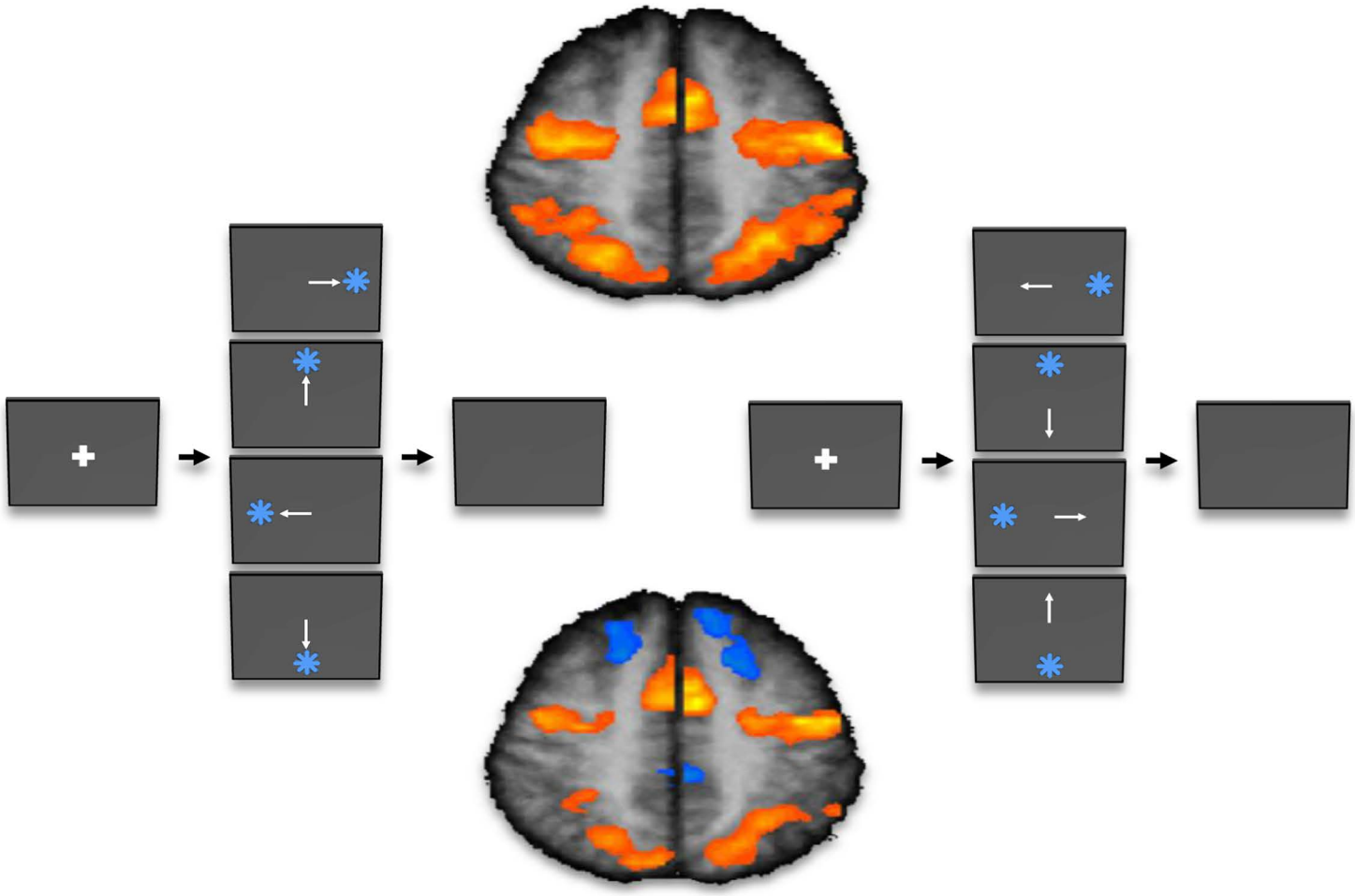

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Imagiologia Funcional das Sacadas Verticais e Horizontais em Síndromes Parkinsónicos

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Functional Imaging of Vertical and Horizontal Saccades in Parkinsonian Syndromes

A thesis submitted to the faculty of University of Coimbra in conformity with the requirements for the degree of Doctor in Health Sciences

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Abstract.

The study of eye movements has been extensively used to gain insight into the pathophysiology of neurodegenerative disorders, over the last 60 years. Among these, Parkinson's disease (PD) and progressive supranuclear palsy (PSP) stand out as a classical example of a clinical disorder where ocular motor examination can not only aid in the diagnosis, but has also helped to unravel the pathomechanisms of each disease. Rapid eye movements (saccades) in PD show mild decreased in amplitude, especially in the vertical plane. This is particularly true for more voluntary saccades (e.g., saccades directed away from the stimulus; antisaccade) in which additional delay and increased number of directional errors (unwanted saccade towards the stimulus) are occasionally seen. Reflexive saccades on the other hand (e.g., saccades directed towards a novel stimulus; prosaccade) seem to be relatively spared in PD. While it has been proposed that these abnormalities are promoted by variable patterns of cortical and subcortical frontostriatal deficit, studies exploring their neural correlates and putative compensatory mechanisms at a functional level are scarce. Importantly, the reason for the predominant affection of vertical saccades in PD is largely unknown, and the cortical mechanisms for the generation of vertical saccades in health and disease have been equally unexplored, when compared with their horizontal counterpart. In PSP, patients show a distinctive ocular motor disorder, consisting of pervasive involuntary back-to-back saccadic horizontal intrusions during attempted steady fixation (square wave jerks, SWJs), marked slowing and restriction of saccades, predominantly in the vertical plane, and increased number of directional errors and delayed reaction times in the horizontal antisaccade task. Although vertical saccade slowing and restriction is primarily attributed to the extensive brainstem damage predominantly affecting the midbrain, much less is known about the cortical and subcortical burden contribution for the saccadic disturbance in PSP, and its influence on the prolonged latency of vertical prosaccades and antisaccades.

Two separate literature searches were performed, to identify articles relevant to supranuclear eye movements disorders and saccadic intrusions, particularly in PD and PSP. Subsequently, two experimental studies were carried out. In the first study, in 19 PD patients off medication and 22 healthy controls, horizontal and vertical prosaccades and antisaccades were measured in an eye tracking behavioural paradigm, followed by a block-design functional Magnetic Resonance Imaging (fMRI) experiment, consisting of two runs (prosaccades, antisaccades) of 6 blocks each (3 vertical and 3 horizontal). In a second study, we use the same behavioural and fMRI protocol to evaluate 8 PSP patients and 10 healthy controls.

Literature review showed that PD studies focusing on the default-mode network (DMN) have demonstrated that an increased DMN connectivity seems to correlate with saccadic hypometria particularly in the vertical plane, possibly reflecting a compensatory mechanism to maintain behavioural saccadic performance. New types of saccadic intrusions have been described in PD, including the apparently highly specific pervasive ocular microtremor. In PSP, SWJs show a characteristic loss of the vertical component

and are abnormally large, possibly reflecting cerebellar and/or brainstem dysfunction. In the first experimental study, while saccade metrics were not significantly different between groups, PD patients showed underactivation of the left frontal eye field (FEF) during horizontal prosaccades and overactivation of the right parietal eye field (PEF) during horizontal and vertical prosaccades and horizontal antisaccades. Moreover, controls showed greater DMN deactivation during antisaccades. Within groups, vertical prosaccades were associated with greater right FEF and cerebellar activity in controls, and bilateral cuneus hypoactivity in PD. Vertical antisaccades were associated with greater DMN deactivation in both groups and left PEF hypoactivity only in PD ($p<0.01$, corrected; GLM). In the second experimental study, PSP patients evidenced slow (horizontal and vertical prosaccades), hypometric (horizontal and vertical prosaccades; vertical antisaccades) and moderately delayed (vertical prosaccades; vertical antisaccades) saccades, compared to controls ($p<0.005$). Concerning neural activation patterns, the PSP group showed decreased frontostriatal blood oxygen level–dependent (BOLD) activation (i.e., left FEF and supplementary eye field [SEF], right thalamus and caudate) during vertical and horizontal prosaccades and vertical antisaccades, relative to controls. In all types of saccades, controls additionally showed greater DMN deactivation. Within groups, controls showed no BOLD differences between the vertical and horizontal prosaccades while PSP patients demonstrated greater DMN deactivation during vertical prosaccades. In antisaccades, both groups evidenced greater DMN deactivation in the vertical plane and PSP further showed relative BOLD hypoactivity in left FEF, left putamen and right thalamus during vertical antisaccades ($p<0.01$, corrected; GLM).

Functional cortical asymmetries between vertical and horizontal saccades seem to occur distinctively in PD patients and healthy controls, particularly in the oculomotor and default mode networks. Putative functional compensatory changes in the parietal eye field in PD patients may help to keep saccadic behaviour at the same level as the healthy controls. However, such compensation may come with the additional cost of enhancing a normal bias between vertical and horizontal saccades' activation in PEF, potentiating performance differences between saccade planes in PD, particularly for more voluntary saccades. In PSP, while previous literature has mainly focused on the brainstem saccadic network involvement, in this work it was found that these patients show marked frontostriatal hipoactivity during saccades, relative to controls, especially for the vertical plane.

In conclusion, frontal hypoactivity during saccades was remarkably greater in PSP than in PD patients, notably extending to the basal ganglia in the former group. Importantly, in PSP patients, parietal cortex putative compensation during saccades seems to be absent, in contrast with PD patients. Both in PD and PSP groups, vertical voluntary saccades were associated with less oculomotor network activity (frontal and/or parietal) than horizontal saccades, particularly in the latter group. These new findings highlight the impact of cortical impairment in saccadic disturbance of PD and PSP, and provide

evidence for distinct involvement of vertical and saccadic network in both diseases at a cortical and subcortical level.

Resumo.

A avaliação dos movimentos oculares tem sido extensamente utilizada no estudo da fisiopatologia das doenças neurodegenerativas, nos últimos 60 anos. Entre estas, a doença de Parkinson (DP) e a paralisia supranuclear progressiva (PSP) sobressaem como um exemplo clássico de um distúrbio clínico no qual a avaliação ocular motora pode não só ajudar no diagnóstico, mas também ajudar na melhor compreensão dos mecanismos patológicos de cada uma destas doenças. Os movimentos rápidos dos olhos (sacadas) na DP, demonstram uma diminuição ligeira da amplitude das sacadas, principalmente no plano vertical. Isto é particularmente verdade para sacadas mais voluntárias (ex. sacadas dirigidas no sentido contrário do estímulo; anti-sacada), nas quais podem também ocorrer um atraso no seu início e um aumento dos erros direccionais (sacadas não intencionais dirigidas ao estímulo). Já as sacadas reflexivas (ex. sacadas dirigidas na direcção de um estímulo não expectável; pró-sacada) parecem estar relativamente preservadas na DP. Ainda que tenha sido proposto que estas anomalias são promovidas por padrões variáveis de défice cortical e subcortical frontoestriatal, são raros os estudos que exploram a correlação destes achados e os seus putativos mecanismos compensatórios a nível neuronal. Mais ainda, a razão porque existe uma afecção sacádica com predomínio no plano vertical na DP é maioritariamente desconhecida, e os mecanismos corticais que geram as sacadas verticais na saúde e na doença têm sido igualmente inexplorados, quando comparados aos das suas congéneres horizontais. Na PSP, os doentes evidenciam um distúrbio ocular motor distinto, consistindo em intrusões sacádicas involuntárias horizontais alternantes durante a fixação ("ondas quadradas", OQs), diminuição marcada da velocidade e restrição das sacadas, principalmente no plano vertical, e no aumento do número de erros direccionais e da latência de início das anti-sacadas. Enquanto que a diminuição da amplitude e restricção sacádica de predomínio vertical é amplamente atribuída a uma extensa e selectiva afecção do mesencéfalo, a contribuição da disfunção cortical e subcortical para o distúrbio sacádico na PSP, e a sua influência no aumento da latência no início das pró-sacadas e anti-sacadas verticais, é pouco conhecida.

Foram realizadas duas revisões da literatura em separado, de modo a identificar artigos relevantes sobre distúrbios supranucleares dos movimentos oculares e intrusões sacádicas, particularmente na DP e PSP. Subsequentemente, 2 estudos esperimentais foram realizados. No primeiro estudo, em 19 doentes com DP e 22 controlos saudáveis, foram analizadas as pró-sacadas e anti-sacadas horizontais e verticais num paradigma comportamental com video-oculografia por infravermelhos, seguido de uma experiência em ressonância magnética funcional (RMNf) com um desenho em blocos, consistindo em duas sequências (pró-sacadas; anti-sacadas), cada uma constituída por dois blocos (3 verticais; 3 horizontais). Num segundo estudo, usaram-se os mesmos paradigmas para avaliar 8 doentes com PSP e 10 controlos saudáveis.

A revisão da literatura demonstrou que estudos na DP, focados na rede neuronal em modo padrão (RMP), mostraram que um aumento da conectividade da RMP se parece correlacionar com a hipometria das sacadas verticais, podendo este aumento da

conectividade traduzir um mecanismo compensatório que mantêm o desempenho comportamental sacádico na DP. Novos tipos de intrusões sacádicas foram descritos na DP, incluíndo o aparentemente específico microtremor ocular contínuo. Na PSP, as OQs parecem demonstrar uma perda característica do componente vertical e são anormalmente largas, podendo isto reflectir uma disfunção cerebelosa e/ou do tronco encefálico. No primeiro estudo experimental, enquanto que a métrica das sacadas não foi significativamente diferente entre grupos, os doentes com DP evidenciaram diminuição da activação da área ocular frontal (aOF) esquerda durante a execução de pró-sacadas horizontais e aumento da activação do área ocular parietal (aOP) direita durante a execução de pró-sacadas verticais e horizontais e anti-sacadas horizontais. Mais ainda, os controlos saudáveis exibiram uma maior desactivação da RMP durante as anti-sacadas. Dentro de cada grupo, as pró-sacadas verticais associaram-se a uma maior activação da aOF direita e cerebelo em controlos saudáveis, e do cúneo em doentes com DP. As anti-sacadas verticais associaram-se a uma maior desactivação da RMP em ambos os grupos e a uma menor activação do aOP esquerda apenas em doentes com DP ($p<0.01$, corrigido; MLG). Na segunda experiência, os doentes com PSP evienciaram sacadas lentas (pró-sacadas horizontais e verticais), hipométricas (pró-sacadas horizontais e verticais; anti-sacadas verticais) e moderadamente atrasadas no seu início (pró-sacadas e anti-sacadas verticais), comparativamente aos controlos saudáveis ($p<0.005$). No que diz respeito aos padrões de activação, o grupo da PSP demonstrou diminuição da activação frontoestriatal (i.e., aOF e área suplementar ocular [aSO] esquerdas, tálamo e caudado direitos) durante a execução de pró-sacadas verticais e horizontais e anti-sacadas verticais, comparativamente aos controlos saudáveis. Tanto nas anti-sacadas verticais como nas horizontais, os controlos demonstraram adicionalmente maior desactivação da RMP. Dentro da cada grupo, o grupo controlo não evidenciou diferenças de activação entre pró-sacadas verticais e horizontais, enquanto que os doentes com PSP demonstraram uma maior desactivação da RMP durante a excução de pró-sacadas verticais. Nas anti-sacadas, ambos os grupos exibiram uma maior desactivação da RMP durante a execução das sacadas no plano vertical e o grupo da PSP demonstrou ainda hipoactivação da aOF esquerda, putamen esquerdo e tálamo direito durante a execução de anti-sacadas verticais ($p<0.01$, corrigido; MLG)

As assimetrias corticais funcionais entre as sacadas verticais e horizontais parecem ocorrer distintamente em doentes com DP e em controlos saudáveis, especificamente na rede ocular motora e na rede neuronal em modo padrão. Mecanismos funcionais, possivelmente compensatórios, na área ocular parietal de doentes com DP, poderão manter o desempenho comportamental sacádico ao mesmo nível dos controlos saudáveis. No entanto, esta compensação pode ter o custo adicional de potenciar uma assimetria pré-existente na activação de sacadas verticais e horizontais na aOP, promovendo assim uma performance diferente entre planos sacádicos na DP, especialmente para as sacadas mais voluntárias. Na PSP, enquanto a literatura prévia se focou maioritariamente no envolvimento da rede sacádica no tronco encefálico, neste estudo foi encontrada evidência funcional de hipoactividade frontoestriatal em doentes

com PSP durante a excecução de saccadas, comparativamente aos controlos saudáveis, particularmente no plano vertical.

Em conclusão, a hipoactividade frontal durante sacadas foi mais marcada em doentes com PSP do que em doentes com DP, estendendo-se até aos gânglios da base no primeiro grupo. De forma importante, em doentes com PSP, a putativa compensação do córtex parietal durante as sacadas parece estar ausente, contrastando com os doentes com DP. Tanto no grupo de DP como no de PSP, as sacadas verticais voluntárias associaram-se a uma menor actividade das áreas oculares motoras (frontal e/ou parietal), comparativamente às sacadas horizontais, principalmente no último grupo. Estes novos achados realçam o impacto do envolvimento cortical no distúrbio sacádico da PD e PSP, e fornecem novos dados que corroboram o envolvimento distinto da rede sacádica vertical e horizontal em ambas as doenças, tanto a nível cortical como subcortical.

