, regarding their genetic profile and their clinical manifestations. The purpose was to examine their cognitive profile, and control for possible correlations with their eye-movement pattern, along with other clinical parameters (Study 4).

More specifically:

Study 1 aimed to investigate the differences between saccadic movements in PD patients and HC, with respect to various disease parameters.

Study 2 aimed to identify differences in the reading pattern between PD and HC.

Study 3 aimed to study gaze parameters like stability and pupil size during sustained fixation and compare them between PD and HC.

Study 4 aimed to characterize the cognitive aspects of a special sub-population of GD patients tht live in the area of Norrbotten, Sweden.

4 MATERIALS AND METHODS

For the purposes of the first two studies, PD patients and healthy volunteers were recruited at the Outpatient Neurology Clinic of Karolinska University Hospital, in Huddinge, Stockholm, Sweden. Recruitment for the third study took place at the Centre of Neurology, Academic Specialist Centrum in Stockholm, Sweden. In studies 1 and 2 patients were clinically assessed in OFF and ON medication status. In study 3 assessments were only done in ON. Cognition was always assessed in ON, in all three eye-movement studies.

During our last project, Study 4, Norrbottnian GD type 3 patients were recruited at Sunderby Regional Hospital of Norrbotten County in Luleå, Sweden.

4.1 CLINICAL EVALUATION

4.1.1 Movement Disorders Society Unified Parkinson's Disease Rating Scale

The PD cohorts of the first three studies were clinically assessed by neurologists who were trained and certified by the MDS to perform clinical evaluations of PD. The MDS-revised version of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS) (88) which was used in Studies 1 and 2, consists of four parts. The first two parts aim to describe the non-motor and motor experiences of daily living. Part IB and part II can be answered by the patient with our without the aid of caregiver. Part IA, however, requires a clinical interview, and so does the last part, IV, which assesses the motor complications of PD. Part III comprises examination of the motor symptoms.

The UPDRS (89) that was used in Study 3 is a previous version of the MDS-UPDRS that also comprises four parts: part I investigates mental dysfunction and mood; part II addresses the motor disability as it affects the activities of daily living; part III describes the motor examination, and part IV describes the treatment-related motor and non-motor complications of PD. In the first two studies, clinical assessments were performed in both ON and OFF medication status. During the third study, evaluation was done only in ON medication status. Patients in OFF had omitted their dopaminergic medication for at least 12 hours. ON status was assessed approximately one hour after the dopaminergic medication intake.

4.1.2 Hoehn and Yahr scale

The classification of PD progression according to disease severity was done with the Hoehn and Yahr (H&Y) (90, 91) scale (Studies 1 and 2) and its modified version (study 3). The H&Y scale classifies PD patients into 5 stages (7 stages in the modified version) according to their motor symptoms. Unilateral symptoms are characterized as stage 1, progression to stage 2 describes patients with bilateral symptoms but without impairment of balance. Stage 3 refers to the presence of postural instability. Loss of physical independence is classified as stage 4, while confinement to wheelchair or bed describes patients at Stage 5, according to the H&Y scale. In the modified version of the scale, stages 1.5 that includes unilateral and axial involvement, and 2.5 that includes recovery on pull test, were added.

4.1.3 Schwab & England activities of daily living scale

The Schwab & England activities of daily living scale was used for the quantification of the level of patients' independence. The scale can be rated by either the patient or the examiner and it uses percentages between 0% for fully dependent and bedridden individuals to 100% for patients totally unaffected in their everyday activities (92).

4.1.4 Levodopa equivalent daily dose

Dopaminergic medication can include levodopa, dopamine agonists, monoaminoxidase-B (MAO-B) inhibitors, and COMT inhibitors. In the first three studies, we converted PD participants' medication, using the most common conversion factors according to the levodopa equivalent evaluation (93). The equation provides a value called 'levodopa equivalent daily dose' (LEDD) that allows for comparisons between treatment effects.

4.1.5 Modified severity scoring tool

The Norrbottnian GD3 population was clinically evaluated with the modified severity scoring tool (mSST) (94) (study 4), an assessment method that is used to rate the neurological manifestations in neuronopathic GD populations.

4.2 COGNITIVE EVALUATION

4.2.1 Montreal Cognitive Assessment

One of the most common tools for the evaluation of the cognitive status of PD patients is the Montreal Cognitive Assessment test (MoCA) (95). It is a quick screening tool, which takes about 10 minutes to apply, that is used in various clinical settings, PD among others. It comprises seven steps that reflect seven cognitive domains with a maximum score of 30 points and a cut-off score of 26 or more to be considered normal (plus one point for less than 12 years of education). MoCA is translated into Swedish and widely used in the clinical praxis. MoCA was used in studies 1, 2, and 3.

4.2.2 Frontal Assessment Battery

The Frontal Assessment Battery (FAB) is a short 10-minute test, consisting of six parts, used to assess frontal functions. It is particularly useful for the cognitive and behavioral evaluation of patients with neurodegenerative diseases that involve the frontal lobes (96). It was used in studies 1 and 3 as a way to quantify executive function and control for association with eye movements that require activation of frontal areas for the evaluation of their generation, such as in the AS and the sustained fixation task.

4.2.3 Mini Mental State Examination

The Mini Mental State Examination (MMSE) is a commonly used screening tool for dementia (97). As the other assessment tools used in our projects, MMSE is not specific for any disease; it serves rather as a brief measurement of different cognitive domains, focused more on verbal skills than visuospatial and/or constructional praxis.

4.2.4 Repeatable Battery for Assessment of Neuropsychological Status

The Repeatable Battery for Assessment of Neuropsychological Status (RBANS) (98) was used for the evaluation of Norrbottnian GD3 patients. It separately assesses immediate memory, visuospatial and constructional function, language, attention, and delayed memory, and provides a total index score as an estimate of global cognition. It has the advantage of providing index scores and normative data from the Swedish population, computed according to age, easily comparable between different age groups. While it takes almost 30 min to complete, it covers many cognitive domains and is therefore an adequate tool for neurocognitive evaluation.

4.3 EYE MOVEMENT EVALUATION

During the first project, the EyeBrain T2 was used (medical device with CE label for clinical use Class IIa, ISO 9001, ISO 13485). It is a head-mounted binocular eye tracker that uses near-infrared light and has an acquisition speed of 300Hz. Oral instructions were given, and the paradigms included horizontal and vertical VGS, and horizontal AS. We measured latency, mean and peak velocity, and gain for all saccades, along with the error rate for AS. Gain is a measure of saccadic accuracy, computed as the ratio of the saccadic amplitude and the distance from the target. A similar protocol was used for the assessment of eye movements in the Norrbottnian GD3 group (study 4), thus at a previous time point than that of the clinical and neuropsychological assessment (results published 2017) (85). For the assessment of text reading in Study 2, we used the RED250 eye tracker (manufactured by SensoMotoric Instruments, SMI; Germany) which is portable and has a capability of 250 Hz. Quantity and quality of fixations and saccades, progressive and regressive, were assessed during silent reading of a text. Last, during the third study, the eye tracker that we used was the Tobii Pro Spectrum, which is screen-based and has a sampling rate of 1200Hz (manufactured by Tobii Pro AB). The participants were orally instructed to focus on a black dot that was presented on a white screen, for eight trials, 15 sec each. During the task we attempted to quantify the ability of the eye to maintain stable fixation both in time and space. Additionally, with the same eye tracker we measured pupil size during the sustained fixation task.

4.4 STATISTICAL ANALYSIS

For studies 1 and 2, Prism 8 (Graph Pad) was used. Normality was checked with the Shapiro-Wilk test, (two-tailed p-values and significance level of < 0.05). Depending on whether the distribution was parametric or not, comparisons between >2 groups were evaluated using ANOVA and Dunn's post-hoc test with Bonferroni correction, or Wilcoxon rank test. Student's t-tests between HC and PD groups, and paired t-tests for comparison within PD subjects in ON and OFF medication states, were used. The Mann Whitney test was used when normal distribution criteria were not fulfilled, and for nonparametric data. Associations between the eye-movement parameters and cognitive/clinical ones were assessed using Pearson's correlations with false-discovery rate correction for multiple testing.

For the purposes of studies 3 and 4, we used the IBM SPSS 25 Statistic Data Editor. Non-parametric tests were used because normality criteria were not fulfilled, as well as for

nonparametric data. The significance level was defined at 0.05. The Mann Whitney test was used for comparisons; Spearman correlation was used to investigate the association between various parameters; logistic regression analysis was performed to investigate the strength of association of clinical and cognitive parameters to those of eye movements, as predictors of diagnosis (HC vs PD). When the final model was decided, Receiver Operating Characteristic (ROC) curve was plotted to visualize its separation potential between the two groups (PD vs HC). The study sample of study 4 allowed for simple descriptive analysis of the population's data along with a between-group Mann Whitney comparison for small groups.

4.5 ETHICAL CONSIDERATIONS

All studies include participants, patients and healthy populations that underwent clinical and cognitive testing. Written and oral informed consent was obtained according to the Helsinki declaration regulations. It was important to explain the process but also take care of the participants when there were unexpected, pathological findings or complications. Participants' data were treated anonymously, all subjects were given a code name, and the key to the files was archived for all studies.

We have applied and received approval for all studies included in the research project. In detail:

Study 1: Diarienummer 2016/348-31/4

Study 2: Diarienummer 2016/19-31/2

Study 3: Diarienummer 2018/437-31/2

Study 4: Diarienummer 2016/19-31/1 and amendment 2017/1957-32/1.

5 RESULTS

5.1 STUDY 1

5.1.1 Participants

Demographics and clinical characteristics of our study groups are shown below. In total 60 participants were included, 20 in each group, HC, PD H&Y2, and H&Y3 (Table 2).

Table 2. Clinical and demographical characteristics of PD patients and HC in Study 1.

	HC	**PD**	**p**	**H&Y2**	**H&Y3**	**p**
n	20	40		20	20	
Gender, Male/Female	12/8	28/12		15/5	13/7	
Age, years	65.9 (7.6)	65.6 (9.1)	0.2	62.2 (9.0)	69.2 (7.7)	**0.01**
MoCA	26.9 (1.9)	25.3 (3.7)	**0.04**	26.0 (3.1)	24.6 (4.1)	0.3
FAB	16.7 (1.4)	15.5 (2.7)	**0.045**	15.8 (2.8)	15.3 (2.5)	0.6
Disease duration, years		4.9 (3.4)		3.5 (2.5)	6.3 (3.6)	**0.007**
LEDD, mg/day		528 (235)		479 (231)	580 (227)	0.2
MDS-UPDRS III OFF		38.4 (11.6)		34.1 (9.8)	42.9 (11.6)	**0.02**
MDS-UPDRS III ON		25.5 (10.8)		21.0 (9.6)	30.5 (9.8)	**0.004**

Values are reported as means (standard deviations). Comparisons between HC and PD were done with the t-test, while the ones between H&Y 2 and 3 were done with the paired t-test. Statistically significant differences at the level of <0.05 are marked with bold. HC: Healthy Controls, PD: Parkinson's Disease, H&Y: Hoehn & Yahr, MoCA: Montreal Cognitive Assessment, FAB: Frontal Assessment Battery, LEDD: Levodopa Equivalent Daily Dose, MDS-UPDRS: Movement Disorders Society Unified Parkinson's Disease Rating Scale.

5.1.2 Saccades

PD participants performed horizontal and vertical VGS with a significantly smaller gain, irrespective of H&Y stage ($p<0.0001$). Gain was not computed for AS. Although the horizontal VGS latency did not differ between groups, vertical VGS latency was prolonged in the PD group. AS latency was also prolonged in the PD group. Interestingly, H&Y2 patients performed less directional errors than the H&Y3 patients in the AS task. A summary of the results is presented in Table 3.

Table 3. Results of ANOVA comparing HC and PD patients in H&Y stage 2 and 3 (in OFF medication state) in Study 1.

	HC	**PD H&Y2**	**PD H&Y3**	**p**
Step horizontal				
Latency, ms	254.7 (40.3)	284.7 (58.6)	279.5 (34.1)	0.1
Gain	0.96 (0.03)	0.90 (0.07)*	0.88 (0.04)*	**<0.0001**
Step vertical				
Latency, ms	245.3 (27.3)	303.8 (68.0)*	303.5 (42.8)*	**0.0002**
Downwards gain	0.98 (0.09)	0.89 (0.10)*	0.85 (0.10)*	**0.0005**
Upwards gain	0.87 (0.11)	0.71 (0.11)*	0.69 (0.13)*	**<0.0001**
Antisaccades				
Latency, ms	282 (39.2)	374.5 (107.2)*	387.3 (58.1)*	**0.0002**
AS error rate	0.18 (0.17)	0.25 (0.14)a	0.47 (0.32)*a	**0.0003**

*Values are reported as means (standard deviations). Between-group differences compared to HC are shown as * using Dunn's post hoc test with Bonferroni correction with a significance level of $p < 0.05$.* a *indicates a significant between-group difference between H&Y2 and H&Y3. Statistically significant differences at the level of $p<0.05$ are flagged with bold. HC: Healthy Controls, PD: Parkinson's Disease, H&Y: Hoehn &Yahr, AS: antisaccades.*

Further analysis to recognize possible correlations between saccadic performance and the clinical and cognitive characteristics of PD patients, revealed a negative correlation between the vertical VGS gain and the MDS-UPDRS score (upwards: $p = 0.01$, downwards: $p = 0.02$). AS latency correlated with the axial MDS-UPDRS ($p=0.043$), AS error rate correlated negatively with MoCA ($p= 0.018$) and FAB ($p= 0.0041$). ROC analysis revealed the greatest

AUC for upwards gain among the other saccade parameters for the discrimination of PD patients from HC individuals (0.85; 95% CI: 0.75–0.95, p<0.0001).

5.1.3 The effect of levodopa

Eye tracking in ON and OFF yielded some interesting results. First, medication had no effect on VGS gain, neither horizontal (p=0.6) nor vertical (downwards p=0.4, upwards p=0.8). Levodopa significantly prolonged the latency of the horizontal (p=0.03), but not the vertical VGS (p=0.2). Despite some prolongation of the vertical VGS latency in ON compared to OFF, the difference did not reach statistical significance (p=0.2).

Contrary to VGS, AS latency was shortened after levodopa intake (p=0.04), but error rate was not affected (p=0.5). A more detailed analysis by H&Y staging revealed that only H&Y2 patients performed the AS task with a shorter latency after levodopa intake (p=0.02), but not H&Y3 patients (p=0.6). Instead, the H&Y3 group had improved AS error rate after levodopa intake (p=0.03). Results are presented in summary in Table 4.

Table 4. Within-subject comparisons with OFF and ON medication state in Study 1.

	PD OFF	**PD ON**	**p**
Step horizontal			
Latency, ms	282.2 (48.6)	291.9 (54.7)	**0.03**
Gain	0.89 (0.06)	0.88 (0.08)	0.6
Step vertical			
Latency, ms	303.7 (57.2)	310.6 (60.3)	0.2
Downwards gain	0.87 (0.10)	0.72 (0.13)	0.4
Upwards gain	0.70 (0.12)	0.69 (0.13)	0.8
Antisaccades			
Latency, ms	372.0 (83.6)	352.8 (86.6)	**0.04**
Express saccade rate	0.07 (0.11)	0.08 (0.15)	0.4
AS error rate	0.35 (0.27)	0.32 (0.23)	0.5

Values are reported as means (standard deviations). PD: Parkinson's Disease, AS: antisaccades. Statistically significant differences at the level of p<0.05 are flagged with bold.

5.2 STUDY 2

5.2.1 Participants

Participants in our second pilot study on reading comprised two groups: 13 HC and 19 PD patients in H&Y≤3. Patients were assessed in both OFF and ON medication status. Their clinical and demographical characteristics are presented in table 5.

Table 5. Clinical and demographical characteristics of PD patients and HC in Study 2.

	HC	**PD**	**p**
n	13	19	
Age, years	69.4 (3.9)	66.1 (8.4)	NS
Education, years	14.1 (2.9)	13.7 (3.6)	NS
Disease duration, years		4.8 (3.7)	
LEDD		547 (207)	
MDS-UPDRS III OFF		41 (11)	
MDS-UPDRS III ON		27 (10)	
MoCA	26.3 (1.7)	25.4 (3.9)	NS

Values are reported as means (standard deviations). HC: Healthy Controls, PD: Parkinson's Disease, LEDD: Levodopa Equivalent Daily Dose, MDS-UPDRS: Movement Disorders Society Unified Parkinson's Disease Rating Scale, MoCA: Montreal Cognitive Assessment. NS: Non-significant.

5.2.2 Reading parameters

PD patients read fewer words per minute than HC, performed more regressive saccades and had longer mean fixation durations. In the OFF state, cognitively impaired patients (MoCA <26) presented with longer fixation durations compared to HC and PD with normal MoCA score. Only the mean fixation duration correlated with MoCA score in PD ($p=0.002$, $R^2=0.46$). Further, disease duration correlated with words per minute in OFF ($p=0.01$; $R^2=0.3$). Levodopa did not affect the reading parameters. Results are presented in Table 6.

Table 6. Reading parameters for HC and PD patients in ON and OFF medication status and comparisons between groups in Study 2.

	PD ON	PD OFF	HC	HC-ON	HC-OFF	ON-OFF
Words per minute	185.6 (67.27)	195.9 (63.31)	243.4 (58.37)	*[1]	*[1]	NS[3]
Number of fixations	156.9 (46.38)	156.7 (57.73)	131.5 (26.2)	NS[1]	NS[1]	NS[3]
Mean fixation duration	269.1 (56.45)	256.5 (38.64)	229.6 (30.41)	*[2]	*[2]	NS[4]
Number of saccades	108.5 (30.7)	106.7 (42.62)	91.92 (21.07)	NS[1]	NS[1]	NS[3]
Mean saccade amplitude	1.709 (0.65)	1.803 (0.71)	2.12 (0.87)	*[2]	NS[2]	NS[4]
Number of regressions	34.89 (20.07)	35.05 (21.33)	22.92 (13.14)	*[1]	*[1]	NS[3]
Mean regression amplitude	0.976 (0.39)	1.032 (0.65)	1.137 (0.43)	NS[2]	NS[2]	NS[4]
Ratio saccades / regressions	68.34 (14.81)	66.64 (14.19)	75.45 (10.52)	NS[2]	NS[2]	NS[4]

*Values are reported as means (standard deviations). Comparisons between HC and PD in ON, and OFF and paired comparisons between PD in ON and OFF. [1] Mann Whitney test, [2] Student's t-test, [3] Wilcoxon rank test, [4] paired t-test. NS: non-significant. SD: standard deviation, PD: Parkinson's Disease, HC: Healthy Controls. * flags statistical significance $p<0.05$.*

5.3 STUDY 3

5.3.1 Participants

For the third study, 43 HC were included along with 50 PD patients of H&Y≤3. Assessments were only performed in ON. Participants' characteristics are summarized in Table 7.

Table 7. Clinical and demographical characteristics of PD patients and HC in Study 3.