Keywords.

Eye movements

Saccades

Functional magnetic resonance imaging

Parkinson’s disease

Progressive supranuclear palsy

Basal ganglia

Co-authorship.

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I, João Lemos, as the author of this thesis was responsible for conducting every stage of the research program documented here, under the supervision of Professor Luís Cunha, Professor Freire Gonçalves and Professor Miguel Castelo-Branco. Specifically, I defined the overall problem, proposed to my supervisors the core scientific idea to solve it, helped to implement the eye-tracking and fMRI paradigms in both experimental studies, together with Drs. João Castelhano and Gil Cunha, and to collect data, together with Drs Daniela Pereira and Luciano Almendra, assisted in data processing and statistical analysis, together with Drs. Daniela Pereira, Diliana Rebelo and Miguel Patrício, interpreted the experimental results, wrote the entire draft version of the papers and carefully revised them according to co-authors comments. Prof. Cristina Januário gave expert advice concerning experimental design and assisted in the preparation of manuscripts for publication. At an earlier phase of this project, I also wrote two review papers under the supervision of Dr. Eric Eggenberger, who assisted in the preparation of manuscripts for publication.

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Dedication.

To Francisco (Chico).

Table of contents.

Title page i

Abstract iii

Resumo vii

Keywords xi

Co-authorship xiii

Acknowledgments xv

Dedication ….....xvii

Table of contents xix

List of tables xxv

List of figures xxvii

List of abbreviations and symbols …….....xxix

Chapter I. Introduction

1. General considerations on eye movements I-3

2. Anatomical and physiological substrate for saccades.....I-5

2.1. Cortex I-6

2.2. Thalamus and basal ganglia.....I-7

2.3. Superior colliculus.....I-10

2.4. Brainstem premotor centers.....I-10

2.5. Cerebellum.....I-12

2.6. Summary of common supranuclear saccadic abnormalities.....I.12

3. Cortical control of the vertical saccades …….....I-14

3.1. The vector theory.....I-14

3.2. Behavioural differences between vertical and horizontal saccades, and upward and downward saccades, and their putative cortical correlates.....I-16

3.3. Cortical lesions promoting impairment of the vertical saccades.....I-17

4. Prosaccades and antisaccades I-18

4.1. Prosaccades.....I-18

4.2. Antisaccades.....I-18

5. Functional magnetic resonance imaging of saccades.....I-20

5.1. The blood-oxygenation-level-dependent (BOLD) signal.....I-20

5.2. Task design.....I-20

5.3. fMRI of the saccadic oculomotor network.....I-21

6. Saccadic disturbance in Parkinson's disease I-26

7. Saccadic disturbance in progressive supranuclear palsy I-29

8. Thesis rationale I-33

References I-37

Chapter II. Supranuclear and internuclear eye movement disorders

Abstract …….....II-3

Keywords II-5

1. Introduction II-7

2. Saccadic disorders II-7

3. Fixation disorders II-10

4. Smooth pursuit disorders II-12

5. Optokinetic disorders II-14

6. Vergence disorders II-16

7. Vestibular disorders II-16

8. Conclusion II-19

References II-25

Chapter III. Saccadic intrusions: review and update

Abstract …….....III-3

Keywords III-5

1. Introduction III-7

2. Saccadic intrusions with normal intersaccadic intervals III-9

2.1. Square wave jerks (SWJ).....III-9

2.2. Square wave pulses (SWP).....III-10

2.3. Macrosaccadic oscillations (MSO).....III-10

2.4. Saccadic pulses (SP).....III-10

3. Saccadic intrusions without normal intersaccadic intervals III-11

3.1. Ocular flutter and opsoclonus (OF and OPS).....III-11

4. Treatment of saccadic intrusions (and oscillations) III-13

5. Comments III-14

References III-19

Chapter IV. Distinct functional properties of the vertical and horizontal saccadic network in health and Parkinson's disease: an eye-tracking and fMRI study

Abstract ……......IV-3

Keywords IV-5

1. Introduction IV-7

2. Methods IV-8

2.1. Participants.....IV-8

2.2. Procedures……IV-9

2.2.1. Behavioral procedure……...…….….IV-9

2.2.1.1 Prosaccades……..……..IV-10

2.2.1.2. Antisaccades……..……IV-10

2.2.2. Imaging procedure……..…….……IV-10

2.2.2.1. fMRI data acquisition.....IV-11

2.2.2.2. fMRI oculomotor task design.....IV-11

2.3. Data processing and analysis……IV-14

2.3.1 Behavioural data……...……IV-14

2.3.2 fMRI data……...…….……..IV-14

2.3.3 Statistical analysis……...……IV-15

3. Results IV-15

3.1. Clinical and demographic data……..……IV-15

3.2. Behavioral data……...……..……..IV-19

3.3. Correlations between behavioral data and demographic and clinical data……..……..…….IV-22

3.4. fMRI data……..……..……IV-22

3.4.1. Prosaccades……..…….…..IV-25

3.4.2. Antisaccades.....IV-28

3.4.3. Vertical > horizontal contrast analysis.....IV-31

3.5. Correlations between behavioral data and functional data…....……..IV-36

4. Discussion IV-36

4.1. The influence of demographic and clinical data on oculomotor behaviour.....IV-36

4.2. Behavioural data.....IV-37

4.3. Imaging data.....IV-38

4.4. Study limitations.....IV-40

4.5. Conclusions.....IV-41

References IV-45

Chapter V. Cortical control of vertical and horizontal saccades in progressive supranuclear palsy: an exploratory fMRI study

Abstract.....V-3

Keywords V-5

1. Introduction V-7

2. Methods V-8

2.1. Participants.....V-8

2.2. Procedures.....V-9

2.2.1. Behavioral procedure.....V-9

2.2.2. Imaging procedure.....V-10

2.3. Data processing and analysis.....V-12

2.3.1. Behavioural data.....V-12

2.3.2. fMRI data.....V-12

2.3.3. Statistical analysis.....V-12

3. Results V-13

3.1. Clinical and demographic data.....V-13

3.2. Behavioral data.....V-13

3.3. fMRI data.....V-16

3.3.1. Prosaccades.....V-16

3.3.2. Antisaccades.....V-19

3.3.3. Vertical > horizontal Saccades.....V-22

4. Discussion V-26

4.1. Behavioural data.....V-26

4.2. Imaging data.....V-27

4.3. Study limitations.....V-29

4.4. Concluding remarks.....V-29

References V-33

Chapter VI. General Discussion

1. Summary of findings VI-3

1.1. Experiment 1.....VI-3

1.2. Experiment 2.....VI-5

2. Thoughts on the field VI-4

2.1. Findings in healthy participants.....VI-5

2.2. Findings in patients.....VI-6

2.3. Additional considerations.....VI-10

3. Future directions VI-11

4. Concluding remarks VI-13

References VI-15

Appendix.

1. Supplemental Table 1. Talairach coordinates (x,y,z) and number of voxels (v) of peak activations in GLM contrast maps for AS-PS contrast (*Chapter IV*) A-3

2. Supplemental Figure 1. T-contrast maps of horizontal PS > baseline contrast in control group and PD group (*Chapter IV*) A-7

3. Supplemental Figure 2. T-contrast maps of vertical PS > baseline contrast in control group and PD group (*Chapter IV*).....A-9

4. Supplemental Figure 3. T-contrast maps of horizontal AS > baseline contrast in control group and PD group (*Chapter IV*).....A-13

5. Supplemental Figure 3. T-contrast maps of vertical AS > baseline contrast in control group and PD group (*Chapter IV*).....A-15

6. Supplemental Figure 1. T-contrast maps of prosaccades (PS) + antisaccades (AS) > baseline contrast in control group (*Chapter V*) A-19

Curriculum vitae ……..……...…..……...……....C-1

List of tables.

Chapter I.

Table 1. Types of eye movements …….……..……..I-5

Table 2. Types of saccades.....I-7

Table 3. Summary of common saccadic abnormalities of supranuclear origin.....I-13

Table 4. Summary of common saccadic abnormalities in PD and PSP.....I-32

Chapter IV.

Table 1. Demographic and Clinical Data……...……IV-9

Table 2. Clinical information for PD patients.....IV-17

Table 3. Behavioral Data.....IV-20

Table 4. Talairach coordinates (x,y,z) and number of voxels (v) of peak activations in GLM contrast maps for horizontal prosaccades (PS) and vertical PS contrasts between controls and PD patients.....IV-26

Table 5. Talairach coordinates (x,y,z) and number of voxels (v) of peak activations in GLM contrast maps for horizontal antisacades (AS) and vertical AS contrasts between controls and PD patients.....IV-29

Table 6. Talairach coordinates (x,y,z) and number of voxels (v) of peak activations in GLM contrast maps for vertical PS-horizontal PS and vertical AS-horizontal AS contrasts in controls and Parkinson's disease patients.....IV-32

Chapter V.

Table 1. Demographic and Clinical Data.....V-9

Table 2. Behavioral Data.....V-15

Table 3. Talairach coordinates (x,y,z) and number of voxels (v) of peak activations in GLM contrast maps for horizontal prosaccades (PS) and vertical PS contrasts between controls (CTL) and PSP group.....V-17

Table 4. Talairach coordinates (x,y,z) and number of voxels (v) of peak activations in GLM contrast maps for horizontal antisaccades (AS) and vertical AS contrasts contrast between controls (CTL) and PSP group.....V-20

Table 5. Talairach coordinates (x,y,z) and number of voxels (v) of peak activations in GLM contrast maps for vertical prosaccades (PS)-horizontal PS and vertical antisaccades (AS)-horizontal AS contrasts within controls (CTL) and PSP group....V-23

Chapter VI.

Table 1. Summary of fMRI findings in patients.....VI-9

List of figures.

Chapter I.

Figure 1. Types of eye movements..... .....I-4

Figure 2. Basal ganglia control of saccades. I-9

Figure 3. Brainstem control of horizontal and vertical saccades.....I-11

Figure 4. Schematic diagram depicting the pathophysiology of saccade abnormalities in Parkinson's disease (PD).....I-28

Figure 5. Schematic diagram depicting the pathophysiology of saccade abnormalities in progressive supranuclear palsy (PSP).....I-31

Chapter II.

Figure 1. Human eye movements……..…...……II-7

Figure 2. Cortical and subcortical anatomic circuitry of horizontal saccades.....II-8

Figure 3. Saccadic behaviour.....II-9

Figure 4. Fixational eye movements.....II-12

Figure 5. Cortical and subcortical anatomic circuitry of horizontal smooth pursuit...II-13

Figure 6. Cortical and subcortical anatomic circuitry of optokinetic response.....II-15

Figure 7. Graviceptive pathway disorders.....II-18

Chapter III.

Figure 1. Schematic illustrations of saccadic intrusions ……...……..III-8

Chapter IV.

Figure 1. Schematic representation of stimuli and time course of the oculomotor tasks.....IV-13

Figure 2. T-contrast maps of prosaccade (PS) and antisaccade (AS) blocks compared with baseline in control group and PD group.....IV-24

Figure 3. Group t-statistical activation maps comparing horizontal prosaccades (PS) and vertical PS contrasts between CTL and PD groups.....IV-27

Figure 4. Group t-statistical activation maps comparing horizontal antisaccades (AS) and vertical AS contrasts between CTL and PD groups.....IV-30

Figure 5. Group t-statistical activation maps comparing vertical prosaccades (PS) to horizontal PS in CTL and PD group.....IV-34

Figure 6. Group t-statistical activation maps comparing vertical antisaccades (AS) to horizontal AS in CTL and PD group.....IV-35

Chapter V.

Figure 1. Schematic representation of stimuli and time course of the oculomotor tasks outside and inside the scanner.....V-11

Figure 2. Group t-statistical activation maps comparing horizontal prosaccades (PS) and vertical PS between controls (CTL) and PSP group.....V-18

Figure 3. Group t-statistical activation maps comparing horizontal antisaccades (AS) and vertical AS contrasts between controls (CTL) and PSP group.....V-21

Figure 4. Group t-statistical activation maps comparing vertical prosaccades (PS)>horizontal PS within PSP group.....V-24

Figure 5. Group t-statistical activation maps comparing vertical antisaccades (AS)>horizontal AS within controls (CTL) and PSP group.....V-25

List of abbreviations and symbols.

Chapter I.

FEF, Frontal eye field

SEF, Supplementary eye field

pre-SMA, Pre-supplementary area

PEF, Parietal eye field

dlPFC, Dorsolateral prefrontal cortex

CEF, Cingulate eye field

DMN, Default-mode network

SC, Superior colliculus

riMLF, Rostral interstitial nucleus of the medial longitudinal fasciculus

PPRF, Pontine paramedian reticular formation

IML, Internal medullary lamina

CN, Caudate nucleus

STN, Subthalamic nucleus

SNpr, Substantia nigra pars reticulata

GPe, Globus pallidus, external segment

SNpc, Sustantia nigra, pars compacta

MLF, Medial longitudinal fasciculus

DV, Dorsal vermis

FN, Fastigial nuclei

PD, Parkinson's disease

PSP, Progressive supranuclear palsy

fMRI, Functional magnetic resonance imaging

BOLD, Blood-oxygenation-level-dependent

HRF, Hemodynamic response function

Chapter II.

BVF, Bilateral vestibular failure

DBS, Deep brain stimulation

ET, Essential tremor

MiS, Microsaccades

OKN, Optokinetic nystagmus

OPS, Opsoclonus

PD, Parkinson's disease

SI, Saccadic intrusions

STN, Subthalamic nucleus

SWJ, Square wave jerks

SVGP, Saccadic vertical gaze palsy

SVV, Subjective visual vertical

VM, Vestibular migraine

Chapter III.

SI, Saccadic intrusions

SO, Saccadic oscillations

SWJ, Square wave jerks

PSP, Progressive supranuclear palsy

FA, Friedereich's ataxia

PD, Parkinson's disease

MSA, Multi-system atrophy

SCA 6, Spinocerebelar ataxia type 6

FCMTE, Familial cortical myoclonic tremor and temporal epilepsy

AOA 2, Ataxia with oculomotor apraxia type 2

OPN, Omnipause neurons

SC, Superior colliculus

SWP, Square wave pulses

MS, Multiple sclerosis

FN, Fastigial nucleus

SNpr, Substantia nigra pars reticulata

MSO, Macrosaccadic oscillations

SCASI, Spinocerebellar ataxia with saccadic intrusions

SP, Saccadic pulses

SSP, Single saccadic pulses

DSP, Double saccadic pulses

OF, Ocular flutter

OPS, Opsoclonus

OMS, Opsoclonus-myoclonus syndrome

CSF, Cerebrospinal fluid

OCB, Oligoclonal bands

mSOLT, microsaccadic oscillations and limb tremor

IVIG, Intravenous immunoglobulin

Chapter IV.

PD, Parkinson’s disease

DMN, Default-mode network

BG, Basal ganglia

SC, Superior colliculus

FEF, Frontal eye field

SEF, Supplementary eye field

PEF, Parietal eye field

dlPFC, Dorsolateral prefrontal cortex

vmPFC, Ventromedial prefrontal cortex

dmPFC, Dorsomedial prefrontal cortex

Chapter V.

PSP, Progressive supranuclear palsy

BOLD, Blood oxygenation-level dependent

fMRI, Functional magnetic resonance imaging

DMN, Default-mode network

FEF, Frontal eye field

SEF, Supplementary eye field

PEF, Parietal eye field

dlPFC, Dorsolateral prefrontal cortex

vmPFC, Ventromedial prefrontal cortex

dmPFC, Dorsomedial prefrontal cortex

Chapter VI.

PD, Parkinson's disease

FEF, Frontal eye field

PEF, Parietal eye field

SEF, Supplementary eye field

dmPFC, Dorsomedial prefrontal cortex

CS, Superior colliculus

DMN, Default-mode network

PSP, Progressive supranuclear palsy

riMLF, Rostral interstitial nucleus of the medial longitudinal fasciculus

PPRF, Pontine paramedian reticular formation

fMRI, Functional magnetic resonance imaging

BOLD, Blood-oxygenation-level-dependent

ROI, Region-of-interest

°, degree

°/s, degree per second

ms, millisecond

Chapter I

Introduction

1. General considerations on eye movements.

Eye movements serve two main purposes: (1) to *stabilize* gaze and keep an image steady on the retina, (2) and to *shift* gaze towards an object of interest [1]. Thus, eye movements can be divided into five different classes, each controlled through separate neural pathways that converge at the level of the ocular motor nuclei in the brainstem [2]. Saccades are rapid eye movements that redirect our gaze to bring the image of an object of interest onto the central region of the retina (fovea) where photoreceptor density is greatest and visual acuity is highest. Depending on the behavioural context in which they are generated, they can be voluntary or reflexive in nature (see below). Smooth pursuit eye movements are much slower and enable us to keep a moving object steady on the fovea. Vergence eye movements consist of disconjugate convergent or divergent movements triggered by either disparity between the location of images on both retinas and/or retinal blur. Vestibular eye movements serve to stabilize the retinal image by compensating brief head movements and/or changes in static orientation of the head. Finally, optokinetic eye movements constitute a slow visually mediated response triggered by large moving visual fields. They complement the vestibular response particularly in the low-frequency range movements and share common neural pathways with smooth pursuit eye movements [1, 2]. Ocular fixation on the other hand, which intuitively could suggest the absence of eye movements, is in fact comprised of small eye movements (<0.5°) (i.e., microsaccades, ocular tremor and drift) which seem to prevent fading of images and further stimulate visual tracking mechanisms [3]. Taken together, during evolution, with the evolvement of foveal vision, binocularity, and frontal vision, voluntary eye movements including saccades, smooth pursuit and vergence became necessary and were "added" to the initial phylogenetic repertoire of eye movements, (i.e., vestibular and optokinetic response). **Figure 1** and **Table 1** provide a general overview of the different types of eye movements and their general characteristics.