	HC	**PD**	**p**
n	43	50	
Gender, Male/Female	16/27	33/17	**0.006**
Age	63 (16)	64 (10.5)	0.728
Education, years	15 (5)	16 (4)	0.895
Age at diagnosis		62 (11.5)	
Years since diagnosis		2 (2.5)	
LEDD		545 (523.75)	
UPDRS part 1		1 (2)	
UPDRS part 2		10 (6)	
UPDRS part 3		21 (15.5)	
UPDRS part 4		2 (3.25)	
UPDRS total		36.5 (21.75)	
Schwab & England		90 (10)	
MoCA	27 (3)	27 (3)	0.2
MMSE	29 (2)	28 (2)	0.4
FAB	18 (2)	17 (3)	0.1

Values are reported as medians and interquartile ranges (IQR), number of participants and gender ratio reported in absolute values. Differences at the level of $p<0.05$ are flagged with bold. HC: Healthy Controls, PD: Parkinson's Disease, LEDD: Levodopa Equivalent Daily Dose, UPDRS: Unified Parkinson's Disease Rating Scale, MoCA: Montreal Cognitive Assessment, MMSE: Mini Mental State Evaluation, FAB: Frontal Assessment Battery.

5.3.2 Fixation

During the fixation task, HC maintained a more stable gaze while focusing on a target and the task was less interrupted compared to PD patients. In more detail: fixation duration, mean ($p=0.007$) and median ($p=0.016$), were shorter in the PD group compared to HC, and the same

was indicated when comparing the longest fixation period between the groups (p=0.008), with less saccades that interrupted the task (p=0.015). Additionally, while fixating on a target in stable light conditions, PD patients presented with smaller pupils than those of HC, as indicated by smaller mean (p = 0.002) and median (p=0.003) pupil diameters. For details see Table 8.

Table 8. Comparisons of eye-movement parameters in PD and HC in Study 3.

	HC	**PD**	**p**
n	43	50	
Mean pupil size	2.5025 (0.3)	2.3527 (0.31)	**0.002**
Median pupil size	2.4925 (0.29)	2.3625 (0.31)	**0.003**
Mean fixation duration	3.026 (4.25)	1.321 (3.93)	**0.007**
Median fixation duration	2.55 (4.48)	0.7336 (5.09)	**0.016**
Longest fixation period	6.0954 (5.46)	4.346 (5.34)	**0.008**
Saccade rate	0.4448 (0.97)	1.1126 (2.49)	**0.015**

Values are reported as medians and interquartile ranges (IQR). Statistically significant differences at the level of p<0.05 are flagged with bold. HC: Healthy Controls, PD: Parkinson's Disease; Longest fixation period: The maximum period of fixation uninterrupted by saccades, blinks or noise (sec); Mean and median pupil size computed in mm; Mean and median fixation duration computed in sec; Saccade rate: The number of detected saccades per second.

Additional analysis with univariate models was performed to explore predictors of diagnosis. The multivariate model with the best predictive ability included the median pupil size (OR 0.811; 95% CI 0.666–0.987; p=0.037), longest fixation period (OR 0.798; 95% CI 0.691–0.921; p=0.002), sex (OR 4.35; 95% CI 1.516–12.483; p=0.006), and visuospatial/executive score in MoCA (OR 0.422; 95% CI 0.233–0.764; p=0.004). The area under the curve of the model in the subsequent Receiver Operating Characteristic (ROC) analysis was 0.817; 95% CI 0.732–0.901 (Fig 2).

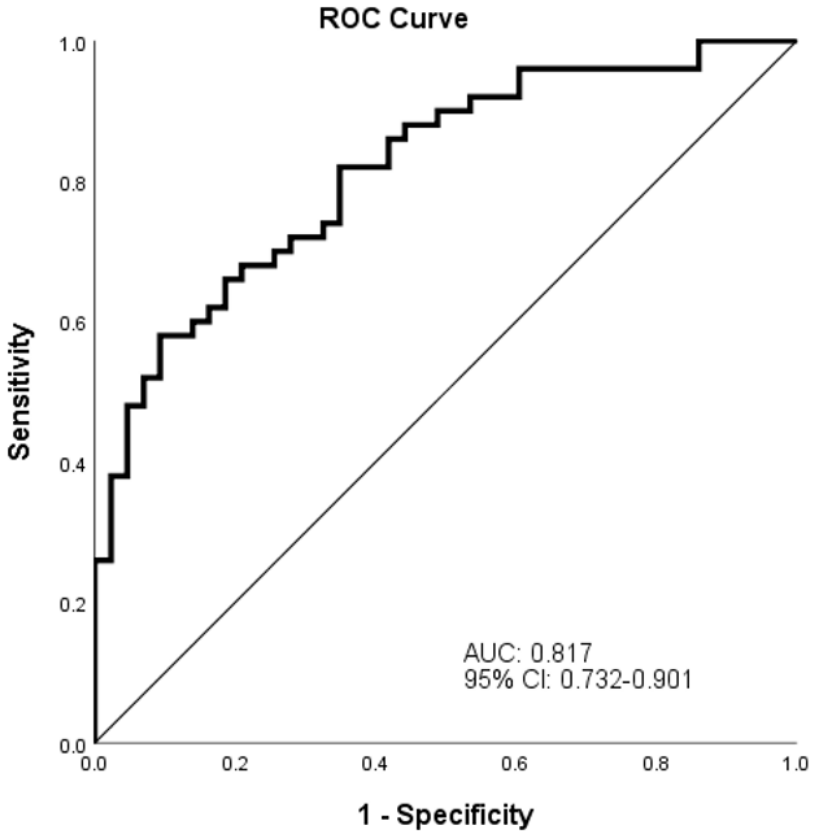

Figure 2. *ROC curve of the multivariate model that includes longest fixation period, median pupil size, sex, and visuospatial/executive subscore of the Montreal Cognitive assessment test. AUC: Area under the curve; CI: Confidence interval, ROC: Receiver Operating Characteristic.*

5.4 STUDY 4

5.4.1 Participants

Ten patients diagnosed with the Norrbottnian type of GD3 were included in Study 4. The patients varied phenotypically, and this is indicated not only by the variability in their disease characteristics (history of epilepsy and mSST score), but also by the different treatments they received (ERT, allo-HSCT, splenectomy) (Table 9). It is important to notice that, although the majority of patients were homozygous for the most common L444P mutation in the *GBA* gene, there was one patient with the L444P/A341T genotype.

Table 9. *Clinical and demographical characteristics of Norrbottnian GD type 3 patients in Study 4.*

	Sex	Age	Mutation	Age at Diagnosis	SPC/Age	Epilepsy/ Age at Diagnosis	Therapy/Age	mSST
1	F	32	L444P/L444P	2	Y/2	Y/16	allo-HSCT /2	7.5
2	F	39	L444P/L444P	7	Y/10	N	ERT	5
3	M	29	L444P/L444P	2	Y/3	Y/17	ERT	11.5
4	M	52	L444P/L444P	5	Y/13	Y/45	ERT	14
5	F	44	L444P/L444P	2	Y/8	Y/23	allo-HSCT /9	18
6	M	30	L444P/L444P	3	N	Y/14	ERT	15
7	F	51	L444P/L444P	1	Y/10	N	ERT	5.5
8	F	57	L444P/L444P	3	Y/3	N	ERT	12.5
9	M	51	L444P/L444P	1	Y/10	N	ERT	13.5
10	M	24	L444P/A341T	1	N	N	ERT	1.5
Median (IQR)		41.5 (21.5)		2 (3)	9 (7)			12 (8.88)

Values are reported as medians and Interquartile Ranges (IQR). Age is presented in years. SPC: splenectomy, ERT: enzyme replacement therapy, allo-HSCT: allogeneic hematopoietic stem cell transplantation, Y: yes, N: no, F: female, M: male, mSST: modified severity scoring tool.

5.4.2 RBANS

The phenotypical variability among patients was reflected on their cognitive results. Individual assessments are schematically shown in Fig 3. At a group level, cognitive evaluation of Norrbottnian GD3 patients showed that they performed better on domains like visuospatial/constructional, language, and delayed memory domains, reaching scores at the lower part of the average, according to qualitative criteria provided by the manufacturer. However, performance on the immediate memory tests was clearly below average, whereas attention, and the total index score, were significantly below average (Fig 4).

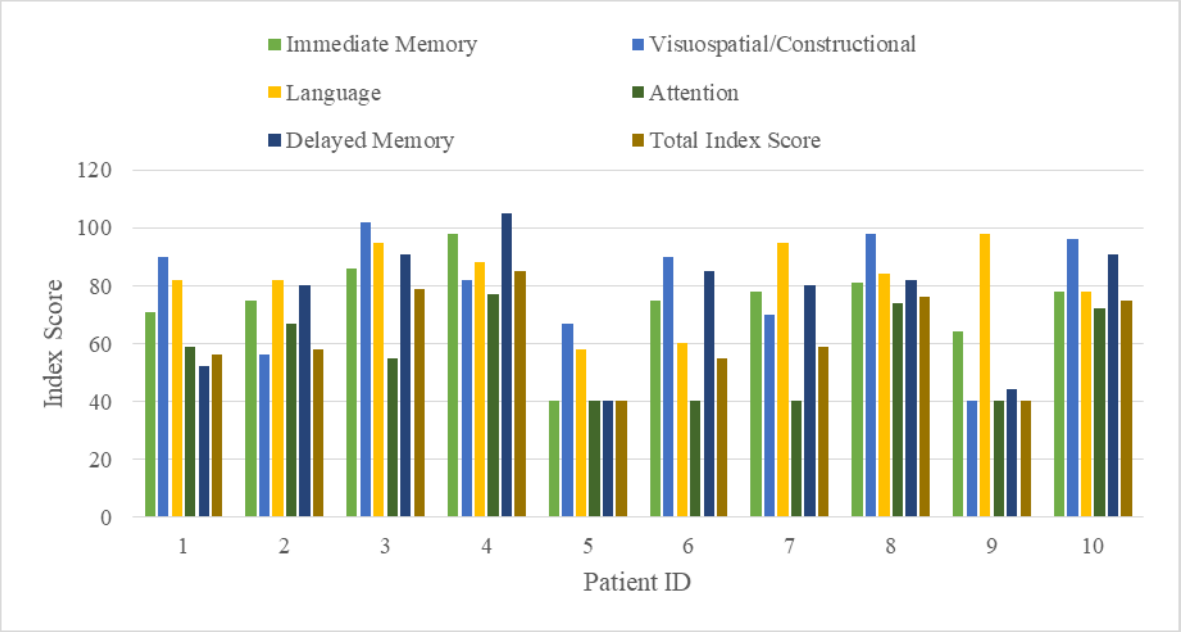

Figure 3. *Individual assessments for each cognitive domain, and the total index score of the RBANS test.*

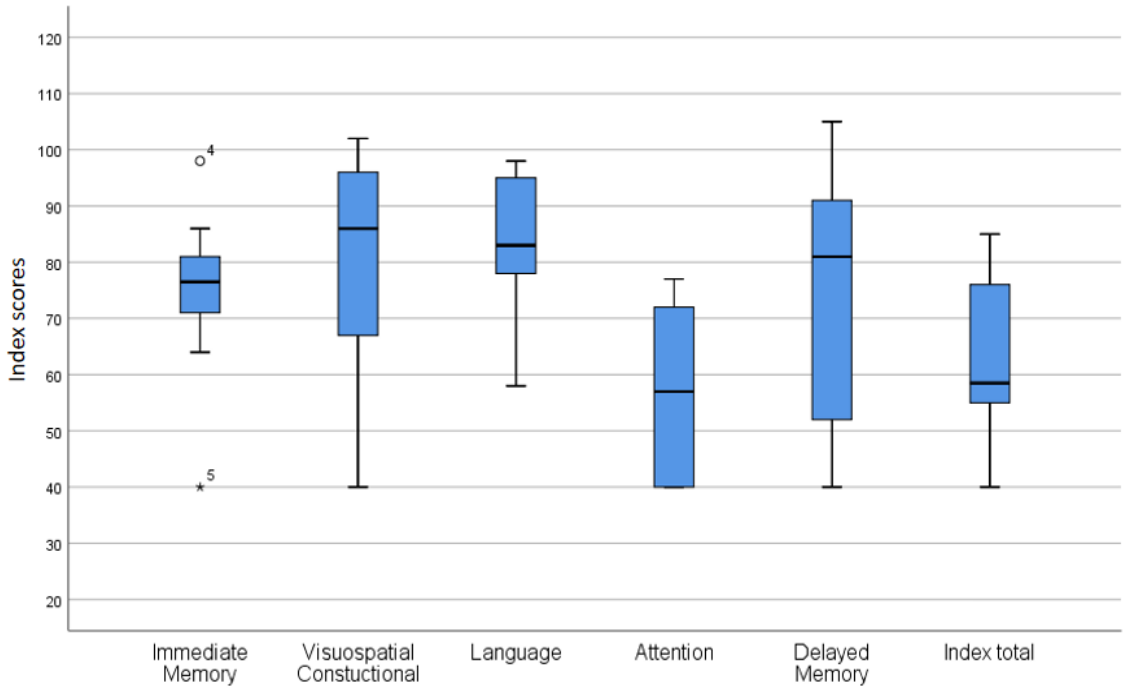

Figure 4. *Boxplots of the RBANS index subscores and the total index score for the Norrbottnian GD3 population. * : extreme outlier, o: mild outlier.*

6 DISCUSSION

One of the most common problems regarding PD is the difficulty to provide early diagnosis and accurate prognosis, and thereby be able to plan appropriate clinical care and support. This PhD project examined whether eye movements can be used as a tool to differentiate between HC and PD patient groups that vary clinically in terms of cognition and disease progression/ H&Y stage. We managed to identify differences between PD and HC, and found that some of the eye-movement parameters were related to disease parameters. This opens the avenue for examining eye movements in persons with very early signs and symptoms of PD thereby facilitating earlier diagnosis. In addition to the traditional assessment of horizontal saccades, our studies indicate that examination of vertical saccades and AS, reading, and sustained fixation, can provide potential biomarkers for PD. We assessed patient groups of different H&Y stages but prospective studies could enable the exploration of oculomotor changes over time, to examine the use of these biomarkers in PD prognosis.

6.1 STUDY 1

Summarizing our results, of all VGS parameters, gain was significantly reduced in PD compared to HC in both horizontal and vertical VGS, and it was correlated with motor performance. Latency was prolonged during vertical VGS and AS in PD compared to HC. The error rate was only worse in the H&Y3 group of PD patients but improved after levodopa intake. Levodopa treatment did not affect the gain, prolonged latency in reflexive saccades, mainly the horizontal ones, and shortened it for AS, in the less affected PD group.

6.1.1 Gain and the BG-SC connection

The hallmark of PD pathology is dopamine denervation in the brain, the imbalance between the direct and indirect pathways, and the subsequent inhibition of movement via the thalamus. It has been suggested that, in a similar way that the dopamine deficit in PD affects motor performance, it also dysregulates the direct and indirect pathway in the BG, and consequently enhances the inhibition on the SC, thus producing saccades with shorter amplitude (45, 99). Implications of a direct projection from zona incerta to the SC support the idea of further dopaminergic control on the SC (100). Our results are in accordance with this hypothesis, because gain was smaller in the PD group in all saccadic movements. Additionally, it was the upwards gain that could discriminate between HC and PD. The fact that gain was correlated with motor performance is not surprising, given that BG function worsens with disease progression, affecting at the same time the SC function.

6.1.2 Latency and the cortex - The antisaccadic movements

Latency control involves the cortex. Before initiating a saccade, multiple processes need to take place, engaging cortical areas like the FEF, PEF, and DLPFC (33). Attention needs to be shifted towards the target, and this takes longer in the case of volitional saccades because the brain needs to inhibit other unwanted eye movements. The eyes need to be disengaged from fixation and compute the amplitude and velocity of the eye movement to reach the target as accurately

as possible (101). The complexity of the process was, therefore, expected to lead to a prolonged latency, mainly in the AS, in the PD group. In our population, latencies of the reflexive saccades were also prolonged in the PD group, although only the difference in vertical reflexive saccade latency between PD and HC was statistically significant.

We also found that the error rate in the AS task was higher for PD patients in a later disease stage. Higher error rates and longer AS latencies in PD have been confirmed with magnetoencephalography studies that showed altered activation of frontal areas during the AS task (102). Additionally, we found that AS latency tended to correlate with the axial MDS-UPDRS subscore, suggesting a common mechanism between postural stability and the AS task. Indeed, this has also been suggested before. The role of the PPN and cuneiform nucleus has been discussed in this context. These nuclei are involved in postural and oculomotor control networks, receive projections from the frontal cortex and project to the SC (103). Last, it is important to mention the correlation of error rate in AS with FAB, which measures executive function and attention, emphasizing the role of cognition in the AS task.

In summary, our finding of affected gain in all saccadic movements in PD while latency is prolonged in the volitional ones, and to a lesser extent to VGS, is in accordance with existing literature that supports that the BG are mainly involved in the volitional saccade generation, which in our studies was tested with AS. Reflexive saccade generation bypasses the BG (44, 99). In addition to previous study results, we found that vertical saccadic gain is a promising marker for diagnosing PD, but this should be further confirmed with studies at earlier stages of the disease.

6.1.3 The role of levodopa

Another important finding was the effect of levodopa on saccadic performance. More specifically, levodopa did not affect the gain, but was associated with prolonged horizontal VGS latency. Contrary to that, AS error rate improved after levodopa intake, while latency only improved in the H&Y2 group but not in the more advanced patients. Our results are in accordance with previous studies that support that levodopa enhances inhibition on the SC and consequently worsens the performance of the reflexive saccades, while it ameliorates volitional saccadic performance due to the reduced inhibition of the frontal cortex by the BG (67). AS generation requires activation of complex pathways: Inhibition of unwanted reflexive saccades from the DLPFC, activation of the FEF and SC with direct FEF-SC projections but also the FEF-BG-SC loop. This last loop is reciprocal and leads to inhibition of frontal areas from the BG during AS in PD, so that eye movement generation is delayed (104). After levodopa intake, the process is facilitated due to the partial removal of inhibition of the frontal areas, leading to a shortening of AS latency. A possible mechanism that could explain the prolongation of the reflexive saccadic latency, is the restricted control of BG-mediated frontal evaluative processes on the PEF, and consequently generation of reflexive saccades with short latencies. In ON, frontal control on the PEF is more effective, and latency is prolonged thus restricting, in a way, their reflexive character.

Vertical saccades, on the other hand, are more complex and their generation and control employ different brain areas than those of the horizontal ones (55). Our results could imply that non-dopaminergic signalling is involved in their initiation since levodopa did not affect their latency, contrary to that of horizontal VGS.

6.1.4 Strengths and limitations

Literature on horizontal eye movements is abundant, probably because their oculomotor pathway is simpler, making results easier to explain. Our study on reflexive and volitional saccades is among the few that examine vertical saccades in PD. In addition to that, we studied AS that seem to serve as a promising marker of frontal function. Last, contrary to most studies on oculomotion, we performed assessments in OFF and ON. Assessments in OFF are practically difficult and require a better planning and cooperation from the patients, therefore literature on the effects of levodopa on ocular motor control is restricted. Our aim was to examine the role of dopaminergic medication on the regulation of neural circuits in the brain. Indeed, our results are very interesting, but need to be confirmed by similar studies.