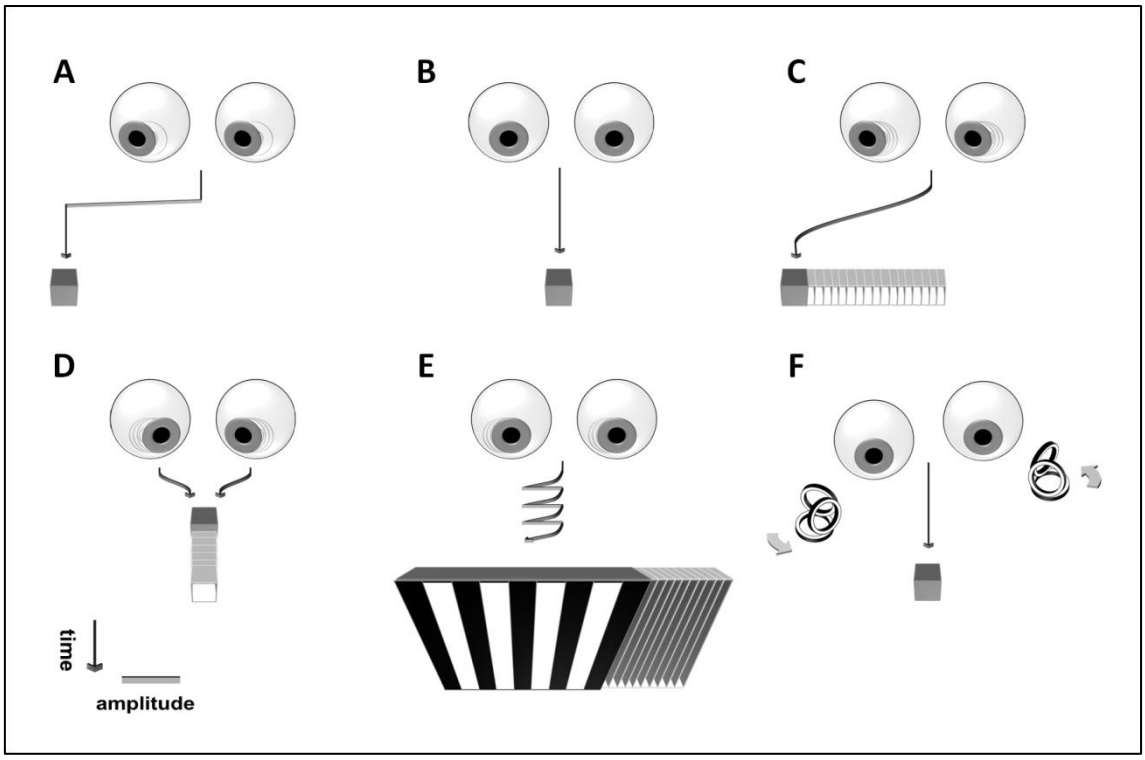

Figure 1. Types of eye movements. A. Saccade is a rapid gaze shift that brings the image of an object of interest onto the fovea. B. Fixation holds the image of a stationary object on the fovea when the head is still. C. Smooth pursuit keeps the image of a small moving target on the fovea. D. Vergence moves the eyes in opposite directions to keep foveating an object of interest. E. Optokinetic response keeps the image of a large moving scene on the fovea. F. Vestibulo-ocular reflex holds the image of a stationary object on the fovea during brief head movements. From reference [4]

Table 1. Types of Eye Movements

Saccades	Rapid, ballistic eye movements which bring images of objects of interest onto the fovea, moving the eyes to a new position; they can be voluntary or present as fast phases of vestibular or optokinetic nystagmus (their velocity can exceed 700°/s while their latency is generally 200–250 ms)
Fixation	Negative feedback system that holds the image of a stationary object on the fovea, keeping gaze steady and minimizing eye drifting
Smooth Pursuit	Slow tracking eye movements (usually less than 50°/s) which keep the image of a small moving stimulus on the fovea
Vergence	Convergent or divergent disjunctive eye movements which align the fovea of each eye with objects located at different distances from the observer (generally small [less than 5°] and slow, although they may be fast if made in conjunction with saccades)
Optokinetic	Slow compensatory eye movements that hold large moving images on the retina; extended head rotation or translation in one direction also produces an optokinetic response
Vestibular	Rapid compensatory eye movements that stabilize images of the visual world on the retina during brief head rotations or translations (up to 800°/s linear movements), compensating for head movements in space

Adapted from reference ref. [1]

2. Anatomical and physiological substrate for saccades.

Saccadic eye movements (saccades) redirect the eyes to foveate a new object of interest, enabling us to explore the visual world in high definition. They are triggered by the cerebral hemispheres and ultimately generated in the brainstem reticular formation. The main cortical areas involved in the generation of saccades include the frontal eye field (FEF), the supplementary eye field (SEF) and pre-supplementary motor area (pre-SMA), and the parietal eye field (PEF) [5]. Particularly for the execution of more voluntary saccades (e.g., antisaccades; see below), additional involvement of the dorsolateral prefrontal cortex (dlPFC) and the posterior part of the anterior cingulate cortex (cingulate eye field, CEF) has been consistently demonstrated [5, 6]. Additionally, the default-mode network (DMN), a network usually active when the brain is not engaged in specific behavioral tasks, is significantly more deactivated during voluntary saccades when compared to reflexive saccades, suggesting greater recruitment of attentional resources during the former type of saccades [7]. Saccadic cortical centers then project their signal to the superior colliculus (SC) and basal ganglia, and from here the saccade command reaches the brainstem [1]. Basal ganglia are thought to play an important role in "gating" saccades in the context of more complex and voluntary behaviours that involve memory, expectations and reward (see below) [8]. In the brainstem, the supranuclear gaze centers are segregated in the midrain for the execution of vertical saccades (i.e., rostral interstitial nucleus of the medial longitudinal fasciculus [riMLF]) and pons for the execution of horizontal saccades (i.e.,

pontine paramedian reticular formation [PPRF]) [9]. The ocular motor nuclei of the third, fourth and sixth cranial nerves receive the final saccadic input from riMLF and/or PPRF, and drive the extraocular muscles [1].

2.1. Cortex.

In animal experiments, when FEF (Brodmann's area 6) is stimulated, a contralateral saccade is elicited [10]. FEF seems to be involved in the generation of more voluntary saccades (e.g., antisaccades, memory-guided saccades, predictive saccades, and intentional visually-guided saccades) (see **Table 2**) [11]. In humans, when FEF is lesioned, contralateral saccades became impaired: memory-guided saccades are delayed and inaccurate, antisaccades become misdirected, and prosaccades are short (hypometric) [12]. Electrical stimulation in the SEF (Brodmann's area 6), located in the dorsomedial frontal lobe, elicits contralateral saccades [13]. SEF seems to play a role in more complex saccadic behaviour such as the execution of a sequence of saccades or antisaccades. Thus, lesions in SEF impair the ability to generate a sequence of memory-guided saccades, more so for left lesions [14]. Immediately anterior to SEF, pre-SMA seems to be involved in behavioural tasks in which a sudden change occurs after a previously established rule [15]. PEF (Brodmann's areas 39 and 40) mainly triggers contralateral reflexive saccades. This is probably conveyed through a direct pathway between PEF and the SC [16]. Thus, reflexive saccades become delayed and short after PEF lesions, more so for right lesions [17, 18]. Similar deficits in memory-guided saccades have been demonstrated after PEF lesions, possibly caused by the interruption of corticocortical networks linking PEF and the frontal lobe [19, 20]. The dlPFC (Brodmann's area 46) lies in the dorsolateral convexity of the frontal lobe, anterior to the FEF, and contrary to the areas described so far, is not an intrinsic motor saccadic area. dlPFC on the other hand, seems to be an executive area involved in the inhibition of unwanted reflexive saccades and working memory. Thus, lesions in dlPFC give raise to misdirected antisaccades (reflexive saccades made in the direction of the target) and inaccurate memory-guided saccades [18, 21]. Particularly in the antisaccade task, it is currently hypothesized that the dlPFC is responsible for the inhibition of reflexive saccades (towards the target), while FEF appears to subsequently trigger correct saccades (against the target) [22]. dlPFC possibly exerts its inhibitory activity via SC [23]. CEF is located in the posterior part of the anterior cingulate cortex (Brodmann areas 23 and 24) and its role in saccades execution is still a matter of debate. It seems to be more involved in the execution of voluntary saccades (e.g., memory-guided saccades and antisaccdes) and its function may include motivation, error monitoring and/or suppression of reflexive behaviour [24, 25]. When CEF is lesioned, antisaccades become misdirected and memory-guided saccades are inaccurate and delayed [24].

Table 2. Types of saccades

Spontaneous saccades	Random saccades that occur when the subject is not engaged in any specific behavioural task
Reflexive saccades	Saccades generated to unexpected novel stimuli within the environment (visual, auditory or tactile)
Express saccades	Very short latency saccades to a novel stimulus, usually elicited in lab environment by using a paradigm in which there is a time gap between fixation stimulus disappearance and target (novel stimulus) appearance (gap paradigm)
Prosaccades*	Voluntary saccades generated in the direction of a suddenly appearing stimulus
Antisaccades	Voluntary saccades generated in the opposite direction of a suddenly appearing stimulus
Memory-guided saccades	Voluntary saccades generated to the location of a previously presented stimulus
Predictive/anticipatory saccades	Voluntary saccades generated to a location where a target is already anticipated/expected
Sequences of saccades	Voluntary memory-guided saccades generated sequentially to a series of locations of previously presented stimuli

Adapted from references [2, 11]

* Prosaccades are usually used in behavioural paradigms in conjunction with other types of saccades, usually more voluntary in nature (e.g., antisaccades). Thus, prosaccades are frequently mentioned in the literature as an example of a reflexive behavioural task, as a result of being included in paradigms in which they represent the less voluntary type of saccade. Nevertheless, they are voluntary in nature. Albeit stimuli location and time appearance during a prosaccade task is usually randomized, thus enhancing the unexpected nature of the stimuli, prosaccades should not be considered purely reflexive saccades [26].

2.2. Thalamus and basal ganglia.

Stimulation of thalamic neurons located in the internal medullary lamina (IML) elicit contralateral saccades [27]. Since IML neurons receive projections from SC, brainstem and cerebellum, project their signal to the basal ganglia and cortical eye fields, but do not seem to have direct pathways to supranuclear brainstem structures (i.e., riMLF and PPRF), thalamus may work as a source of efference copy (i.e., an update of the visual space across saccades, providing accurate information about eye position, this way ensuring visual stability) to the cortical eye fields [28]. Not surprisingly, thalamic lesions have been associated with inaccurate saccades in the double step task, a paradigm in which the execution of a saccade is crucially dependent on the information about eye position at the end of the saccade performed immediately before [29]. The specific involvement of *cerebellar*-thalamo-cortical pathways within thalamus seems to be instrumental for causing such poor saccade adaptation [30].

Basal ganglia, particularly the caudate nucleus (CN), the subthalamic nucleus (STN) and the substantia nigra pars reticulata (SNpr), participate in complex saccadic behaviour (i.e., memory-guided saccades, antisaccades) performed mainly to the contralateral side (see **Figure 2**). Three distinct pathways within basal ganglia play a role in saccade generation, projecting their final output to the SC. The direct pathway (CN; SNpr) facilitates saccades while the indirect (CN; external segment of the globus pallidus, GPe; SNpr) and hyperdirect (cortex; STN; SNpr) pathways suppress saccades [31]. Specifically, the SNpr tonically inhibits the SC, which seems to counteract the excitatory signals from FEF, PEF and dlPFC to SC [32–34]. Immediately before a saccade, the CN sends phasic inhibitory signals directly to the SNpr (direct pathway), which removes the sustained SNpr-induced inhibition on SC and facilitates the initiation of a saccade. CN is activated on its turn by several cortical areas, including FEF, SEF and dlPFC [33, 35, 36]. In contrast, saccades may be suppressed if the spontaneous inhibition on SC is enhanced by activation of the indirect and/or hyperdirect pathway and subsequent excitation of the SNpr neurons [37–39]. The hyperdirect pathway (cortex; STN; SNpr) seems to be particularly relevant in maintaining a stable fixation immediately before a saccade. Similarly to CN, the STN also receives inputs from the FEF, SEF and dlPFC, albeit cortical signals seem to reach STN earlier, when compared to CN [31, 40–42]. Taken together, basal ganglia act as "gating" mechanism for saccade generation, only facilitating saccade execution when sustained inhibition on SC is reduced by the activation of the direct pathway. This mechanism probably prevents the convergent excitatory cortical signals originated in PEF, SEF and FEF from triggering motor output of the SC. Importantly, basal ganglia seem to select an appropriate behaviour not only on the basis of memory (see above) but also of expectation. Thus, reward expectation modulates basal ganglia saccade-related activity. Animal studies on reward have elegantly shown that the efficacy of corticocaudate synapses can be enhanced by dopamine reward-related inputs from substantia nigra pars compacta (SNpc) to CN, if reward is expected after the saccade. Specifically, saccades that were rewarded previously were less delayed and faster than non-rewarded saccades [8].

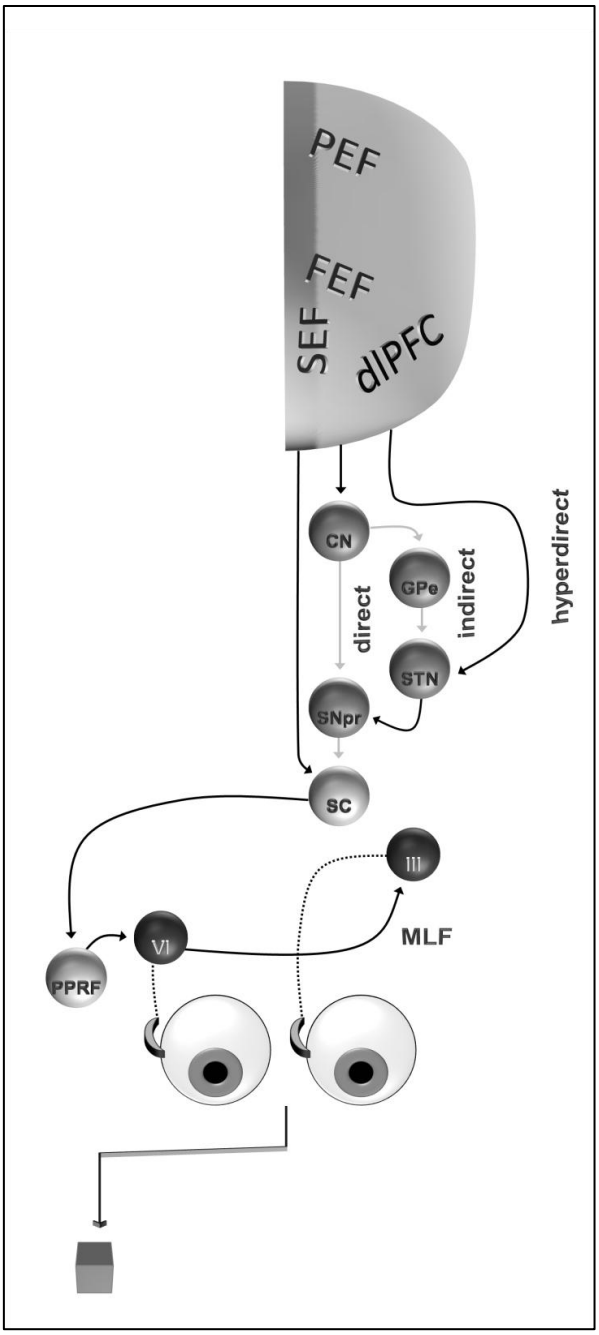

Figure 2. Basal ganglia control of saccades. Basal ganglia mainly "gate" contralateral saccades. While the direct pathway (CN --> SNpr) facilitates saccades, the indirect (CN --> GPe --> STN --> SNpr) and hyperdirect (cortex --> STN --> SNpr) pathways suppress saccades (see text for further details). Black lines represent excitatory connections. Gray lines represent inhibitory connections. The image represents from top to bottom: cortical oculomotor network (FEF, SEF, PEF, dlPFC); subcortical oculomotor network (basal ganglia: CN, GPe, STN, SNpr); brainstem oculomotor network (SC, PPRF, MLF, VI and III nucleus). In the example above, the activation of the left hemisphere promotes a contralateral rightward saccade. (for sake of clarity, substantia nigra pars compacta and the correspondent dopaminergic input to CN were omitted). FEF, frontal eye field; SEF, supplementary eye field; PEF, parietal eye field; dlPFC, dorsolateral prefrontal cortex; CN, caudate nucleus; GPe, external component of globus pallidus; STN, subthalamic nucleus; SNpr, substantia nigra pars reticulata; SC, superior colliculus; PPRF, paramedian pontine reticular formation; VI, sixth nucleus; III, third nucleus; MLF, medial longitudinal fasciculus. Adapted from references [1, 31]

2.3. Superior colliculus.

The SC is a multi-layered structure which contains a visuomotor map. The dorsal layers (visual map) receive retinal projections which orderly project onto its surface, mapping the contralateral visual field. The ventral layers (motor map) participate in the generation of contralateral saccades and project to premotor structures in the brainstem, including the PPRF and the riMLF [43]. This motor map is retinotopically organized: large saccades are represented in the inferior segment while short saccades are represented in the superior segment; saccades with a predominant upward component are mediated medially while those with a predominant downward component are mediated laterally [44]. The rostral pole of the SC suppresses saccades instead, since it contains "fixation" neurons which project to pontine omnipause neurons, which on their turn inhibit PPRF and riMLF neurons [45]. Isolated SC lesions in humans are rare, but according to experimental animal data, rostral pole lesions may promote very short latency saccades (express saccades) (see **Table 2**) and frequent saccadic intrusions, while more caudal lesions are possibly associated with delayed, slow and short contralateral saccades, and a paucity of spontaneous contralateral saccades [46–49]. Nevertheless, enduring deficits from SC lesions are rare, unless if associated with lesions of other critical areas including FEF [50]. SC seems to contribute to the selection of a target to be foveated, showing no direct control on saccadic amplitude, direction or trajectory [51].

2.4. Brainstem premotor centers.

Brainstem control of saccades has been extensively studied. As previously mentioned, the SC relays saccadic commands from the cortical oculomotor network onto the brainstem premotor centers (PPRF; riMLF). Currently, it is well established that PPRF in pons provides the premotor saccadic signal for horizontal saccades while riMLF in midbrain plays the equivalent role for vertical saccades [52]. Specifically, stimulation of the PPRF promotes ipsilateral saccades [53]. And as expected, an isolated lesion of the PPRF abolishes ipsilateral saccades [54]. The PPRF projects to the ipsilateral VI nucleus. The sixth nucleus contains two types of neurons: (1) motoneurons which innervate the ipsilateral lateral rectus muscle and (2) interneurons, which project to the medial rectus subgroup of the contralateral III nucleus by ascending in the medial longitudinal fasciculus (MLF) (see **Figure 3.a,b**) [55, 56]. Neurons in the riMLF encode vertical and torsional saccadic signals [57]. The pathways projecting from riMLF to oculomotor nuclei (III and IV nucleus) seem to differ with respect to the direction being coded (upward vs. downward saccades). Thus, pathways for upward saccades project bilaterally to the III nucleus while pathways for downward saccades project ipsilaterally to the III and IV nucleus (see **Figure 3.c,d**) [58, 59]. Torsional saccades on the other hand, are encoded ipsilaterally [57]. This unique architecture may explain why unilateral lesions of the riMLF only partially affect vertical saccades (downward saccades are usually more compromised, since these lack bilateral riMLF

projections), while completely abolishing ipsitorsional quick eye movements [60]. Bilateral riMLF lesions on the other hand completely abolish torsional and vertical saccades [60, 61].

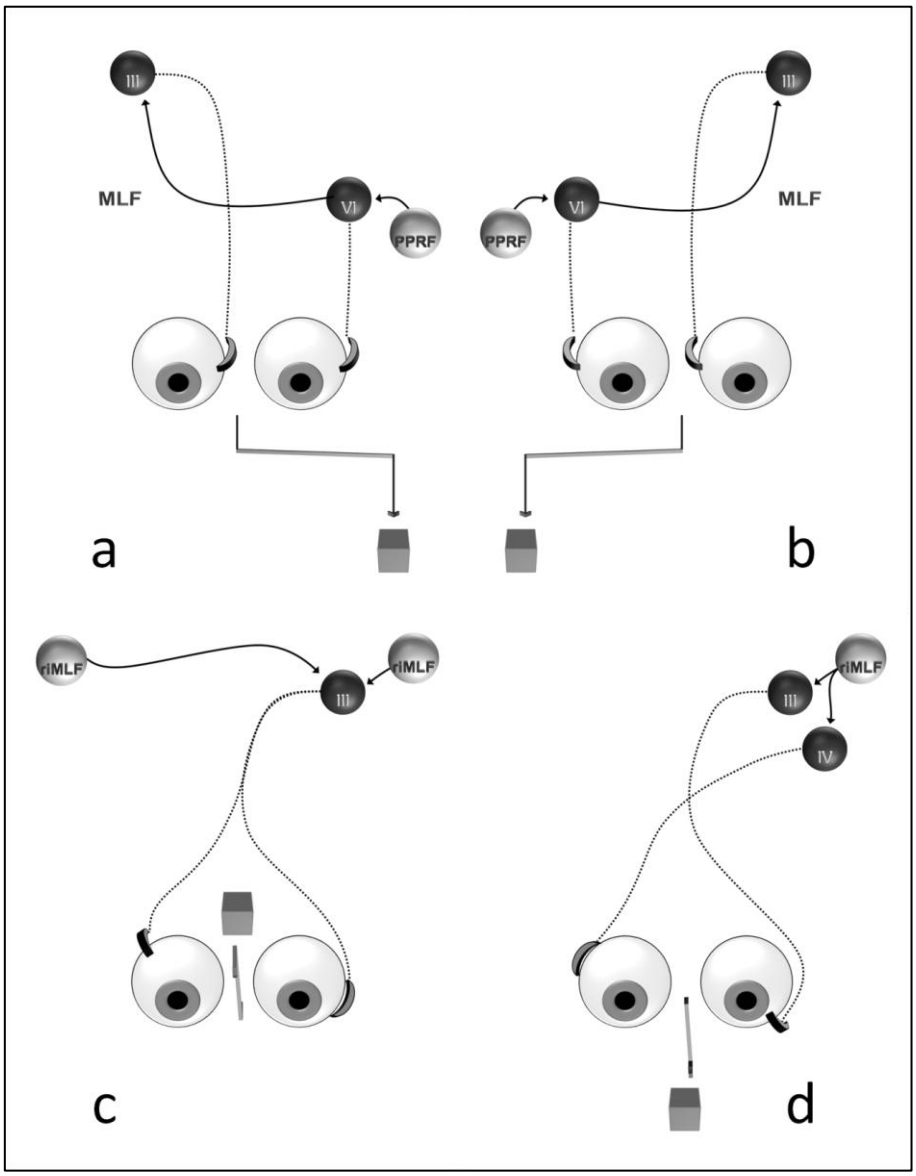

Figure 3. Brainstem control of horizontal (**a**, leftward; **b**, rightward) and vertical (**c**, upward; **d**, downward) saccades. Each PPRF excites the ipsilateral VI nucleus. From here, motoneurons will innervate the ipsilateral lateral rectus muscle and interneurons will ascend in the MLF and project to the contralateral medial rectus subgroup in the III nucleus. The latter structure will innervate the ipsilateral medial rectus muscle (**a,b**). Each riMLF contains neurons conveying upward and downward saccades. However, during upward saccades, the III nucleus seems to receive bilateral riMLF projections (**c**), while during downward saccades, the III and IV nucleus only receive ipsilateral riMLF projections (**d**) (see text for further details). Contralateral ocular motor nuclei and/or saccade generator centers have been omitted for sake of clarity. MLF, medial longitudinal fasciculus; PPRF, pontine paramedian reticular formation; riMLF, rostral interstitial nucleus of the medial longitudinal fasciculus; III, third nucleus; IV, fourth nucleus; VI, sixth nucleus

2.5. Cerebellum.

The cerebellum receives and projects several pathways related to saccadic performance. Here we highlight the projections from FEF, SEF and PEF via the SC and pontine nuclei, to the dorsal vermis (DV) and fastigial nuclei (FN). The cerebellum on its turn projects to these same structures directly and/or via thalamus [62, 63]. The DV and FN play an important role in the accuracy of saccades. Thus, Purkinje cells in the DV are active immediately before an ipsilateral saccade and towards the end of a contralateral saccade [64]. Cell axons of the DV project exclusively to the FN, inhibiting it [65]. FN cells on its turn discharge immediately prior to onset of contralateral saccades and towards the end of ipsilateral saccades [66]. Among several efferent pathways, FN cells send their projections to the PPRF and riMLF in the brainstem [67]. Taken together, cerebellum role in the control of saccades includes: (1) to provide an additional drive to increase early saccade acceleration; (2) to monitor saccade progress by detecting errors and updating saccade commands to accurately move the eyes; (3) to assure that a saccade terminates at the right time. Thus, the DV receives updated information about saccade initiation, progress and termination and adjusts its inhibition upon the FN to optimally reprogram the correct amplitude, direction, and speed [68, 69]. Unilateral lesions of the DV cause hypometric ipsilateral saccades and hypermetric contralateral saccades [70]. The opposite pattern occurs with unilateral FN lesions [71]. Bilateral lesions of the DV and FN cause bilateral hipometria and hypermetria, respectively [71, 72]. Interestingly, cerebellar dysfunction also leads to impairments of reflexive and voluntary saccades usually seen in lesions affecting the cortical and/or subcortical oculomotor network. This supports the view that cerebellum influences the cortical mechanisms related to the control of saccades. Thus, in cerebellar patients, saccadic gain is variable and saccade latency is prolonged. Antisaccades are misdirected and memory-guided saccades and sequences of saccades are inaccurate [73]. The cerebellar control of vertical saccades is not well understood. The posterior interpositus nucleus appears to show an influence on vertical saccades, since its inactivation causes hypermetria of upward saccades and hypometria of downward saccades [74].

2.6. Summary of common supranuclear saccadic abnormalities.

Table 3 lists common saccadic abnormalities related to the dysfunction of supranuclear oculomotor structures.

Table 3. Summary of common saccadic abnormalities of supranuclear origin*

Abnormality	Site of lesion
Hypometric saccades	Dorsal vermis (+ipsilateral[§]); fastigial nucleus (+contralateral[§]); FEF (+contralateral[§]); PEF (+contralateral[§]); superior colliculus (+contralateral[§]); basal ganglia (vertical>horizontal)
Hypermetric saccades	Fastigial nucleus (+ipsilateral[§]); dorsal vermis (+contralateral[§])
Delayed saccades	FEF (+contralateral[§]; +voluntary saccades[§]); PEF (+contralateral[§]; +reflexive saccades[§]); CEF (+voluntary saccades); superior colliculus (+contralateral[§]); basal ganglia (+voluntary saccades)
Short latency saccades	Superior colliculus (+rostral pole)
Slow saccades	riMLF (vertical and torsional[§§]; ipsitorsional>vertical[§]); PPRF (+horizontal[§§]; ipsilateral[§]); superior colliculus (+caudal lesion; +contralateral[§]); omnipause neurons (vertical and horizontal)
Misdirected AS	dlPFC; FEF; SEF; CEF; basal ganglia
Inaccurate MGS	FEF; SEF; dlPFC; CEF; basal ganglia
Inaccurate sequences of MGS	SEF
Inaccurate DSS	Thalamus; cerebellum

Adapted from reference [11, 75]

*This list is not supposed to be exhaustive and some lesion locations have been rarely demonstrated in humans

[§]If unilateral lesion; [§§] if bilateral lesion

AS, antisaccades; MGS, memory-guided saccades; DSS, double-step saccades; FEF, frontal eye field; SEF, supplementary eye field; PEF, parietal eye field; CEF, cingulate eye field; dlPFC, dorsolateral prefrontal cortex; riMLF, rostral interstitial nucleus of the medial longitudinal fasciculus; PPRF, paramedian pontine reticular formation

3. Cortical control of the vertical saccades.

It is clear from the last section that most of the research on cortical/subcortical control of saccadic eye movements has been focused on horizontal saccades, while the investigation of vertical saccades has deserved far less attention. In sharp contrast, the brainstem control of vertical and horizontal saccades has been extensively investigated [76]. This could be due to several of the following reasons: (1) vertical saccades are mostly spared in cortical lesions, which has made them less attractive for investigating the cortical oculomotor network [77]; (2) some of the physiological mechanisms raised to explain common saccadic paradigms along the horizontal plain (e.g., antisaccades; dlPFC influence upon FEFs, facilitating one FEF's activation over the FEF on the other hemisphere) cannot be used to fully explain the same paradigms along the vertical plane [78]; (3) age-related impairment of vertical saccades (e.g., upward hypometria) made them a less specific parameter at bedside assessment, when trying to differentiate patients with known impairment of the amplitude of vertical saccades (e.g., Parkinson's disease) from controls [79, 80]; (4) bilateral cortical stimulation, which is technically more challenging than unilateral stimulation, is required to elicit pure vertical eye movements in animal experiments [81, 82]; (5) the recording of vertical eye movements has posed technical limitations in the past (e.g., lid artifacts), when the use of electro-oculography was standard practice [1].

Still, there are strong arguments derived from both normal individuals and patients' population which stress the urgent need for studying vertical saccades. First, vertical saccades assume equal relevance to their horizontal counterpart in daily activities, contributing to locomotion and social interaction [77]. Second, normal individuals show subtle but reproducible differences between vertical and horizontal saccades (e.g., vertical saccades are more delayed and less accurate) which suggests that saccade plane and direction may be distinctively driven not only within the brainstem, but also at a cortical level [83–86]. In certain neurodegenerative disorders (e.g., progressive supranuclear palsy, PSP), vertical gaze impairment may be the only presenting sign, highlighting the importance of evaluating vertical saccades in a consistent basis and the need for further elucidate their cortical and subcortical mechanisms in detail [87].

3.1. The vector theory.

Early studies largely based on extrapolation from monkey data have provided important insight into the cortical control of vertical saccades. Thus, unilateral frontal cortex (precentral sulcus) or occipital cortex faradization was followed by pure contralateral deviation of the eyes. Unilateral stimulation immediately above and below those points still promoted contralateral deviation of the eyes, but now with an associated downward and upward component, respectively. Bilateral simultaneous excitation of the same points caused no eye movements (if stimulation was performed on two similar points which had previously given on unilateral stimulation, pure lateral conjugate deviation of the eyes), purely downward (if stimulation was performed on two similar points located

immediately above), and upward eye movements (if stimulation was performed on two similar points located immediately below). If stimulation was simultaneously given on one point of the frontal cortex (which had previously given pure contralateral deviation of the eyes) and on a point of the opposite occipital cortex (which had previously given the same movement of the eyes but in a contrary direction) the action of the frontal cortex invariably preponderates (contralateral deviation of the eyes) [81, 82]. Purely vertical (up or down) movements were rarely obtained under unilateral cortical stimulation [82]. Frontal cortex preponderance over occipital cortex could be due to fact that, especially in occipital cortex, conjugate eye movements elicited by stimulation could reflect the excitation of a sensory system that ultimately reached the oculomotor system, and not necessarily a primary oculomotor effect [82]. These experiments were pivotal in showing that the execution of vertical saccades required bilateral cortical activation, in contrast with horizontal saccades, which are mainly driven by the contralateral cortex. Still, the pathways conveying vertical signals between the cortex and the brainstem premotor centers (i.e., riMLF) and their dynamic functioning are largely unknown. Probably, these cortico-brainstem pathways are the same that subserve horizontal eye saccades. What changes during vertical saccades is the signal being conveyed [82]. According to the vector theory, each saccadic movement corresponds to a vector quantity with a specific amplitude and direction. During unilateral cortical stimulation, horizontal vectors are activated in one hemisphere and are probably reciprocally inhibited on the other, which produces a predominant horizontal eye displacement. During bilateral stimulation, the opposing right and left horizontal vectors cancel each other and the pure vertical vector (down or up) remains, producing pure vertical saccades [82, 88, 89]. Taken together, unilateral oculomotor pathways probably carry vertical and horizontal vectors information. Whether these signals are decomposed into separate and independent horizontal and vertical reference signals or instead are conveyed as a single vector comparator, before reaching the SC is largely unknown. Other studies have refined the above-mentioned observations by using microstimulation over the FEF. In Bruce et al's study it was shown that saccade direction, instead of showing a global organization across FEF, was coded in a systematic fashion at a local level at different tangential depths (small advances of the microelectrode [unilateral FEF stimulation] promoted saccades progressing from oblique upward to oblique downward, always directed to the opposite side) [10]. Subsequent animal studies on monkeys have confirmed that polar direction in FEF is probably mapped in columns [90]. Contemporaneous work in humans, using electrical stimulation on FEF and clearly separating generated saccadic from smooth pursuit eye movements, has shown in human patients that unilateral FEF stimulation causes mainly contralateral pure horizontal or oblique upward saccades. Contralateral oblique saccades with a downward component were only seen when the eyes were initially moved onto a vertical eccentric position. The authors proposed that vertical upward bias could suggest: (1) a more superficial representation of saccade neurons coding for upward directions since stimulation was applied superficially; (2) greater number and/or lower saccade thresholds of neurons coding for upward direction in FEF [91]. Still, early work

in humans has also shown contralateral oblique *downward* responses under unilateral FEF stimulation [92].