Study 1 has several limitations. First, our groups did not only differ in PD stage but also in age and cognition. Second, we only included patients with a relatively narrow spectrum of symptom severity. Patients with PDD were not included, nor patients in late stages of the disease (H&Y 4 and 5). This would possibly provide interesting results, however, planning such a study would be more resource consuming given the practical difficulties of OFF evaluation in patients with advanced disease, along with the difficulties expected to be encountered in following instructions and using a head-mounted eye tracker. Nevertheless, more modern, screen-based eye trackers are more comfortable. Furthermore, detailed neuropsychological evaluation was not part of the study, but it could provide important insights for the interpretation of our results regarding the role of cognition on the saccadic generation. Regarding evaluation in ON and OFF, patients that used dopamine agonists and MAO-B inhibitors, agents that have a long half-life, were instructed to refrain from their use for at least 12 hours, as they were instructed to do with levodopa. Consequently, the OFF assessment for these patients might not have been true OFF. Practically, though, it would have been impossible to ensure a total lack of dopaminergic medication in our PD patients for the purposes of our study. Last but not least, a larger sample size that would include patients with prodromal symptoms, at risk for PD, would have provided more robust conclusions.

6.2 STUDY 2

During this small pilot study, we examined differences in eye movements between HC and PD in a more pragmatic environment during reading. In summary, fixation duration was significantly longer in PD compared to HC, especially when cognition was impaired, regression frequency was higher in PD, and fixation duration correlated with MoCA score. The medication state did not seem to make any difference in the reading performance of PD patients.

6.2.1 Reading

Reading is a demanding task that requires a sequence of fixations and saccades to see the words, but also cognitive processes to understand the text. While the eyes fixate on a word, cognitive processes take place in order for new information to be acquired (39). Studies that compared pseudo-reading with natural reading have shown that, in natural reading saccades are less reflexive, fixation is under greater control, and a high automatic phonological processing is employed, requiring less attentional resources compared to pseudo-reading (105). Longer fixation duration during reading could be related to deficits in decision-making processes, attention, and working memory, which are known to be affected in PD, thus reflecting the cognitive load that prolongs word processing (106). Moreover, sentence comprehension is impaired in PD, probably due to deficits in working memory (107). Interestingly, reading in Alzheimer's disease is slower due to longer fixation durations, short saccadic amplitudes while the eyes move to the next word, as well as a higher number of regressions both to the previous words and within the same word (108). In conclusion, our results suggest that PD patients' reading difficulties might be attributed to cognitive deficits, rather than pure oculomotor impairment.

6.2.2 Strengths and limitations

At the time of the recruitment of participants for this project, literature on reading in PD was extremely limited. Since then, a few more studies have been published describing reading assessments in PD patients. Our study is, therefore, among the first that examined reading in PD in ON and OFF.

Our results have to be interpreted while keeping in mind the limitations of the project. This was a pilot study and due to the small sample size, our results have limited generalizability. Additionally, given that cognition plays an important role, a more detailed evaluation and characterization of the cognitive status of the participants would add to the strength of our conclusions.

6.3 STUDY 3

Our third project aimed to describe the features of stable gaze and the differences between PD and HC. Our main findings indicate that PD patients make more interruptions during sustained fixation as indicated by the higher saccade rate and the shorter fixation durations in the PD group compared to HC. Additionally, we found that while focusing on this simple task, PD participants did not dilate their pupils to the same extent as HC.

6.3.1 Sustained fixation and attention

Sustained fixation has been discussed as an indicator for attention and cognition, as well as visual perception, and mechanisms that control it do not differ from those of saccadic and smooth pursuit control (35). Both gaze stability and corrective movements are important parameters of sustained fixation, and the extent to which HC and PD succeed in maintaining focus or break this process can contribute to PD diagnosis. This was obvious in our study where

PD patients interrupted the task more easily than HC participants did. Saccadic intrusions are well documented in PD, usually identified as square wave jerks (SWJ), attributed both to impaired inhibition of unwanted movements from the DLPFC, but also from a compensatory FEF activity due to excessive inhibition of the SC (27, 69, 99). The nature of the movements that interfered with sustained fixation in our study was not studied, therefore we can only speculate that they were SWJ, although they could also have been microsaccades or express saccades. Whether these deficits in fixation control are attributed to attentional deficits in the PD group cannot be concluded from our results as PD and HC did not differ in any cognitive test. A more thorough cognitive evaluation focused on attention, though, might have answered this question.

6.3.2 Pupil size during sustained fixation

Pupil size was smaller in PD than in HC, a finding that possibly reflects deficits in cognitive processes related to attention, as previously suggested (109). We hypothesize that the role LC as a regulatory for the ANS nucleus which is affected in PD and is also related to cognitive impairment, can partly explain our findings (54, 110). Recent research in mice suggests that LC alone cannot be accounted for the pupil diameter during stable luminance, but the role of arousal and attention is equally signfficant (111). It would be oversimplifying to attribute our findings solely to the LC degeneration in PD. Other parameters, that include both pre- and postganglionic defects of the pupil innervation (112), along with cognitive processes, should be taken into consideration.

6.3.3 Can the sustained fixation task identify PD?

Our study suggests that, fixation duration, pupil diameter, sex, and the visuospatial score in MoCA can serve as discriminators between PD and HC, when put together in a model. However promising, this model is not optimal. The visuospatial score in MoCA is a simple measurement, and the only one that differed between PD and HC groups. Our group of participants was tested clinically and cognitively with MoCA, FAB, and MMSE and we could only identify weak correlations between fixation parameters and the MMSE score. Our explanation for this finding, apart from the fact that MMSE is not optimal for the cognitive assessment of PD and its ceiling effect (113), was that we only included participants with overall good performance in cognitive tests and none that was diagnosed with dementia. There are previous studies that have also pointed out that gaze distractibility in PD is common, without identifying any direct dependence on disease stage and cognition, discussing however the role of executive dysfunction (114). Using the fixation task alone to identify PD, is rather optimistic, it could, nevertheless, together with other parameters, serve as a possible biomarker.

6.3.4 Strengths and limitations

Although studying eye movements has been popular in the field of neurodegenerative diseases, studies on fixed gaze are scarce. The same applies to studies on the psychosensory pupil reflex where luminance conditions (examining PLR) are stable and the need for accommodation (examining PNR) is minimal. Moreover, pupils are usually studied using pupilometers and not

eye trackers. Our study has the advantage of studying both parameters, gaze stability and pupil response at the same time. We used a simple paradigm that requires minimal cognitive and emotional effort, excluding, to a large extent, distractors and factors that can affect pupil size.

Despite its strengths, however, this study has some limitations. As already discussed in our publication, differences in sex distribution between HC and PD may have an effect on our results regarding pupil size, although there is inconsistency in previous study findings on the topic (115, 116). Additionally, we only examined our patients in ON medication status, and didn't identify correlations between LEDD and performance on the task. Assessment in OFF would have been interesting because it cannot be excluded that our results were affected by medication side effects. These can include affection of the sympathetic or parasympathetic systems that may affect pupil size. Our study groups did not differ in the cognitive testing, which was only investigational and not thorough. MMSE correlations with some of the parameters are problematic as the test is not optimal for evaluating PD patients, due to a lack of adequate executive function evaluation. Finally, our study did not include characterization of the eye movements that interrupted fixation. Given that eye movements were recorded with a screen-based tracker and that patients were allowed to move their heads to some extent, it is questionable whether this setting would allow for the discrimination of the type of eye movements that disrupted fixation.

6.4 STUDY 4

Our last project studied cognition in a special subpopulation of GD, namely the Norrbottnian GD3 patients. This special, small group of patients (only ten patients in our study) has a relatively good quality of life and life expectancy, partly due to new therapeutic options.

6.4.1 Cognitive profile of Norrbottnian GD3

The results of Study 4 indicate that Norrbottnian GD3 patients have a relatively spared language and visuospatial ability, while they present deficits in immediate memory and attention domains. Similar results have been published on young GD3 patients (117) where language performance was intact, but processing speed was affected. Their findings however also included visuospatial deficits, while our group performed well on visuospatial testing. Age and methodology differences could explain this discrepancy. Attentional deficits and overall good language skills were also described in a large longitudinal study on GD3 (118), despite methodological differences between this and our study. We also tested for differences between patient groups based on previous splenectomy, antiepileptic medication, and previous allo-HSCT or ERT, but the small number of patients and heterogeneity of their symptoms did not allow for safe conclusions.

6.4.2 How is Norrbottnian GD3 linked to PD?

Norrbottnian GD3 patients share a common genetic background with some PD patients who carry mutations in the *GBA* gene. The missense mutation L444P, which is the most common for Norrbottnian GD3, is associated with worse cognitive performance for PD patients that

carry it, especially regarding performance in working memory and executive function (119). Our findings confirmed worse attention and executive function in the Norrbottnian GD3 group, but did not confirm the visuospatial deficit. A direct comparison, however, between PD and the Norrbottnian type of GD3 is impossible and not important.

6.4.3 Strengths and limitations

The greatest advantage of our study is the characterization of an interesting population that is impossible to find elsewhere. These patients have been followed up longitudinally concerning other disease parameters, and information on their genetic background, comorbidities, and treatment is registered in detail. Neurocognitive evaluation adds to their detailed follow-up.

At the same time, our results are difficult to generalize, not only because the population is so small but also very unique. Such rare conditions present with distinct phenotypes. Similarities with other conditions, like PD in our case, may, however, provide insights into pathophysiological mechanisms that concern these clinical entities. A cross-sectional evaluation of cognition is rather indicative of the patients' profile at the time of the assessment than informative regarding their overall cognitive performance in time. Longitudinal assessments with RBANS would add a lot to the results of the present study.