3.2. Behavioural differences between vertical and horizontal saccades, and upward and downward saccades, and their putative cortical correlates.

Vertical saccades gain in normal individuals seems to be lower than that of horizontal saccades. And within vertical saccades, upward saccades tend to be *hypometric* while downward saccades tend to be *hypermetric* [83, 84, 86, 93]. Both prosaccades and antisaccades seem to demonstrate such behaviour [86]. Since most daily activities imply making horizontal saccades (e.g., reading), the greater accuracy of horizontal saccades could reflect an adaptative behaviour. Similarly, the up-down asymmetry of saccades may correspond to adaptative changes in response to environmental demands, as most saccades in normal, daily vision are made to the inferior visual hemispace. Alternatively, such asymmetries (horizontal-vertical; up-down) could be inate and reflect a physiological asymmetry within cortical, subcortical, and/or cerebellar pathways regulating the amplitude of horizontal versus vertical saccades and downward versus upward saccades.

Saccade latency also seems to show both a horizontal-vertical and up-down asymmetry. The majority of oculomotor studies have shown that upward saccades are usually initiated faster than downward saccades and vertical saccades are more delayed than horizontal saccades [85, 86, 94–96]. This applies both to prosaccades and antisaccades, although up-down asymmetry is less marked in the latter type [85, 86, 97]. Of note, one large contemporary study demonstrated similar latencies for upward and downward saccades [93]. Up-down asymmetry may be due to a physiological asymmetry within the attentional and motor preparation cortical networks [86]. In a recent study using magnetoencephalography, it was demonstrated that clues appearing in the superior visual field seem to access earlier the left frontal cortex (motor preparation area) while clues appearing in the inferior visual field access earlier the right parietal cortex (attentional network). Immediately before saccade onset, this tendency reverts and the left frontal cortex seems to be more active during the preparation of downward saccades [94]. Thus, upper stimuli promote early activation of motor preparation areas (left frontal cortex), which could result in faster latencies for upward saccades. Lower stimuli on the other hand require more visual processing and thus, downward saccades take longer to be initiated [98]. Transcranial magnetic stimulation over the right parietal cortex prolongs the latency of vertical saccades, particularly for more voluntary saccades and for downward saccades. This finding further highlights the role of the right parietal cortex in the generation of saccadic up-down asymmetries [95]. One additional factor that may promote the above-mentioned asymmetry is that visual information in the cortex seems to be processed distinctively for upper and lower visual stimuli (e.g., extrastriate visual cortex receives asymmetric projections from primary visual cortex, in what regards to upper and lower object representations) [99].

Horizontal-vertical asymmetry (horizontal saccades demonstrate shorter latency than vertical saccades) on its turn could reflect the importance of the horizontal meridian in our quotidian [86, 100].

Oblique saccades have been used in several behavioural paradigms in order to study possible interactions between the vertical and horizontal saccadic system. Oblique saccades show a systematic tendency to curve towards the horizontal meridian. The horizontal component dominance may reflect a faster onset of the horizontal saccadic network (cortex and/or brainstem), and/or the unique architecture of the extraocular muscles (only one pair of muscles are needed to perform pure horizontal saccades, while more muscles are needed to perform vertical saccades) [101].

A visual distractor placed nearby a target object has been shown to influence a saccade's trajectory. Interestingly, vertical saccades seem to be more prone to such influence than horizontal saccades. This may reflect the different representation of horizontal and vertical saccades in the SC (horizontal saccades, contralateral SC; vertical saccades, bilateral SC) and how inhibitory processes needed to avoid the influence of the visual distractor are implemented within the SC (a bilateral representation of the motor command in the SC [such as the case for vertical saccades] allows for a more efficient and coarse inhibition of the visual distractor's representation) [102]. Another factor that may play a role on the distinct influence of a visual distractor on vertical and horizontal saccades is the underrepresentation of the vertical meridian in the SC [103, 104].

3.3. Cortical lesions promoting impairment of the vertical saccades.

Unilateral cortical lesions causing impairment of the vertical saccades are scarcely reported. Vertical saccades in these cases seem to be only mildly affected when compared to horizontal saccades. The latter are usually hypometric and/or delayed when executed to the opposite side, especially for right unilateral lesions [105]. Since vertical saccades require bilateral cortical activation to be executed, it is reasonable to think that in case of unilateral damage, the unaffected hemisphere might still compensate a potential vertical saccadic deficit. On the other hand, the few cases reporting dysfunction of the vertical saccades following unilateral cortical lesions raise one very important question: Could the cortical control of the vertical saccades be functionally asymmetric? In other words, could some oculomotor-related areas in the right (or left) hemisphere be more important for the generation of vertical saccades than their contralateral homologue area (e.g., right FEF over left FEF)? This could explain why right FEF lesions are more prone to cause vertical saccadic deficits. Within the same line of thought, could the hypothetical greater number of saccade neurons coding the upward direction in cortex explain the predominant upward saccadic deficit following unilateral cortical lesions? Indeed, Pflugshaupt et al reported a patient with a strictly unilateral right FEF lesion who demonstrated a significant reduction of exploratory vertical saccades and additional hypometria of upward saccades, which were also less

frequent than downward saccades [77]. The authors further added one other potential form of functional asymmetry concerning the cortical control of vertical saccades: a possible compensation by the unaffected ipsilateral PEF, which appears to be enhanced for downward as opposed to upward saccades, could explain the predominant affection of upward saccades in this case [94]. Averbuch-Heller et al. described 3 patients with right hemispheric acute infarction (middle cerebral artery territory, posterior limb of the right internal capsule and frontoparietal area) who showed upgaze palsy. Importantly, none of these patients had brainstem involvement. This again, provided anecdotal evidence that the cortical control of the vertical gaze may be partly lateralized. Moreover, the pathway carrying vertical gaze signals may descend in the posterior limb of the internal capsule [106]. Of note, the up-down asymmetry of saccade latency seen on normal individuals seems to be spared in patients with unilateral cortical lesions [105]. Bilateral cortical lesions involving FEF can also disturb vertical saccades, but here with the additional impairment of horizontal saccades [107].

4. Prosaccades and antisaccades.

4.1. Prosaccades.

A prosaccade consists of a gaze shift *in the direction* of an object of interest (i.e., visual, auditory stimulus, etc). Once the visual information related to a relevant stimulus reaches the striate and extrastriate visual cortex, it travels to: (1) the PEF where sensorymotor transformations occur and triggering of more reflexive saccades is most probable, taking into account its direct connections to SC [32, 108]; (2) the FEF, more so to the *lateral* aspect of the *contralateral* FEF, where initiation of more automatic saccades is promoted, exerting a direct influence on saccade reaction time [109, 110]; (3) the SEF, albeit its activity is consistently greater for more complex saccades [111]; (4) the SC, which ultimately receives projections from all the above-mentioned areas, and also influences saccadic reaction time [112]; (5) the oculomotor cerebellum (vermis and fastigial nucleus) [5]. Of note, most of these areas not only mediate saccadic eye movements but are also involved in attentional processes, since saccades made towards a specific object are preceded by a shift in attention to that object [26]. This attentional network seems to be disengaged if a time gap (e.g., 200 milisseconds) is introduced between the extinction of the central fixation point and the onset of the peripheral target in a prosaccade trial [113]. This will consistently reduce prosaccades latency – the *gap effect* [114]. One other theory accounting for the gap effect argues that the introduction of a time gap deactivates fixation neurons in the SC thus resulting in disinhibition of saccade neurons in the same structure and facilitation of saccades [114].

4.2. Antisaccades.

An antisaccade consists of a initial suppression of an (automatic) prosaccade towards the target followed by a vector inversion of the location of the object, so that the subject shifts his gaze *away from* the target to a mirror location [78]. In a antisaccade trial, if an initial unwanted prosaccade is made towards the peripheral target, this is considered a directional error, reflecting either a failure to inhibit the incorrect response (prosaccade) and/or to sufficiently activate the correct response (antisaccade) [26, 115]. Working memory is essential to assure a correct antisaccade performance [116]. Still, normal individuals are capable of correcting the majority of the directional errors by reverting the direction of a initially misdirected saccade, although discrepant results have been published [93, 117]. Additional processing concerning the application of the inhibitory processes and vector inversion may explain the greater latency of antisaccades when compared to prosaccades [118, 119]. The network underlying the generation of prosaccades also mediates antisaccades, albeit with some notable differences. First, PEF in antisaccades seems to play a role in the inhibition of an unwanted prosaccade and at the same time it may provide the basis for the vector inversion required to perform a gaze shift away from the target [120, 121]. *Lateral* and *medial* FEF hyperactivity immediately before antisaccade execution may reflect an enhanced level of inhibition to this region (e.g., by dlPFC) [5, 122]. Animal electrophysiological data corroborates this assumption [123]. SEF activity during antisaccades may indicate its preferential bias for overweighting the signal to generate antisaccades versus the signal to generate a prosaccade [124]. Thus, in general, PEF, SEF and FEF show greater activation during antisaccades than prosacades in functional neuroimaging studies (see below) [5]. These differences seem to arise predominately from the preparatory period [111, 125]. One other important structure is consistently recruited during the performance of antisaccades: the dlPFC [5]. The latter structure may exert a top-down inhibitory influence on FEF in order to prevent an unwanted prosaccade [111]. The role of CEF on antisaccades requires further investigation, but this structure probably monitors the likelihood of a directional error before, during and after an antisaccade [111]. As previoulsly detailed in this chapter, basal ganglia play a prominent role during the performance of more voluntary saccades, particularly antisaccades [120]. Basal ganglia probably exert their influence in paradigms in which reward after an antisaccade trial is manipulated. As an example, Blaukopf and DiGirolamo (2006) found that both highly rewarded and punished antisaccade trials showed greater latency than moderately rewarded or punished trials [126]. One rarely mentioned network also involved in the generation of more complex saccades such as antisaccades is the default mode network (DMN). Areas corresponding to this network have been shown to exhibit greater BOLD activity during rest than during a cognitive task [127]. Not surprisingly, Herweg et al. (2014) found that DMN areas (i.e., "parts of the medial prefrontal cortex, precuneus, cingulate cortex, medial temporal lobe, middle temporal gyrus, operculum, and insula") were more deactivated during antisaccades than prosaccades, implying that antisaccades are a more demanding cognitive task [7]. Others had previously found this pattern [128, 129]. Interestingly, during prosaccades, DMN (medial prefrontal cortex) deactivation has also been documented, but here, just for centripetal saccades (and not centrifugal saccades), favouring the requirement for greater attentional demands when saccadic eye

movements are executed away from the center [130]. A recommended standardized protocol of the antisaccade task has been recently published [131].

5. Functional magnetic resonance imaging of saccades.

5.1. The blood-oxygenation-level-dependent (BOLD) signal.

Functional magnetic resonance imaging (fMRI) is a relatively recent technique widely used to probe brain function, by measuring haemodynamic changes after enhanced neural activity [132, 133]. Due to the different magnetic properties of oxygenated and deoxygenated haemoglobin, changes in local perfusion influence the relative percentage of these two forms of haemoglobin, which will be readily detected by fMRI in the form of a BOLD (blood-oxygenation-level-dependent) signal/contrast (areas with high concentration of oxygenated hemoglobin give a greater BOLD signal than areas with low concentration – positive BOLD signal) [134, 135]. The increase in blood flow on its turn is related and proportional to neuronal activity (i.e., local field potential), particularly with the input and intracortical processing of a given area (*neurovascular coupling*) [136–138]. These events produce a complex BOLD signal function in time, called the hemodynamic response function (HRF), which represents a limitation in the temporal resolution of fMRI [134, 135]. However, BOLD signal interpretation is anything but straightforward and important shortcomings have been recently highlighted [132]. Thus, positive BOLD signal does not always means an increase of activity of task- or stimulus-specific neurons. Actually, any change in excitation–inhibition balance leading to net excitation, inhibition, or simple sensitivity adjustment may influence local perfusion and promote an increase of the BOLD signal. This is particularly true when neural inhibition is obtained through an increased synaptic inhibition or shunting of the cortical output through the axo-axonic connections of the chandelier cells [132]. Negative BOLD signal during specific tasks on the other hand is usually interpreted as decrease in neuronal activity under its basal level, although is still a matter of debate if negative BOLD response represents mainly a neuronal and/or a vascular effect [139].

5.2. Task design.

In an fRMI experiment, modulation of brain response (BOLD signal) resulting from various stimuli or task challenges is observed [135]. Current fMRI experimental approaches can be categorized into two main categories: blocked and event-related designs [135]. In a blocked design, multiple trials of the condition A are clustered within a block (~20 seconds is the typical duration) and alternate with blocks from the condition B (a resting state or a comparison task) over the course of a scan (run). In contrast, in an event-related design, trials from both conditions A and B may be randomized [134]. Thus, event-related designs allow to detect changes in BOLD signal

related to individual trials as opposed to blocks of trials, thus reducing the bias arising from blocked designs, regarding trial anticipation and habituation [140, 141]. Nevertheless, blocked designs have higher statistical power [142]. Thus, the choice of experimental design (e.g. blocked or event-related) has to take into account the advantages and disadvantages of each approach. Blocked design experiments are usually simple, and their analysis is robust and does not depend on an accurate HRF model. Event-related design experiments are more sophisticated, allow for separation of the different types of trials, and carry higher temporal resolution. Of note, the choice of a baseline condition is also an important consideration, since the use of different baseline conditions (e.g., picture naming, passive viewing, rest, etc) may give raise to different patterns of BOLD activation across studies [143]. Importantly, the existence of several areas overlapping with the DMN, which often shown BOLD deactivation during the cognitive task when compared to baseline, further complicate BOLD signal interpretation [144]. Thus, by adding a baseline condition, it is then possible to discern if differential activity between condition A and B is due to a relative increase in activation in one condition or a relative decrease in the other [129].

5.3. fMRI of the saccadic oculomotor network.

fMRI studies have consistently demonstrated FEF activation at the intersection between the precentral and superior frontal sulci during the execution of saccade tasks [5, 134, 145]. FEF can be further divided into two parts: superior and inferior FEF [5, 134, 145]. Inferior (lateral) FEF might be more concerned with the generation of antisaccades [146], while superior (medial) FEF seems to mediate both prosaccades and antisaccades [5]. Overall, FEF is more active in antisaccade tasks than in prosaccade tasks across studies, although anecdotal exceptions have been published [5, 147]. FEF BOLD activity is highly correlated with saccade frequency, but not with saccade amplitude [148]. The latter finding supports animal electrophysiological data demonstrating a strictly topological coding of saccade amplitudes in FEF [10]. FEF BOLD response correlates positively with saccade latency, speaking in favour of its control over saccade reaction time, namely by conveying inhibitory signals to the eye-movement system (i.e., SC) during the performance of more complex saccades such as antisaccades [110, 112, 149]. Animal electrophysiological data has provided strong evidence for a contralateral bias in FEF neural activity concerning the coding of saccade direction along the horizontal plane [10, 82]. Surprisingly, the majority of human fMRI studies on saccadic eye movements do not demonstrate such bias between rightward and leftward saccades [150, 151]. The few reports showing contralaterality were not able to detail the relative participation of attention, visual encoding, working memory and/or motor execution in the generation of the FEF BOLD signal [152–155]. Importantly, the difference observed in the degree of contralateral organization between monkeys and humans studies might be related to an actual difference between species and not from the theoretical discrepancy between imaging and electrophysiology techniques (i.e., the fact that fMRI resolution may be insufficient for differentiating between contralateral and return

saccade FEF BOLD activity, or between increased versus decreased neuronal activity in FEF) [136, 150, 154]. One recent fMRI study has elegantly reconciled Bender's and Bruce et al.'s views (1980; 1985) on the cortical topographic organization of saccade directions [10, 82, 153]. In ten normal subjects, Kastner et al (2007) showed that FEF activity during the execution of memory-guided saccades grossly followed a topographic pattern resembling Bender's findings (1980) (there was a lateral-medial progression from the upper vertical meridian to the lower vertical meridian, albeit a reverse pattern was also noted in a minority of individuals). Additionally, specific saccade directions were often represented in multiple locations across FEF, supporting a columnar organization of saccade direction in FEF, as demonstrated by Bruce et al. (1985) [153]. Leoné and colleagues (2014) further expanded these findings. As Kastner et al. (2007), the authors documented the existence of a contralateral bias and repeated phase shifts (switching between the upper and lower vertical meridians) in the representation of the saccade direction along the FEF (and PEF). Importantly, saccade amplitude coding in these areas also seems to follow a gradient (medial to lateral), but independent and not influenced by the direction coding. In summary, saccade location in FEF (and PEF) seems to be decomposed in separate direction and amplitude dimensions [156]. Although FEF contralaterality bias has been rarely evidenced in fMRI studies investigating human saccadic function, FEF does seem to show a motor predominance, irrespective of the direction of horizontal saccades [157]. Thus, during visually-guided saccades, several areas in the right hemisphere, including the lateral FEF, showed greater BOLD activity than the homologue areas in the opposite hemisphere [157]. This is probably related to the additional role that FEF has on attention, since spatial attention modulation is known to be lateralized to the right frontoparietal network [157, 158].

Human SEF activation during saccades is localized bilaterally in the dorsomedial frontal cortex, adjacent to the posterior limit of the pre-supplementary motor area [134]. SEF activation is consistently greater for antisaccades than prosaccades, particularly just before their execution, which speaks in favour of its role in the presetting of complex saccades [5, 110, 111]. The correlation of SEF activity with saccade latency has given discrepant results [110, 112, 149]. In animal studies, similarly to FEF, it appears that saccade direction coding progressively shifts across the SEF, suggesting a topographical columnar organization [13]. However, this has not been corroborated by fMRI studies so far [153]. As in FEF, saccade frequency, but not amplitude, seems to modulate BOLD activity in SEF [148]. Taken together, it is still highly debated if there any systematic organization of saccade direction or amplitude in SEF [159].

According to fMRI studies, PEF seems to be located along the intraparietal sulcus, more so in its posterior part [134, 160]. The presence of BOLD activity early in the preparation for a saccade has been rarely demonstrated in PEF, contrasting to what has been reported in FEF and SEF [5, 125, 150, 161]. This supports the traditional view that PEF mainly mediates the process of vector inversion in antissacades, not being critical for saccade execution [162]. Thus, PEF seems to code the target location for an

upcoming saccade (prosaccade versus antisaccade), rather than the location of the visual stimulus *per se* [163]. In line with what was found in FEF, PEF also shows predominant contralateral activity during the execution of a saccade [164, 165]. Importantly, this activity can be "shifted" to the opposite PEF (vector inversion) when an antisaccade is required [163]. Domagalik et al. (2012) extended the findings that link the parietal cortex with vector inversion. The authors suggested that vector inversion is driven by a pathway which includes not only the PEF, but also the precuneus, posterior cingulate gyrus, bilateral retrosplenial cortices and the parahyppocampal cortices [166]. Saccade frequency is also correlated with PEF BOLD activity [148]. Similarly to FEF and SEF, PEF BOLD activity is consistently higher for antisaccades than prosaccades [5, 146]. Since PEF's role has traditionally been linked to the execution of more reflexive saccades (e.g., prosaccades), the fMRI evidence for greater PEF activity during antisaccades might reflect its additional role in covert attention (i.e., greater attentional demands during antisaccades) [12, 146]. According to human fMRI studies, PEF seems to code target direction/location (regardless of whether a saccade is actually made) in retinocentric coordinates while amplitude coding probably follows a lateral-medial gradient (i.e., central visual field is represented laterally and peripheral visual field is represented medially) (see above) [164, 167]. In one study, PEF BOLD activity was positively correlated with saccade latency, suggesting that also PEF may convey inhibitory signals to the eye-movement system (e.g., SC), especially when generating goal-directed saccades [149].

Two important areas are consistently activated during antisaccades but not prosaccades: the CEF and the dlPFC [5]. CEF has been involved in monitoring voluntary saccadic eye movements, but its exact location remains controversial [24, 168]. It is possibly located immediately ventral to SEF, but even the distinction between CEF and SEF in the medial surface of the brain can be difficult [168]. The rostral and dorsal parts of the anterior cingulate cortex are thought to mediate different processes of the antisaccade task. Thus, an accurate performance (less directional errors) requires deactivation of the rostral part early in the trial, while error responses activate both parts later in the trial (response evaluation) [129]. The rostral part of the anterior cingulate cortex in probably integrated in the default mode network and its deactivation optimizes performance by allocating resources to task-necessary regions [127, 129]. These data are in agreement with the critical role of the anterior cingulate cortex in conflict detection [169]. This is probably why this region shows greater activation during antisaccades (i.e., high conflict scenario) than prosaccades (i.e., low conflict scenario) [111].

In the majority of fMRI studies, dlPFC activity in the posterior part of the middle frontal gyrus has only been found during antisaccades, although its activity during prosaccades has been anecdotally reported [5, 170]. The dlPFC (as the CEF) is not an oculomotor area *per se*. Instead, the dlPFC is thought to exert a top-down modulation over the oculomotor-related areas (i.e., FEF, SC). possibly providing saccade-suppression signals to support the task-appropriate behavior [23, 111, 150, 171]. Animal data demonstrates that dlPFC, similarly to FEF and PEF, has predominantly

contralateral response fields [154, 172, 173]. As previously mentioned, this contralaterality is far less obvious in human fMRI studies [154]. As in FEF, dlPFC possibly has a columnar organization coding for a particular saccade direction [172, 173]. The dlPFC response during saccades seems to occur during the preparatory period, and BOLD activity is higher for correctly performed antissacades. Reports on hemispheric predominance (right versus left predominant activation) have been contradictory [111, 120, 125, 150].

The basal ganglia (caudate and putamen) and thalamus BOLD activations are mainly seen during antisaccades > baseline contrast [5]. Intriguingly, this difference is not usually seen in the antisaccades > prosaccades contrast, although exceptions have been reported [151, 174]. Given these findings, Cameron et al. (2009) has suggested that the basal ganglia (caudate nucleus) BOLD activation cannot be solely attributed to a general suppression mechanism preventing the execution of automatic unwanted prosaccades, during antisaccades [174]. Instead, the authors proposed that caudate nucleus activation is probably related to *switching to a most effortful* response within a given trial (e.g., the subject is suddenly told to perform an antisaccade [non-dominant response], when a prosaccade was initially required [dominant response]) [174]. Additionally, basal ganglia role in the inhibiton of an unwanted prosaccade during the antisaccade task may not be strictly lateralized (e.g., SNpr exclusively inhibiting the ipsilateral SC so that a contralateral prosaccade is prevented). Accordingly to De Weijer et al. (2010), during an antisaccade, the FEF in the hemisphere contralateral to the intended saccade direction projects directly and inferiorly to the SC in order to execute it (lateralized activation). In parallel, the same FEF may activate the ipsilateral SNpr through the indirect pathway, resulting in tonic inhibition not only of the ipsilateral SC, but also of the contralateral SC (nonlateralized inhibition) in order to prevent unwanted prosaccades [155, 175]. Presumingly, the sum of these forces (i.e., nonlateralized inhibiton *and* lateralized activation) will ultimately generate an antisaccade [155]. Of note, the role of caudate versus putamen in saccades has been contradictory, since caudate neuronal activation is usually seen in monkeys while putamen BOLD activation is usually seen in humans [151]. The coding of saccade direction in basal ganglia is not well understood and fMRI studies have not shown a clear contralateral bias in caudate and/or putamen. Still, an interesting model was advanced by Watanabe and Munoz (2009), derived from caudate neurophysiological data on monkeys. In the direct pathway (see above), two types of saccade-related neurons seem to exist: “automatic” neurons responding to contralateral stimuli and “voluntary” neurons showing higher activity for contralateral saccades (thus, not influenced by stimulus direction). Both types of neurons seem to work congruently when a contralateral prosaccade is required (e.g., left caudate “voluntary” and “automatic” neurons activation drives the execution of a rightward prosaccade). In a different scenario, when a rightward antisaccade is needed, then the right caudate “automatic” neurons (responding to the visual stimulus located in the left and thus facilitating a leftward saccade) probably enter in conflict with the left caudate voluntary neurons (facilitating a rightward saccade). To resolve this conflict, a third group of neurons, probably within the indirect pathway, seem to

suppress the activity of the ipsilateral “automatic” neurons, and ultimate drive the correct response [176]. The authors further speculate that the “automatic”, “voluntary” and “suppressive” neurons activity might be driven by the PEF/FEF, SEF and dlPFC, respectively [176].

The thalamic activity observed particularly during antisaccades is considered to reflect both its involvement in visual attention and the continuous update about the saccade vector that thalamus provides to the cortical oculomotor network (i.e., FEF; PEF) [177–180]. Additionally, active suppression of visual input during saccades in order to prevent blurring of the visual scene is probably mediated by the thalamus [181]. Interestingly, right thalamic BOLD activity also seems to correlate with saccade latency [177].

Superior colliculus BOLD activity is stronger for contralateral saccades, particularly centrifugal saccades, which is consistent with previous animal data [130, 182, 183]. Furthermore, the magnitude of SC BOLD responses correlates negatively with saccade latency, which supports its role in the control of saccadic latency, together with FEF and PEF [112].

Cerebellar posterior vermis and hemispheres are usually activated during fMRI studies employing saccade paradigms [184]. Vermis BOLD activation may be related to ocular motor performance exclusively while the hemispheric activations are possibly ascribed to attention and visuospatial working memory [158, 184, 185]. Middle cerebellar penduncle and dentate nucleus BOLD activation on the other hand, may reflect either attentional or ocular motor processing. In line with the previous findings, posterior vermis activation may be related to execution of more reflexive saccades (e.g., prosaccades), while the cerebellar hemispheres and tonsil, may be involved in the generation of more complex saccades [5, 185]. However, this latter finding has not been consistent among studies [186]. Cerebellum also plays a relevant role in saccadic adaptation and motor learning. Thus, this structure is thought to process saccadic errors (i.e., inaccurate saccades, in which the endpoint of gaze either does not reach the target – hypometric saccades, or goes beyond it – hypermetric saccades) in order to improve subsequent motor performance, by modifying saccadic amplitude. Liem et al. (2013) has nicely shown that while small amplitude saccadic errors seem to be processed in the oculomotor vermis, large saccadic errors promoted greater BOLD activity in the cerebellar hemispheres [187]. These results have called into question the apparently strict cognitive role of cerebellar hemispheres. Cerebellar activity seems to show a right predominance (lateralization), independently of the stimulus direction [184].

The oculomotor nuclei in the brainstem receive their input from premotor saccade generator centers (riMLF in the midbrain for generating vertical saccades and PPRF in the pons for the horizontal counterpart) which on their turn are activated by the SC [1]. Basically, the SC neurons send a position code signal to the premotor gaze centers, which transform this signal into a temporal code [9]. Brainstem fMRI has been rarely performed to investigate the oculomotor network within this area due to technical

constraints including the small size of these structures and motion artefacts caused by large vessel pulsations [183]. Still, Linzenbold et al. (2011) were able to show part of this network in a fMRI experiment in which participants were told to execute horizontal saccades. As expected, the superior colliculi, the PPRF, the abducens and the oculomotor nuclei were detected [183].

From the above mentioned fMRI studies, it becomes clear that in sharp contrast with the extensive investigation performed over the last decades on the functional imaging of the cortical control of horizontal saccades, their vertical counterpart has been completely neglected. Ironically, the almost inexistent evidence available on functional cortical BOLD differences between the execution of vertical and horizontal saccades comes from one study exclusively investigating cerebellar BOLD activity during saccades [188]. Unfortunately in this study, targets had different amplitudes between the horizontal and vertical plane (i.e., 10° and 7.5°, respectively), which may preclude a reliable fMRI BOLD comparison between saccades, since saccadic amplitude is known to influence BOLD signal topography in FEF and PEF, and BOLD signal extent in the primary visual cortex [156, 157, 188]. Moreover, statistical analysis was not detailed regarding this comparison, although "slight differences in signal intensity" between planes were briefly mentioned. Whether these differences were located in the cerebellum and/or cerebrum, this was not specified [188]. Dietereich et al. (2000) found no cerebellar BOLD differences between vertical and horizontal saccades in 3 normal individuals, but the cortex was not assessed [184]. Neggers et al. (2012) used both vertical and horizontal saccades in a fMRI blocked design, but the authors did not compared BOLD activity between saccade planes, since this was not the aim of the study [151]. Several fMRI studies using retinotopic techniques have mapped the cortical topography of saccades executed to different target positions, including purely vertical positions. Again in these studies, comparison between saccade planes was not the main purpose of the investigation, but rather mapping the coding of saccade direction and amplitude in oculomotor areas [153, 156, 164]. Bodis-Wollner et al. (1999) used vertical saccades in a fMRI paradigm, but a formal comparison with their horizontal counterpart using data from a previous study by the same authors was not performed [189, 190].

6. Saccadic disturbance in Parkinson's disease.

Parkinson's disease (PD) is a neurodegenerative disorder comprising asymmetric hypokinesia, tremor, rigidity, postural imbalance, and additional non-motor symptoms [191]. Neurodegeneration seems to follow a specific ascending pattern in PD, beginning in the lower brainstem towards midbrain to include the basal ganglia (particularly SNpc) and finally reaching the cortex [192]. Albeit rostral progression has been called into question recently, marked neurpathological involvement of the basal ganglia is a prominent and classical feature [193, 194]. Since the output of basal ganglia greatly influences saccadic behaviour, it is not surprising that an extensive body of research on

saccades has been focused on PD patients [31]. PD saccadic abnormalities mainly include the following: (1) *hypometria* of reflexive (e.g., prosaccades, particularly in the vertical plane [80, 195–197]) and voluntary saccades (e.g., antisaccades, along the horizontal [and vertical?] plane [198]); (2) *prolonged latency* of saccades, more evident in voluntary (e.g., antisaccades along the horizontal [and vertical?] plane [199]) than reflexive saccades (horizontal and vertical prosaccades [197, 200, 201]); increased number of antisaccade *directional errors* (horizontal [199, 202] and vertical? [201]). Of note, in most studies, horizontal (and vertical?) prosaccades latency has been documented as normal or shorter in PD patients, relative to controls [199, 202]. Saccade velocity is usually normal in PD patients relative to healthy controls, since the premotor saccade generator centers are spared until late stages of the disease [193, 203, 204]. Hypometria is due to an excessive tonic supression of SC by SNpr probably heightened by the existence of a weak frontostriatal (i.e., FEF) pre-oculomotor drive and additional basal ganglia involvement [200, 205–207]. Increased latency presupposes the above-mentioned mechanisms and possibly entails additional disruption of the parieto-collicular drive (i.e., PEF) later in the disease [200, 205]. Short latency prosaccades on the other hand, when present may represent either transient interruptions of SC inhibition by abnormal synchronous oscillatory activity within basal ganglia or malfunctioning of prefrontal areas (i.e., dlPFC) responsible for suppressing automatic saccades [200, 202, 208, 209]. Likewise, the increased number of directional errors in antisaccade task is thought to arise from dLPFC impairment and consequent loss of its inhibitory action over FEF and SC (see **Figure 4** and **Table 4**) [202]. Whether frontal and parietal dysfunction reflects additional cortical impairment or only deficient BG output to these areas is still a matter of debate [210, 211]. The reason why vertical and horizontal saccades seem to be distinctively affected in PD (e.g., greater hypometria along the vertical plane) is not well understood. Moreover, to date there is no formal comparison of vertical antisaccades parameters between PD patients and healthy controls [201].

Two functional magnetic resonance (fMRI) studies addressed cortical impairment of the saccadic network in PD patients to date, including only horizontal saccades in their paradigms. Rieger et al. (2008) showed BOLD "perisaccadic" frontal (i.e., FEF and SEF) hipoactivity and relative posterior (i.e., posterior cingulate gyrus; parahippocampal gyrus; inferior parietal lobule; precuneus; and middle temporal gyrus) hyperactivity in nine PD participants when compared to controls, while performing voluntary (self-paced) horizontal saccades in a block-design paradigm [212]. In an event-related fMRI study with 13 PD patients, Cameron et al. (2012) further showed that this relative frontal hypoactivation occurred in the preparatory stage rather than during the execution of horizontal saccades [213]. These studies point to a probable executive failure in presetting the oculomotor network in PD with subsequent impairment of saccades. No fMRI study so far has addressed separately the cortical activation of vertical and horizontal saccades in PD patients.