7 CONCLUSIONS

Our studies on eye movements were mainly focused on examining oculomotor alterations, a feature that is commonly described in PD but not routinely assessed on a clinical basis. The main challenge since the beginning of the project was to examine if these assessments could be done in a practical and easy manner, comfortable for both the participant and the examiner, and still provide useful data. Our results confirm most of the already known findings, which confirms the initial idea of reproducibility in the clinical setting. Most important though, they have highlighted novel aspects of eye movement examination, using paradigms that have not been used to a great extent that provided interesting results. We can, therefore, hypothesize that eye tracking could safely be integrated in the overall clinical examination of patients with PD and other neurodegenerative disorders. Further studies that would examine patients of various disease stages and cognitive states are needed to confirm our results. Longitudinal assessments can reveal the usefulness of these results in evaluating PD prognosis.

Studies on rare disorders are of great importance, despite small patient groups and limited generalizability. GD has been an important source of information for PD mechanisms, and the longitudinal follow-up of Norrbottnian GD3 patients is very valuable in terms of shedding light to the evolution of neurological and cognitive symptoms of patients. In addition, these patients, as previously mentioned, live longer compared to other neuronopathic forms of GD. A deeper understanding of their disease would help ameliorate their quality of life with appropriate interventions.

8 FUTURE PERSPECTIVES

8.1 EYE TRACKERS

Literature on the field of eye movements is vast, especially regarding saccades, and less regarding fixation and reading. Hence, it is surprising that eye tracking is not popular in the clinical setting. One of the reasons is that older eye trackers are impractical to use and they require a lot of calibration. In addition, the patient's cooperation is necessary: patients often need to wear a rather uncomfortable head-mounted device (like the one used in Study 1), or even stabilize the head using other techniques. Then, the recorded data need to be analyzed, and this is time- and effort-consuming.

New-generation eye trackers are usually screen-based (like the ones used in study 2 and 3), more comfortable for the patient, have higher acquisition speed, which provides better data quality, and they need less calibration. However, filtering and analyzing the enormous amount of recorded data, remains a basic problem. The use of artificial intelligence might help resolve this problem, and efforts are already made towards this direction.

In our third study, apart from evaluation of eye movement, we also evaluated pupil size, without using a pupilometer, but with a high-end eye tracker. Our results seem to agree with previous studies on pupil reflex that used pupilometers. In the future, and if our results become replicated by similar studies, pupil evaluation and eye movements could be integrated in the same examination.

8.2 A DIFFERENT APPROACH OF THE ASSESSMENT OF READING

Literature regarding reading in PD is rather restricted, and for this reason not conclusive. More studies in the field are needed as reading difficulties are a commonly expressed problem, and its assessment provides conclusions on the oculomotor function during a complex but natural task. Combination with functional neuroimaging studies for PD would add to our knowledge. Eye movements during reading could also be assessed using a more linguistic approach, controlling for specific parameters, like fixation duration on words with different semantic value, regressions in areas of the text with conflicting information etc. Eye tracking while reading numbers, musical notes, and symbols, comparisons between different languages with different reading patterns and nonsense texts would also be of great interest.

8.3 INTEGRATION OF EYE TRACKING IN EPIDEMIOLOGICAL STUDIES

To date, there are multiple PD databases on national and international level, uni- or multicentric, that include PD patients at various clinical and cognitive stages: de novo, untreated patients, or medicated under various treatments, pharmacological or surgical, and at-risk individuals that carry known mutations or suffer prodromal symptoms. Most of these studies assess the participants clinically and cognitively, gather information on wet biomarkers, for example from blood and CSF, as well as imaging biomarkers. The evaluation of eye movements with a modern eye tracker takes less than 30 minutes (including calibration and

multiple paradigms). It is non-invasive and comfortable and can be done at the clinic, as part of a clinical assessment, even by non-medical staff. It could, therefore, easily be considered as an additional biomarker to be included in the thorough assessments of study participants. This would provide large-scale data including longitudinal evaluations of high importance. Common eye-tracking protocols among the databases and careful characterization of the groups are, however, prerequisites for comparable results. This would be the best way to confirm the usefulness of eye tracking in PD diagnosis and prognosis, and consequently use it as an everyday tool in the assessment of patients, even at a primary-care level.

8.4 WHAT ABOUT THE REST OF EYE MOVEMENTS?

There are multiple paradigms of eye-movement examination. Smooth pursuit, horizontal and vertical, vergence, and nystagmus can be integrated in the assessments, along with the pupil examination of the PLR and PNR. Fixational movements like ocular drift, SWJ and microsaccades can also provide interesting information.

More than simple tasks, though, oculomotor assessments can reveal a lot during more complex tasks. Eye-movement examination during visual scanning of faces, scenes, or during presentation of auditory stimuli are some of the examples. Moreover, simultaneous evaluation of cognition together with oculomotor assessments, performed on screens rather than paper, could reveal differences between PD and HC that cannot be solely explained by the discrepancies in the numerical scores in cognitive testing. The same can obviously be done not only in PD but other diseases, or even healthy populations with different characteristics.

8.5 LONGITUDINAL EVALUATION OF NORRBOTTNIAN GD3

Follow-up of cognition in Norrbottnian GD3 patients should accompany their regular clinical and biochemical follow-up. Longitudinal assessments are more representative of a disease profile, and they help us reach safer conclusions. In addition to that, the effect of older and novel treatments, can only be evaluated in time by regularly repeated assessments. Our group is planning to re-evaluate cognition with RBANS in the same group of patients five years after the initial evaluation.

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10 REFERENCES

1. Lees AJ, Hardy J, Revesz T. Parkinson's disease. Lancet. 2009;373(9680):2055-66.
2. Tarakad A, Jankovic J. Diagnosis and Management of Parkinson's Disease. Semin Neurol. 2017;37(2):118-26.
3. Balestrino R, Schapira AHV. Glucocerebrosidase and Parkinson Disease: Molecular, Clinical, and Therapeutic Implications. Neuroscientist. 2018:1073858417748875.
4. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry. 1992;55(3):181-4.
5. Postuma RB, Berg D, Stern M, Poewe W, Olanow CW, Oertel W, et al. MDS clinical diagnostic criteria for Parkinson's disease. Mov Disord. 2015;30(12):1591-601.
6. Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkmann J, et al. Parkinson disease. Nat Rev Dis Primers. 2017;3:17013.
7. Aarsland D, Creese B, Politis M, Chaudhuri KR, ffytche DH, Weintraub D, et al. Cognitive decline in Parkinson disease. Nature Reviews Neurology. 2017;13:217.
8. Emre M, Aarsland D, Brown R, Burn DJ, Duyckaerts C, Mizuno Y, et al. Clinical diagnostic criteria for dementia associated with Parkinson's disease. Mov Disord. 2007;22(12):1689-707; quiz 837.
9. Litvan I, Goldman JG, Troster AI, Schmand BA, Weintraub D, Petersen RC, et al. Diagnostic criteria for mild cognitive impairment in Parkinson's disease: Movement Disorder Society Task Force guidelines. Mov Disord. 2012;27(3):349-56.
10. Svenningsson P, Westman E, Ballard C, Aarsland D. Cognitive impairment in patients with Parkinson's disease: diagnosis, biomarkers, and treatment. Lancet Neurol. 2012;11(8):697-707.
11. Schrag A, Siddiqui UF, Anastasiou Z, Weintraub D, Schott JM. Clinical variables and biomarkers in prediction of cognitive impairment in patients with newly diagnosed Parkinson's disease: a cohort study. Lancet Neurol. 2017;16(1):66-75.
12. Collins LM, Williams-Gray CH. The Genetic Basis of Cognitive Impairment and Dementia in Parkinson's Disease. Front Psychiatry. 2016;7:89.
13. Kehagia AA, Barker RA, Robbins TW. Cognitive impairment in Parkinson's disease: the dual syndrome hypothesis. Neurodegener Dis. 2013;11(2):79-92.
14. O'Callaghan C, Lewis SJG. Cognition in Parkinson's Disease. Int Rev Neurobiol. 2017;133:557-83.
15. Williams-Gray CH, Evans JR, Goris A, Foltynie T, Ban M, Robbins TW, et al. The distinct cognitive syndromes of Parkinson's disease: 5 year follow-up of the CamPaIGN cohort. Brain. 2009;132(Pt 11):2958-69.
16. Botha H, Carr J. Attention and visual dysfunction in Parkinson's disease. Parkinsonism Relat Disord. 2012;18(6):742-7.
17. Kudlicka A, Clare L, Hindle JV. Executive functions in Parkinson's disease: systematic review and meta-analysis. Mov Disord. 2011;26(13):2305-15.
18. Cosgrove J, Alty JE, Jamieson S. Cognitive impairment in Parkinson's disease. Postgrad Med J. 2015;91(1074):212-20.
19. Luo TZ, Maunsell JHR. Attention can be subdivided into neurobiological components corresponding to distinct behavioral effects. Proc Natl Acad Sci U S A. 2019;116(52):26187-94.