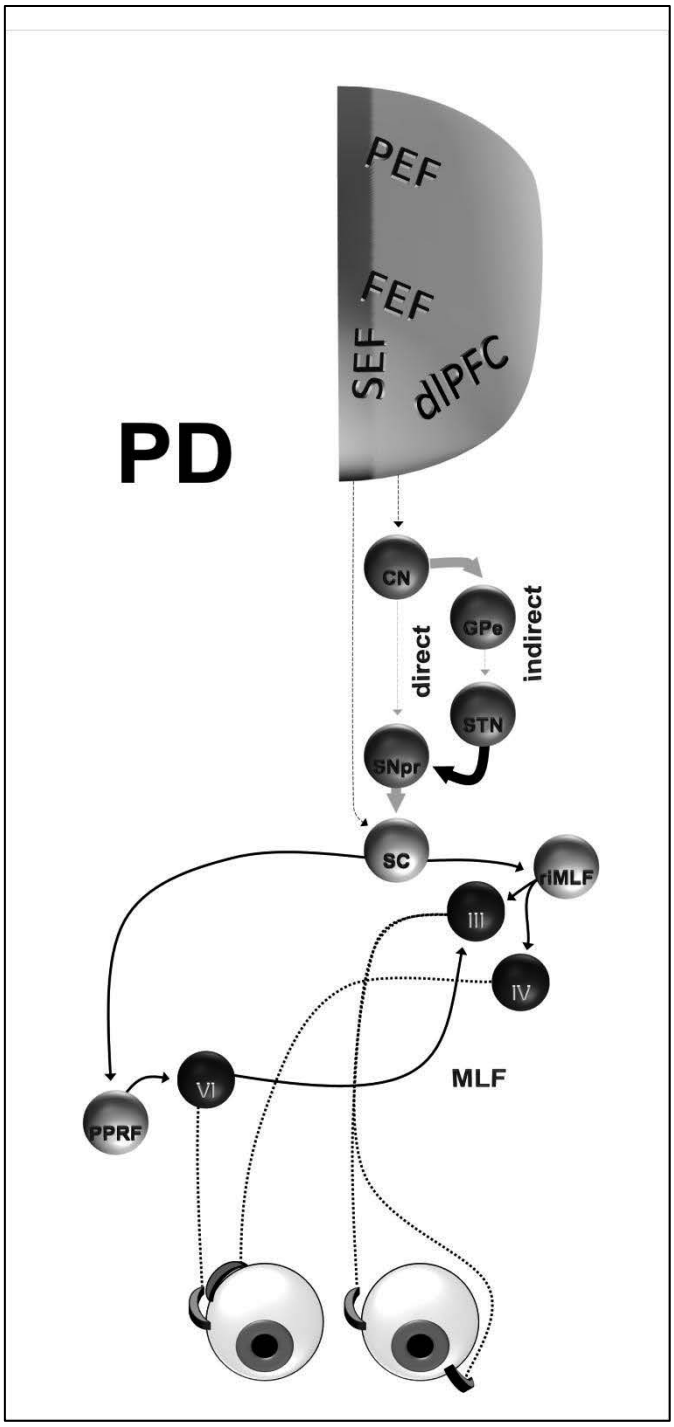

Figure 4. Schematic diagram depicting the pathophysiology of saccade abnormalities in Parkinson's disease (PD). Caudate nucleus in PD lacks its dopaminergic input from SNpc (not shown for clarity). This promotes a functional imbalance within basal ganglia, favouring the activity of the indirect pathway (thick lines). The latter bias will greatly enhance the tonic inhibition that SNpr exerts over the SC, leading to the suppression of saccades. This pathomechanism probably underlies the hypometria and prolonged latency of saccades found in PD. Descending inputs from the oculomotor cortex to the basal ganglia and SC (thin dashed lines) may further aggravate the saccadic deficit. If the enhanced suppression generated by a relatively hyperactive indirect pathway becomes intermittently "leaky", saccades with extremely short latency will occur (reflexive saccades). Finally, dlPFC intrinsic damage and/or decreased input from basal ganglia to dlPFC (not depicted) probably underlies the increased number of directional errors during antisaccades. The direct pathway (CN --> SNpr) facilitates saccades, while the indirect (CN --> GPe -->

STN --> SNpr) and hyperdirect (cortex --> STN --> SNpr) (not shown) pathways suppress saccades. Black lines represent excitatory connections. Gray lines represent inhibitory connections. The image represents from top to bottom: cortical oculomotor network (FEF, SEF, PEF, dlPFC); subcortical oculomotor network (basal ganglia: CN, GPe, STN, SNpr); brainstem oculomotor network (SC, riMLF PPRF, MLF, VI, IV and III nucleus). Similarly, only the ipsilateral projection from SC to riMLF is shown, and only one riMLF and one PPRF and their afferent/efferent conections are depicted. FEF, frontal eye field; SEF, supplementary eye field; PEF, parietal eye field; dlPFC, dorsolateral prefrontal cortex; CN, caudate nucleus; GPe, external component of globus pallidus; STN, subthalamic nucleus; SNpr, substantia nigra pars reticulata; SNpr, substantia nigra pars compacta; SC, superior colliculus; riMLF, rostral interstitial nucleus of the medial longitudinal fasciculus; PPRF, paramedian pontine reticular formation; VI, sixth nucleus; III, third nucleus; IV, fourth nucleus; MLF, medial longitudinal fasciculus. Adapted from references [1, 31, 204, 214].

7. Saccadic disturbance in progressive supranuclear palsy.

Progressive supranuclear palsy (PSP) is a severe neurodegenerative disorder characterized by symmetric hypokinesia, postural imbalance, impairment of saccades and variable frontal behavioural dysfunction [215, 216]. Marked involvement of the midbrain and pons in the brainstem including the premotor saccade generator centers (riMLF; PPRF) greatly accounts for the saccadic deficits. Thus, both reflexive and voluntary saccades (e.g., vertical and horizontal prosaccades [217, 218], [and antisaccades?]) in PSP are characteristically *slow and hypometric, especially along the vertical plane*, reflecting greater neurodegeneration and neuronal loss in the midbrain (containing riMLF), relative to pons (containing PPRF) [87]. While velocity disturbance is reasonably explained by the damage of the riMLF and PPRF, additional involvement of the interstitial nucleus of Cajal in the midbrain seems to better account for the amplitude deficits seen in PSP [219]. Previously hypothesized involvement of the omnipause neurons in the pons to explain saccadic velocity deficits is not currently supported [87]. The latency of more reflexive saccades in PSP may be *normal or increased* (e.g., horizontal [217, 220–222] and vertical prosaccades [223]), depending on the variable involvement of areas known to modulate saccade latency including dlPFC, SC, PEF, and SNpc [221, 222]. The latency of more voluntary saccades is usually *prolonged* (e.g., horizontal [and vertical?] antisaccades [221, 222]) possibly due to the reasons mentioned above. Characteristically, these patients show an increased number of *directional errors* during the horizontal (and vertical?) antisaccade task [221, 222], suggesting involvement of cortical areas exerting a top-down inhibitory control to prevent the execution of unwanted saccades (e.g., dlPFC) (see **Figure 5** and **Table 4**). To the authors' best knowledge, no study has used vertical antisaccades to evaluate PSP patients and data on velocity and amplitude of horizontal antisaccades is at best scarce. Taken together, while there are several lines of evidence showing extensive brainstem oculomotor network disease in PSP, the status of the cortical and subcortical saccadic network is largely unknown.

We did not find any fMRI study addressing the cortical/subcortical control of saccades in PSP. Recently, Amtage et al. (2014) in a FDG-PET study found a significant

correlation between 20° horizontal saccades velocity and neuronal activity of a non-oculomotor area (rostral vermis [lobules V, VI]) in patients with PSP. Additionally, the authors found an association between right CEF hypometabolism and downward vertical saccadic palsy (saccadic amplitude < 20°). While no justification was advanced for the first finding, concomitant reduced blinking (and not downward saccadic palsy) could potentially explain CEF hypometabolism in these patients [224].

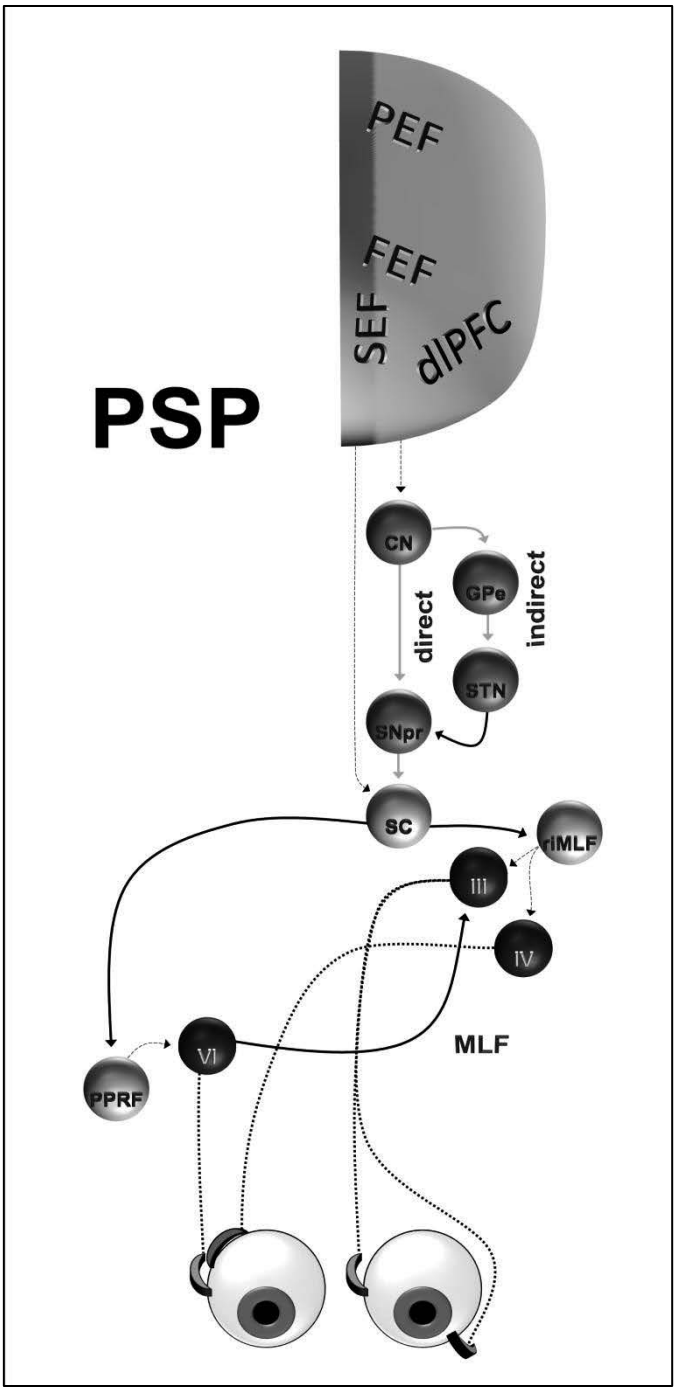

Figure 5. Schematic diagram depicting the pathophysiology of saccade abnormalities in progressive supranuclear palsy (PSP). riMLF and PPRF in PSP patients undergo severe neurodegenerative changes and neuronal loss, and their input to the ocular motor nuclei is dramatically decreased (thin dashed lines). This promotes marked slowing and shortening of saccades. Additional iNC involvement (not shown for clarity) possibly plays a role in the saccade amplitude deficits. Putative decrease of the cortical drive input to the basal ganglia and/or SC may play a role in saccadic latency deficits (thin dashed lines). While several lines of evidence have shown pre- and post-synaptic dysfunction of basal ganglia in PSP, its role in saccadic impairment has not been detailed, mostly because the velocity and amplitude deficits promoted by brainstem disease probably overshadow saccadic abnormalities caused by intrinsic impairment of basal ganglia. Thus, a relatively hyperactive indirect pathway partially accounting for saccadic deficits in PSP cannot be ruled out. The same applies to cortical impairment. Finally, dlPFC intrinsic damage and/or decreased input from basal ganglia to dlPFC (not depicted) probably underlie the

increased number of directional errors during antisaccades. The direct pathway (CN --> SNpr) facilitates saccades, while the indirect (CN --> GPe --> STN --> SNpr) and hyperdirect (cortex --> STN --> SNpr) (not shown) pathways suppress saccades. Black lines represent excitatory connections. Gray lines represent inhibitory connections. The image represents from top to bottom: cortical oculomotor network (FEF, SEF, PEF, dlPFC); subcortical oculomotor network (basal ganglia: CN, GPe, STN, SNpr); brainstem oculomotor network (SC, riMLF PPRF, MLF, VI, IV and III nucleus). Similarly, only the ipsilateral projection from SC to riMLF is shown, and only one riMLF and one PPRF and their afferent/efferent conections are depicted. FEF, frontal eye field; SEF, supplementary eye field; PEF, parietal eye field; dlPFC, dorsolateral prefrontal cortex; CN, caudate nucleus; GPe, external component of globus pallidus; STN, subthalamic nucleus; SNpr, substantia nigra pars reticulata; SC, superior colliculus; riMLF, rostral interstitial nucleus of the medial longitudinal fasciculus; iNC, interstitial nucleus of Cajal; PPRF, paramedian pontine reticular formation; VI, sixth nucleus; III, third nucleus; IV, fourth nucleus; MLF, medial longitudinal fasciculus. Adapted from references [76, 87, 217, 219]

Table 4. Summary of common saccadic abnormalities in PD and PSP

	Horizontal Prosaccades	Vertical Prosaccades	Horizontal Antisaccades	Vertical Antisaccades
PD				
Latency	normal or ↑	normal or ↑	↑ or normal	?
Velocity	normal	normal	normal	?
Gain	↓	↓	↓	?
Directional errors	normal	normal	↑ or normal	?
PSP				
Latency	normal or ↑	normal or ↑	↑↑ or normal	?
Velocity	↓↓	↓↓↓	?	?
Gain	↓↓	↓↓↓	?	?
Directional Errors	normal	normal	↑↑↑	?

↓ mild decrease; ↓↓ moderate decrease; ↓↓↓ marked decrease; ↑ mild increase; ↑↑ moderate increase; ↑↑↑ marked increase; ? scarce or no evidence

PD, Parkinson's disease; PSP, progressive supranuclear palsy

8. Thesis rationale

In the preceding sections, several points concerning saccadic eye movements have been outlined, from general considerations on reflexive and voluntary saccades, to the extensive network generating them, their investigation by functional imaging, and the saccadic dysfunction in two specific parkinsonian disorders. From the studies mentioned above, it is clear that the precise cortical mechanisms generating the execution of vertical saccades are not well understood and their investigation in neuroscience is only just beginning. Our work was driven by the numerous questions these studies left unanswered. We started our research by asking a simple question: is cortical/subcortical oculomotor network distinctively activated for vertical and horizontal saccades at a functional level? Indeed, previous saccadic behavioural data in health and disease supports the possibility of such asymmetry, and both a behavioural strategy favouring the execution of horizontal saccades and/or distinctive involvement of vertical and horizontal cortical saccadic network in aging and neurodegeneration may be playing a role in those findings [80, 86]. Animal electrophysiological studies and human fMRI studies investigating the representation of saccade position and amplitude in oculomotor cortex (e.g., FEF and PEF) have provided evidence for the existence of a topographical map for saccade direction, further highlighting the neuronal segregation in cortex for the coding of vertical and horizontal saccades [10, 82, 153, 156, 164]. Moreover, fMRI studies investigating the dynamic interaction between the two hemispheres during the execution of horizontal saccades have demonstrated the possibility of a contralateral bias (i.e., right hemisphere mostly drives leftward saccades; the opposite situation for the left hemisphere) and a predominance bias (right hemisphere activity is relatively higher, regardless of saccade direction) [153, 157, 163]. The existence of such biases during the execution of vertical saccades has never been addressed with fMRI, although studies using transcranial magnetic stimulation support that possibility [95]. To effectively tackle our main question, we designed an fMRI experiment in which we compared blocks of vertical saccades to blocks of horizontal saccades. Although in fMRI experiments, blocked designs have been progressively replaced by event-related designs, the former are still an extremely important technique due to the robustness of results, increased statistical power and large BOLD signal change related to baseline [135]. On the other hand, by choosing a blocked design, we were aware that overlapping neuronal networks in the cortex subserving the execution of vertical and horizontal saccades could go unnoticed due to spatial averaging, and potential BOLD differences might not be detected [132]. Surprisingly, since the advent of fMRI in 1991, no study so far has mainly focused on the comparison between BOLD activity during the execution of vertical versus horizontal saccades [225]. Moreover, the distinct pattern of BOLD activity between horizontal reflexive (e.g., prosaccades) and voluntary (e.g., antisaccades) saccades has been greatly detailed over the last two decades [5]. But also here the vertical plane has been largely neglected and consequently, vertical antisaccades are rarely used in behavioural paradigms although they may constitute a promising biomarker, and their BOLD activity is not known [93,

226]. Therefore, we also intermingled antisaccades blocks with prosaccades blocks in our fMRI experiment.

Previous fMRI work concerning saccades has focused on specific structures and components of the process individually, [e.g., FEF's BOLD activity during the fixation cue period). Since there are no previous comparisons between vertical and horizontal saccades employing fMRI, we chose to approach this subject as a whole using fMRI whole-brain analysis. Finally, in our research we decided to investigate healthy volunteers, Parkinson's disease and progressive supranuclear palsy patients. It has long been shown that several parkinsonian/basal ganglia disorders are ideally suited for studying the saccadic oculomotor network, since basal ganglia output has a strong influence on saccadic performance and neurodegeneration in certain disorders including PSP selectively affects other critical areas for the execution of saccades, such as the midbrain and pontine saccade generator centers [87, 204]. Importantly, we specifically selected two clinical entities which are known to affect predominantly vertical saccades. While in PSP, greater vertical impairment reflects predominant brainstem damage, in PD, intrinsic cortical/subcortical damage may substantially account for such asymmetry [87, 200]. Thus, our initial expectations were that fMRI could potentially demonstrate functional physiological asymmetries between vertical and horizontal saccades in normals. Additionally, these asymmetries should be exacerbated in PD patients due to putative greater involvement of cortical/subcortical oculomotor sub-areas mediating vertical saccades. Already in PSP, such asymmetries should be even more evident, if one takes into account the greater cortical impairment in these patients relative to PD, potentially participating in the saccadic deficit and ultimately failing to compensate for the brainstem-induced saccadic deficit.