20. Dujardin K, Tard C, Duhamel A, Delval A, Moreau C, Devos D, et al. The pattern of attentional deficits in Parkinson's disease. Parkinsonism Relat Disord. 2013;19(3):300-5.
21. Gilbert CD, Li W. Top-down influences on visual processing. Nat Rev Neurosci. 2013;14(5):350-63.
22. Glaholt MG, Wu MC, Reingold EM. Evidence for top-down control of eye movements during visual decision making. J Vis. 2010;10(5):15.
23. Buhmann C, Kraft S, Hinkelmann K, Krause S, Gerloff C, Zangemeister WH. Visual Attention and Saccadic Oculomotor Control in Parkinson's Disease. Eur Neurol. 2015;73(5-6):283-93.
24. Armstrong RA. Visual Dysfunction in Parkinson's Disease. Int Rev Neurobiol. 2017;134:921-46.
25. van der Lijn I, de Haan GA, Huizinga F, van der Feen FE, Rutgers AWF, Stellingwerf C, et al. Self-Reported Visual Complaints in People with Parkinson's Disease: A Systematic Review. J Parkinsons Dis. 2022.
26. Ekker MS, Janssen S, Seppi K, Poewe W, de Vries NM, Theelen T, et al. Ocular and visual disorders in Parkinson's disease: Common but frequently overlooked. Parkinsonism Relat Disord. 2017;40:1-10.
27. Gorges M, Pinkhardt EH, Kassubek J. Alterations of eye movement control in neurodegenerative movement disorders. J Ophthalmol. 2014;2014:658243.
28. Raskin SA, Borod JC, Wasserstein J, Bodis-Wollner I, Coscia L, Yahr MD. Visuospatial orientation in Parkinson's disease. Int J Neurosci. 1990;51(1-2):9-18.
29. Levin BE, Llabre MM, Reisman S, Weiner WJ, Sanchez-Ramos J, Singer C, et al. Visuospatial impairment in Parkinson's disease. Neurology. 1991;41(3):365-9.
30. Del Pino R, Acera M, Murueta-Goyena A, Lucas-Jiménez O, Ojeda N, Ibarretxe-Bilbao N, et al. Visual dysfunction is associated with cognitive impairment in Parkinson's disease. Parkinsonism Relat Disord. 2021;92:22-5.
31. Leyland LA, Bremner FD, Mahmood R, Hewitt S, Durteste M, Cartlidge MRE, et al. Visual tests predict dementia risk in Parkinson disease. Neurol Clin Pract. 2020;10(1):29-39.
32. Lal V, Truong D. Eye movement abnormalities in movement disorders. Clin Park Relat Disord. 2019;1:54-63.
33. Gaymard B. Cortical and sub-cortical control of saccades and clinical application. Rev Neurol (Paris). 2012;168(10):734-40.
34. Yamagishi S, Furukawa S. Factors Influencing Saccadic Reaction Time: Effect of Task Modality, Stimulus Saliency, Spatial Congruency of Stimuli, and Pupil Size. Front Hum Neurosci. 2020;14:571893.
35. Krauzlis RJ, Goffart L, Hafed ZM. Neuronal control of fixation and fixational eye movements. Philos Trans R Soc Lond B Biol Sci. 2017;372(1718).
36. Martinez-Conde S, Macknik SL, Hubel DH. The role of fixational eye movements in visual perception. Nat Rev Neurosci. 2004;5(3):229-40.
37. Aytekin M, Victor JD, Rucci M. The visual input to the retina during natural head-free fixation. J Neurosci. 2014;34(38):12701-15.
38. Mathôt S. Pupillometry: Psychology, Physiology, and Function. J Cogn. 2018;1(1):16.
39. Rayner K. Eye movements in reading and information processing: 20 years of research. Psychol Bull. 1998;124(3):372-422.
40. Henderson JM, Choi W, Luke SG, Desai RH. Neural correlates of fixation duration in natural reading: Evidence from fixation-related fMRI. Neuroimage. 2015;119:390-7.

41. Radach R, Kennedy A. Theoretical perspectives on eye movements in reading: Past controversies, current issues, and an agenda for future research. European Journal of Cognitive Psychology. 2004;16(1-2):3-26.
42. Luke SG, Darowski ES, Gale SD. Predicting eye-movement characteristics across multiple tasks from working memory and executive control. Mem Cognit. 2018;46(5):826-39.
43. Pa J, Dutt S, Mirsky JB, Heuer HW, Keselman P, Kong E, et al. The functional oculomotor network and saccadic cognitive control in healthy elders. Neuroimage. 2014;95:61-8.
44. Terao Y, Fukuda H, Ugawa Y, Hikosaka O. New perspectives on the pathophysiology of Parkinson's disease as assessed by saccade performance: a clinical review. Clin Neurophysiol. 2013;124(8):1491-506.
45. Hikosaka O, Takikawa Y, Kawagoe R. Role of the basal ganglia in the control of purposive saccadic eye movements. Physiol Rev. 2000;80(3):953-78.
46. Hodgson T, Chamberlain M, Parris B, James M, Gutowski N, Husain M, et al. The role of the ventrolateral frontal cortex in inhibitory oculomotor control. Brain. 2007;130(Pt 6):1525-37.
47. Basso MA, Bickford ME, Cang J. Unraveling circuits of visual perception and cognition through the superior colliculus. Neuron. 2021;109(6):918-37.
48. Taouali W, Goffart L, Alexandre F, Rougier NP. A parsimonious computational model of visual target position encoding in the superior colliculus. Biol Cybern. 2015;109(4-5):549-59.
49. Ajasse S, Benosman RB, Lorenceau J. Effects of pupillary responses to luminance and attention on visual spatial discrimination. J Vis. 2018;18(11):6.
50. van der Wel P, van Steenbergen H. Pupil dilation as an index of effort in cognitive control tasks: A review. Psychon Bull Rev. 2018;25(6):2005-15.
51. Chan-Palay V, Asan E. Alterations in catecholamine neurons of the locus coeruleus in senile dementia of the Alzheimer type and in Parkinson's disease with and without dementia and depression. J Comp Neurol. 1989;287(3):373-92.
52. Scatton B, Javoy-Agid F, Rouquier L, Dubois B, Agid Y. Reduction of cortical dopamine, noradrenaline, serotonin and their metabolites in Parkinson's disease. Brain Res. 1983;275(2):321-8.
53. Bohnen NI, Albin RL. The cholinergic system and Parkinson disease. Behav Brain Res. 2011;221(2):564-73.
54. Bari BA, Chokshi V, Schmidt K. Locus coeruleus-norepinephrine: basic functions and insights into Parkinson's disease. Neural Regen Res. 2020;15(6):1006-13.
55. Lemos J, Pereira D, Almendra L, Rebelo D, Patrício M, Castelhano J, et al. Distinct functional properties of the vertical and horizontal saccadic network in Health and Parkinson's disease: An eye-tracking and fMRI study. Brain Res. 2016;1648(Pt A):469-84.
56. Anderson TJ, MacAskill MR. Eye movements in patients with neurodegenerative disorders. Nat Rev Neurol. 2013;9(2):74-85.
57. Termsarasab P, Thammongkolchai T, Rucker JC, Frucht SJ. The diagnostic value of saccades in movement disorder patients: a practical guide and review. J Clin Mov Disord. 2015;2:14.
58. Jung I, Kim JS. Abnormal Eye Movements in Parkinsonism and Movement Disorders. J Mov Disord. 2019;12(1):1-13.
59. MacAskill MR, Anderson TJ, Jones RD. Adaptive modification of saccade amplitude in Parkinson's disease. Brain. 2002;125(Pt 7):1570-82.
60. Kimmig H, Haussmann K, Mergner T, Lücking CH. What is pathological with gaze shift fragmentation in Parkinson's disease? J Neurol. 2002;249(6):683-92.

61. Blekher T, Weaver M, Rupp J, Nichols WC, Hui SL, Gray J, et al. Multiple step pattern as a biomarker in Parkinson disease. Parkinsonism Relat Disord. 2009;15(7):506-10.
62. Terao Y, Fukuda H, Yugeta A, Hikosaka O, Nomura Y, Segawa M, et al. Initiation and inhibitory control of saccades with the progression of Parkinson's disease - changes in three major drives converging on the superior colliculus. Neuropsychologia. 2011;49(7):1794-806.
63. Briand KA, Strallow D, Hening W, Poizner H, Sereno AB. Control of voluntary and reflexive saccades in Parkinson's disease. Exp Brain Res. 1999;129(1):38-48.
64. Heuer HW, Mirsky JB, Kong EL, Dickerson BC, Miller BL, Kramer JH, et al. Antisaccade task reflects cortical involvement in mild cognitive impairment. Neurology. 2013;81(14):1235-43.
65. Mirsky JB, Heuer HW, Jafari A, Kramer JH, Schenk AK, Viskontas IV, et al. Anti-saccade performance predicts executive function and brain structure in normal elders. Cogn Behav Neurol. 2011;24(2):50-8.
66. Antoniades CA, Demeyere N, Kennard C, Humphreys GW, Hu MT. Antisaccades and executive dysfunction in early drug-naive Parkinson's disease: The discovery study. Mov Disord. 2015;30(6):843-7.
67. Hood AJ, Amador SC, Cain AE, Briand KA, Al-Refai AH, Schiess MC, et al. Levodopa slows prosaccades and improves antisaccades: an eye movement study in Parkinson's disease. J Neurol Neurosurg Psychiatry. 2007;78(6):565-70.
68. Rivaud-Péchoux S, Vidailhet M, Brandel JP, Gaymard B. Mixing pro- and antisaccades in patients with parkinsonian syndromes. Brain. 2007;130(Pt 1):256-64.
69. Shaikh AG, Xu-Wilson M, Grill S, Zee DS. 'Staircase' square-wave jerks in early Parkinson's disease. Br J Ophthalmol. 2011;95(5):705-9.
70. Hall CA, Chilcott RP. Eyeing up the Future of the Pupillary Light Reflex in Neurodiagnostics. Diagnostics (Basel). 2018;8(1).
71. You S, Hong JH, Yoo J. Analysis of pupillometer results according to disease stage in patients with Parkinson's disease. Sci Rep. 2021;11(1):17880.
72. Granholm E, Morris S, Galasko D, Shults C, Rogers E, Vukov B. Tropicamide effects on pupil size and pupillary light reflexes in Alzheimer's and Parkinson's disease. Int J Psychophysiol. 2003;47(2):95-115.
73. Yu CY, Lee T, Shariati MA, Santini V, Poston K, Liao YJ. Abnormal eye movement behavior during reading in Parkinson's disease. Parkinsonism Relat Disord. 2016;32:130-2.
74. Jehangir N, Yu CY, Song J, Shariati MA, Binder S, Beyer J, et al. Slower saccadic reading in Parkinson's disease. PLoS One. 2018;13(1):e0191005.
75. Stock L, Krüger-Zechlin C, Deeb Z, Timmermann L, Waldthaler J. Natural Reading in Parkinson's Disease With and Without Mild Cognitive Impairment. Front Aging Neurosci. 2020;12:120.
76. Sidransky E, Nalls MA, Aasly JO, Aharon-Peretz J, Annesi G, Barbosa ER, et al. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. N Engl J Med. 2009;361(17):1651-61.
77. Alcalay RN, Levy OA, Waters CC, Fahn S, Ford B, Kuo SH, et al. Glucocerebrosidase activity in Parkinson's disease with and without GBA mutations. Brain. 2015;138(Pt 9):2648-58.
78. Anheim M, Elbaz A, Lesage S, Durr A, Condroyer C, Viallet F, et al. Penetrance of Parkinson disease in glucocerebrosidase gene mutation carriers. Neurology. 2012;78(6):417-20.
79. Roshan Lal T, Sidransky E. The Spectrum of Neurological Manifestations Associated with Gaucher Disease. Diseases. 2017;5(1).

80. Stirnemann J, Belmatoug N, Camou F, Serratrice C, Froissart R, Caillaud C, et al. A Review of Gaucher Disease Pathophysiology, Clinical Presentation and Treatments. Int J Mol Sci. 2017;18(2).
81. Machaczka M, Kämpe Björkvall C, Wieremiejczyk J, Paucar Arce M, Myhr-Eriksson K, Klimkowska M, et al. Impact of imiglucerase supply shortage on clinical and laboratory parameters in Norrbottnian patients with Gaucher disease type 3. Arch Immunol Ther Exp (Warsz). 2015;63(1):65-71.
82. Dahl N, Lagerström M, Erikson A, Pettersson U. Gaucher disease type III (Norrbottnian type) is caused by a single mutation in exon 10 of the glucocerebrosidase gene. Am J Hum Genet. 1990;47(2):275-8.
83. Machaczka M, Paucar M, Bjorkvall CK, Smith NJC, Cox TM, Forsgren L, et al. Novel hyperkinetic dystonia-like manifestation and neurological disease course of Swedish Gaucher patients. Blood Cells Mol Dis. 2018;68:86-92.
84. Dreborg S, Erikson A, Hagberg B. Gaucher disease--Norrbottnian type. I. General clinical description. Eur J Pediatr. 1980;133(2):107-18.
85. Blume J, Beniaminov S, Kämpe Björkvall C, Machaczka M, Svenningsson P. Saccadic Impairments in Patients with the Norrbottnian Form of Gaucher's Disease Type 3. Front Neurol. 2017;8:295.
86. Wong K, Sidransky E, Verma A, Mixon T, Sandberg GD, Wakefield LK, et al. Neuropathology provides clues to the pathophysiology of Gaucher disease. Mol Genet Metab. 2004;82(3):192-207.
87. Orvisky E, Park JK, LaMarca ME, Ginns EI, Martin BM, Tayebi N, et al. Glucosylsphingosine accumulation in tissues from patients with Gaucher disease: correlation with phenotype and genotype. Mol Genet Metab. 2002;76(4):262-70.
88. Goetz CG, Tilley BC, Shaftman SR, Stebbins GT, Fahn S, Martinez-Martin P, et al. Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): scale presentation and clinimetric testing results. Mov Disord. 2008;23(15):2129-70.
89. Fahn S, Elton RL, UPDRS program members. Unified Parkinsons Disease Rating Scale. In: Fahn S, Marsden CD, Goldstein M, Calne DB, editors. Recent developments in Parkinsons disease, vol 2. Florham Park, NJ: Macmillan Healthcare Information;1987. p. 153-63.
90. Goetz CG, Poewe W, Rascol O, Sampaio C, Stebbins GT, Counsell C, et al. Movement Disorder Society Task Force report on the Hoehn and Yahr staging scale: status and recommendations. Mov Disord. 2004;19(9):1020-8.
91. Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. Neurology. 1967;17(5):427-42.
92. Schwab J, England A. Projection technique for evaluating surgey

in Parkinson’s disease. In: Gillingham F, Donaldson M, eds

Third Symposium on Parkinson’s Disease. 1969;Vol. 232. Edinburgh:

Livingston:152-7.
93. Tomlinson CL, Stowe R, Patel S, Rick C, Gray R, Clarke CE. Systematic review of levodopa dose equivalency reporting in Parkinson's disease. Mov Disord. 2010;25(15):2649-53.
94. Davies EH, Mengel E, Tylki-Szymanska A, Kleinotiene G, Reinke J, Vellodi A. Four-year follow-up of chronic neuronopathic Gaucher disease in Europeans using a modified severity scoring tool. J Inherit Metab Dis. 2011;34(5):1053-9.
95. Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc. 2005;53(4):695-9.

96. Dubois B, Slachevsky A, Litvan I, Pillon B. The FAB: a Frontal Assessment Battery at bedside. Neurology. 2000;55(11):1621-6.
97. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12(3):189-98.
98. Randolph C, Tierney MC, Mohr E, Chase TN. The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS): preliminary clinical validity. J Clin Exp Neuropsychol. 1998;20(3):310-9.
99. Pretegiani E, Optican LM. Eye Movements in Parkinson's Disease and Inherited Parkinsonian Syndromes. Front Neurol. 2017;8:592.
100. Bolton AD, Murata Y, Kirchner R, Kim SY, Young A, Dang T, et al. A Diencephalic Dopamine Source Provides Input to the Superior Colliculus, where D1 and D2 Receptors Segregate to Distinct Functional Zones. Cell Rep. 2015;13(5):1003-15.
101. Yang Q, Bucci MP, Kapoula Z. The latency of saccades, vergence, and combined eye movements in children and in adults. Invest Ophthalmol Vis Sci. 2002;43(9):2939-49.
102. Waldthaler J, Vinding MC, Eriksson A, Svenningsson P, Lundqvist D. Neural correlates of impaired response inhibition in the antisaccade task in Parkinson's disease. Behav Brain Res. 2022;422:113763.
103. Ewenczyk C, Mesmoudi S, Gallea C, Welter ML, Gaymard B, Demain A, et al. Antisaccades in Parkinson disease: A new marker of postural control? Neurology. 2017;88(9):853-61.
104. Railo H, Olkoniemi H, Eeronheimo E, Pääkkönen O, Joutsa J, Kaasinen V. Dopamine and eye movement control in Parkinson's disease: deficits in corollary discharge signals? PeerJ. 2018;6:e6038.
105. Choi W, Desai RH, Henderson JM. The neural substrates of natural reading: a comparison of normal and nonword text using eyetracking and fMRI. Front Hum Neurosci. 2014;8:1024.
106. Aarsland D, Bronnick K, Williams-Gray C, Weintraub D, Marder K, Kulisevsky J, et al. Mild cognitive impairment in Parkinson disease: a multicenter pooled analysis. Neurology. 2010;75(12):1062-9.
107. Grossman M. Sentence processing in Parkinson's disease. Brain Cogn. 1999;40(2):387-413.
108. Fernández G, Laubrock J, Mandolesi P, Colombo O, Agamennoni O. Registering eye movements during reading in Alzheimer's disease: difficulties in predicting upcoming words. J Clin Exp Neuropsychol. 2014;36(3):302-16.
109. Wang CA, Munoz DP. A circuit for pupil orienting responses: implications for cognitive modulation of pupil size. Curr Opin Neurobiol. 2015;33:134-40.
110. Doppler CEJ, Kinnerup MB, Brune C, Farrher E, Betts M, Fedorova TD, et al. Regional locus coeruleus degeneration is uncoupled from noradrenergic terminal loss in Parkinson's disease. Brain. 2021;144(9):2732-44.
111. Megemont M, McBurney-Lin J, Yang H. Pupil diameter is not an accurate real-time readout of locus coeruleus activity. Elife. 2022;11.
112. Hori N, Takamori M, Hirayama M, Watanabe H, Nakamura T, Yamashita F, et al. Pupillary supersensitivity and visual disturbance in Parkinson's disease. Clin Auton Res. 2008;18(1):20-7.
113. Chou KL, Lenhart A, Koeppe RA, Bohnen NI. Abnormal MoCA and normal range MMSE scores in Parkinson disease without dementia: cognitive and neurochemical correlates. Parkinsonism Relat Disord. 2014;20(10):1076-80.
114. Gorges M, Muller HP, Lule D, Pinkhardt EH, Ludolph AC, Kassubek J. The association between alterations of eye movement control and cerebral intrinsic functional connectivity in Parkinson's disease. Brain Imaging Behav. 2016;10(1):79-91.

115. Toth AJ, Campbell MJ. Investigating sex differences, cognitive effort, strategy, and performance on a computerised version of the mental rotations test via eye tracking. Sci Rep. 2019;9(1):19430.
116. Campbell MJ, Toth AJ, Brady N. Illuminating sex differences in mental rotation using pupillometry. Biol Psychol. 2018;138:19-26.
117. Goker-Alpan O, Schiffmann R, Park JK, Stubblefield BK, Tayebi N, Sidransky E. Phenotypic continuum in neuronopathic Gaucher disease: an intermediate phenotype between type 2 and type 3. J Pediatr. 2003;143(2):273-6.
118. Steward AM, Wiggs E, Lindstrom T, Ukwuani S, Ryan E, Tayebi N, et al. Variation in cognitive function over time in Gaucher disease type 3. Neurology. 2019;93(24):e2272-e83.
119. Mata IF, Leverenz JB, Weintraub D, Trojanowski JQ, Chen-Plotkin A, Van Deerlin VM, et al. GBA Variants are associated with a distinct pattern of cognitive deficits in Parkinson's disease. Mov Disord. 2016;31(1):95-102.