In summary, the main goal of our thesis was to gain a better understanding of the cortical and subcortical mechanisms underlying the generation of vertical saccades. Additionally, we investigated these mechanisms both for reflexive and voluntary saccades, by adding a more voluntary task, also along the vertical plane (vertical antisaccades). Finally, we concentrated on two disease groups that share a predominant vertical saccadic impairment (vertical saccades are slower than horizontal saccades in PSP; vertical saccades are shorter and possibly more delayed than horizontal saccades in PD). Importantly, the above deficit seems to be mainly caused by brainstem disease in one group (PSP), and cortical/subcortical disease in the other (PD). This was accomplished through the following work:

Chapters 2 and **3** constitute two published reviews which have strongly inspired the subsequent experimental work detailed in **Chapters 4** and **5**. The work conducted in the latter 2 chapters is summarized and discussed in **Chapter 6**. In this last chapter we review the main experimental findings of the thesis, provide further thoughts on the field and highlight future directions.

Specifically, in **Chapter 2** we review supranuclear and internuclear eye movement disorders, highlighting relevant work from the last three years. Recent data on vertical

saccades and their cortical and subcortical control certainly drove us to pursue this topic in our research. Specifically, we found evidence showing that bilateral cortical lesions (sparing midbrain vertical gaze centers) could dramatically impair vertical saccades in one patient. Also, in PD patients, is now becoming clear that certain cortical areas (i.e., posterior cingulate gyrus; medial temporal lobe) seem to compensate for their vertical saccadic deficits (i.e., vertical hypometria), by increasing the connectivity between each other. Interestingly, basal ganglia's dual role on inhibiting reflexive behaviour (i.e., prosaccades) and facilitating more intended behaviour (i.e., antisaccades) has now been corroborated neurophysiologically *in vivo* by recording deep brain stimulation field potentials in PD patients during the execution of saccades.

In a similar way, some of the ideas for our research stemmed from the work reviewed in **Chapter 3**. The material in this chapter mainly addresses new findings on involuntary saccadic eye movements present in ocular fixation (saccadic intrusions). It is striking that the same brain network that generates reflexive and voluntary saccades, when defective, may also promote saccadic instability during fixation in several disorders, including PSP. Remarkably in these patients, the characteristic asymmetry between vertical and horizontal saccades propagates to ocular fixation. Thus, miniature fixational eye movements called microsaccades (<0.5° amplitude), also lack a normal vertical component in patients with PSP.

In **Chapter 4** we describe in full detail Experiment 1. In this experiment we compared the execution of vertical and horizontal prosaccades and antisaccades blocks between healthy participants and PD patients. We first measured behavioural performance outside MRI and this was followed by a block-design fMRI task using similar paradigms. Saccadic behavioural performance was not significantly different between groups, which may have reflected the highly selected PD population for this work, in an early stage of the disease, showing mild motor dysfunction and mild or no cognitive impairment. Still, vertical antisaccades tended to be more prolonged in PD, which highlights its potential use as biomarker in larger studies. During the fMRI task however, PD patients showed clear frontal (FEF) hypoactivity during vertical and horizontal saccades, possibly compensated by right parietal (PEF) hyperactivity, when compared to controls. Strikingly, controls were able to deactivate the DMN during antisaccades at a greater extent than PD patients. Importantly, these findings stress that putative functional compensatory changes occur in PD patients before clinically denoting significant saccadic changes in amplitude and/or latency. Our main contrast, the comparison of BOLD activity between vertical and horizontal saccadic performance, provided several new interesting findings concerning the cortical control of saccades. Thus, vertical prosaccades in controls promoted greater right frontal (FEF) and cerebellar activity than their horizontal counterpart. PD patients on the other hand showed less extrastriate cortex activity during vertical saccades. Vertical antisaccades, when compared to horizontal antisaccades, were associated with greater DMN deactivation in both groups and left parietal hypoactivity (PEF) only in PD patients. This data provides first time evidence showing that there are functional physiological

cortical asymmetries during the execution of vertical versus horizontal saccades in normal individuals. There might be several, non-exclusive, reasons for these novel findings. Vertical prosaccades may require higher attentional demands than horizontal prosaccades, as reflected by their greater latency. Alternatively, certain topographical "clusters" within FEF and cerebellum may be exclusively/preferentially concerned with the execution of vertical prosaccades, and importantly these seem to be lateralized to the right hemisphere. Vertical antisaccades also seem to cognitively more demanding than horizontal antisaccades. Importantly, BOLD asymmetries between vertical and horizontal saccades in PD patients seem to be either lost or changed, which supports previous evidence showing cortical frontal, parietal and visual dysfunction in these patients.

Proceeding directly from this work, in **Chapter 5**, we sought to address in a pilot study if the cortical asymmetries associated with the execution of vertical and horizontal saccades were equally lost or changed in another parkinsonian disorder also predominantly affecting vertical saccades, but here due to extensive brainstem disease: PSP. Experiment 2 was conducted using the same paradigms, outside and inside the MRI scanner. As expected, behavioural data evidenced marked slowing and restriction of saccades in PSP patients relative to controls, predominantly along the vertical plane, which mainly reflects the severe brainstem damage in patients. Interestingly during fMRI, patients showed decreased frontal (FEF) and basal ganglia activity during prosaccades (horizontal and vertical) and antisaccades (vertical), relative to controls. Moreover, PSP patients showed less DMN deactivation than controls for all types of saccades. This data indicates that the physiopathology of the saccadic disturbance in PSP possibly extends beyond the brainstem, affecting critical cortical and subcortical structures involved in the generation of reflexive and voluntary saccades. Cortical and subcortical impairment may indeed participate in the amplitude and latency deficits previously demonstrated in these patients. Controls showed no BOLD differences between vertical and horizontal prosaccades, which in comparison with Experiment 1, may have been related to the substantially smaller number of subjects included in Experiment 2. However patients did show greater DMN deactivation during vertical prosaccades, relative to horizontal prosaccades, which may indicate greater requirement of cognitive resources in patients, when reflexive saccades are executed along the vertical plane. Both groups evidenced greater DMN deactivation during vertical antisaccades when compared to their horizontal counterpart, but patients further showed frontal (FEF) and basal ganglia hypoactivity during vertical antisaccades. Again here, frontostriatal relative hypoactivity during vertical voluntary saccades in patients could be related to their predominantly vertical saccadic deficit, stressing the impact of cortical impairment in saccadic disturbance of PSP.

References.

1. Leigh RJ, Zee DS (2015) The neurology of eye movements, 5th ed. Oxford University Press, New York
2. Büttner U, Büttner-Ennever JA (2006) Present concepts of oculomotor organization. Prog Brain Res 151:1–42. doi: 10.1016/S0079-6123(05)51001-X
3. Martinez-Conde S, Macknik SL, Hubel DH (2004) The role of fixational eye movements in visual perception. Nat Rev Neurosci 5:229–240. doi: 10.1038/nrn1348
4. Lemos J, Eggenberger E (2014) Supranuclear eye movement disorders. Curr Opin Ophthalmol 25:471–9. doi: 10.1097/ICU.0000000000000104
5. Jamadar SD, Fielding J, Egan GF (2013) Quantitative meta-analysis of fMRI and PET studies reveals consistent activation in fronto-striatal-parietal regions and cerebellum during antisaccades and prosaccades. Front Psychol 4:749. doi: 10.3389/fpsyg.2013.00749
6. McDowell JE, Dyckman KA, Austin BP, Clementz BA (2008) Neurophysiology and neuroanatomy of reflexive and volitional saccades: evidence from studies of humans. Brain Cogn 68:255–70. doi: 10.1016/j.bandc.2008.08.016
7. Herweg NA, Weber B, Kasparbauer A, et al (2014) Functional magnetic resonance imaging of sensorimotor transformations in saccades and antisaccades. Neuroimage 102 Pt 2:848–60. doi: 10.1016/j.neuroimage.2014.08.033
8. Hikosaka O, Nakamura K, Nakahara H (2006) Basal ganglia orient eyes to reward. J Neurophysiol 95:567–84. doi: 10.1152/jn.00458.2005
9. Sparks DL (2002) The brainstem control of saccadic eye movements. Nat Rev Neurosci 3:952–964. doi: 10.1038/nrn986
10. Bruce CJ, Goldberg ME, Bushnell MC, Stanton GB (1985) Primate frontal eye fields. II. Physiological and anatomical correlates of electrically evoked eye movements. J Neurophysiol 54:714–34.
11. Leigh RJ, Kennard C (2004) Using saccades as a research tool in the clinical neurosciences. Brain 127:460–77. doi: 10.1093/brain/awh035
12. Gaymard B, Ploner CJ, Rivaud S, et al (1998) Cortical control of saccades. Exp brain Res 123:159–63.
13. Russo GS, Bruce CJ (2000) Supplementary eye field: representation of saccades and relationship between neural response fields and elicited eye movements. J Neurophysiol 84:2605–21.
14. Gaymard B, Rivaud S, Pierrot-Deseilligny C (1993) Role of the left and right supplementary motor areas in memory-guided saccade sequences. Ann Neurol 34:404–6. doi: 10.1002/ana.410340317
15. Hikosaka O, Isoda M (2008) Brain mechanisms for switching from automatic to controlled eye movements. Prog Brain Res 171:375–82. doi: 10.1016/S0079-6123(08)00655-9
16. Lynch JC, Graybiel AM, Lobeck LJ (1985) The differential projection of two cytoarchitectonic subregions of the inferior parietal lobule of macaque upon the deep layers of the superior colliculus. J Comp Neurol 235:241–54. doi: 10.1002/cne.902350207
17. Braun D, Weber H, Mergner T, Schulte-Mönting J (1992) Saccadic reaction times in patients with frontal and parietal lesions. Brain 115:
18. Pierrot-Deseilligny C, Rivaud S, Gaymard B, Agid Y (1991) Cortical control of reflexive visually-guided saccades. Brain 114:
19. Bressler SL, Tang W, Sylvester CM, et al (2008) Top-down control of human visual cortex by frontal and parietal cortex in anticipatory visual spatial attention. J Neurosci 28:10056–61. doi: 10.1523/JNEUROSCI.1776-08.2008
20. Cavada C, Goldman-Rakic PS (1989) Posterior parietal cortex in rhesus monkey: II. Evidence for segregated corticocortical networks linking sensory and limbic areas with the frontal lobe. J Comp Neurol 287:422–45. doi: 10.1002/cne.902870403
21. Pierrot-Deseilligny C, Rivaud S, Gaymard B, Agid Y (1991) Cortical control of memory-guided

saccades in man. Exp brain Res 83:607–17.
22. Pierrot-Deseilligny C, Müri RM, Ploner CJ, et al (2003) Decisional role of the dorsolateral prefrontal cortex in ocular motor behaviour. Brain 126:1460–73.
23. Gaymard B, François C, Ploner CJ, et al (2003) A direct prefrontotectal tract against distractibility in the human brain. Ann Neurol 53:542–5. doi: 10.1002/ana.10560
24. Gaymard B, Rivaud S, Cassarini JF, et al (1998) Effects of anterior cingulate cortex lesions on ocular saccades in humans. Exp brain Res 120:173–83.
25. Johnston K, Levin HM, Koval MJ, Everling S (2007) Top-Down Control-Signal Dynamics in Anterior Cingulate and Prefrontal Cortex Neurons following Task Switching. Neuron 53:453–462. doi: 10.1016/j.neuron.2006.12.023
26. Hutton SB (2008) Cognitive control of saccadic eye movements. Brain Cogn 68:327–340. doi: 10.1016/j.bandc.2008.08.021
27. Schlag-Rey M, Schlag J (1989) The central thalamus. Rev Oculomot Res 3:361–90.
28. Wurtz RH (2008) Neuronal mechanisms of visual stability. Vision Res 48:2070–2089. doi: 10.1016/j.visres.2008.03.021
29. Gaymard B, Rivaud S, Pierrot-Deseilligny C (1994) Impairment of extraretinal eye position signals after central thalamic lesions in humans. Exp brain Res 102:1–9.
30. Gaymard B, Rivaud-Péchoux S, Yelnik J, et al (2001) Involvement of the cerebellar thalamus in human saccade adaptation. Eur J Neurosci 14:554–60.
31. Hikosaka O, Takikawa Y, Kawagoe R (2000) Role of the basal ganglia in the control of purposive saccadic eye movements. Physiol Rev 80:953–78.
32. Paré M, Wurtz RH (2001) Progression in neuronal processing for saccadic eye movements from parietal cortex area lip to superior colliculus. J Neurophysiol 85:2545–62.
33. Shook BL, Schlag-Rey M, Schlag J (1991) Primate supplementary eye field. II. Comparative aspects of connections with the thalamus, corpus striatum, and related forebrain nuclei. J Comp Neurol 307:562–583. doi: 10.1002/cne.903070405
34. Segraves MA, Goldberg ME (1987) Functional properties of corticotectal neurons in the monkey's frontal eye field. J Neurophysiol 58:1387–419.
35. Stanton GB, Goldberg ME, Bruce CJ (1988) Frontal eye field efferents in the macaque monkey: I. Subcortical pathways and topography of striatal and thalamic terminal fields. J Comp Neurol 271:473–92. doi: 10.1002/cne.902710402
36. Selemon LD, Goldman-Rakic PS (1988) Common cortical and subcortical targets of the dorsolateral prefrontal and posterior parietal cortices in the rhesus monkey: evidence for a distributed neural network subserving spatially guided behavior. J Neurosci 8:4049–68.
37. Hikosaka O, Sakamoto M, Miyashita N (1993) Effects of caudate nucleus stimulation on substantia nigra cell activity in monkey. Exp brain Res 95:457–72.
38. Nakanishi H, Kita H, Kitai ST (1987) Intracellular study of rat substantia nigra pars reticulata neurons in an in vitro slice preparation: electrical membrane properties and response characteristics to subthalamic stimulation. Brain Res 437:45–55. doi: 10.1016/0006-8993(87)91525-3
39. Hammond C, Deniau JM, Rizk A, Feger J (1978) Electrophysiological demonstration of an excitatory subthalamonigral pathway in the rat. Brain Res 151:235–244. doi: 10.1016/0006-8993(78)90881-8
40. Monakow KH, Akert K, Künzle H (1978) Projections of the precentral motor cortex and other cortical areas of the frontal lobe to the subthalamic nucleus in the monkey. Exp Brain Res 33:395–403. doi: 10.1007/BF00235561
41. Huerta MF, Kaas JH (1990) Supplementary eye field as defined by intracortical microstimulation: connections in macaques. J Comp Neurol 293:299–330.
42. Huerta MF, Krubitzer LA, Kaas JH (1987) Frontal eye field as defined by intracortical microstimulation in squirrel monkeys, owl monkeys, and macaque monkeys. II. Cortical connections. J Comp Neurol 265:332–61. doi: 10.1002/cne.902650304
43. Robinson DA (1972) Eye movements evoked by collicular stimulation in the alert monkey. Vision Res 12:1795–1808. doi: 10.1016/0042-6989(72)90070-3

44. Sparks DL, Gandhi NJ (2003) Single cell signals: an oculomotor perspective. Prog Brain Res 142:35–53. doi: 10.1016/S0079-6123(03)42005-0
45. Gandhi NJ, Keller EL (1997) Spatial distribution and discharge characteristics of superior colliculus neurons antidromically activated from the omnipause region in monkey. J Neurophysiol 78:2221–5.
46. Schiller PH, Sandell JH, Maunsell JH (1987) The effect of frontal eye field and superior colliculus lesions on saccadic latencies in the rhesus monkey. J Neurophysiol 57:1033–49.
47. Hikosaka O, Wurtz RH (1985) Modification of saccadic eye movements by GABA-related substances. I. Effect of muscimol and bicuculline in monkey superior colliculus. J Neurophysiol 53:266–91.
48. Pierrot-Deseilligny C, Rosa A, Masmoudi K, et al (1991) Saccade deficits after a unilateral lesion affecting the superior colliculus. J Neurol Neurosurg Psychiatry 54:1106–9.
49. Carasig D, Paul K, Fucito M, et al (2006) Irrepressible saccades from a tectal lesion in a Rhesus monkey. Vision Res. doi: 10.1016/j.visres.2005.05.024
50. Schiller PH, True SD, Conway JL (1980) Deficits in eye movements following frontal eye-field and superior colliculus ablations. J Neurophysiol 44:1175–89.
51. Optican LM (2005) Sensorimotor transformation for visually guided saccades. Ann N Y Acad Sci 1039:132–48. doi: 10.1196/annals.1325.013
52. Henn V (1992) Pathophysiology of rapid eye movements in the horizontal, vertical and torsional directions. Baillière's Clin Neurol 1:373–91.
53. Cohen B, Komatsuzaki A (1972) Eye movements induced by stimulation of the pontine reticular formation: Evidence for integration in oculomotor pathways. Exp Neurol 36:101–117. doi: 10.1016/0014-4886(72)90139-2
54. Henn V, Lang W, Hepp K, Reisine H (1984) Experimental gaze palsies in monkeys and their relation to human pathology. Brain 107:
55. Steiger H-J, Büttner-Ennever J (1978) Relationship between motoneurons and internuclear neurons in the abducens nucleus: a double retrograde tracer study in the cat. Brain Res. doi: 10.1016/0006-8993(78)90387-6
56. Büttner-Ennever JA, Akert K (1981) Medial rectus subgroups of the oculomotor nucleus and their abducens internuclear input in the monkey. J Comp Neurol 197:17–27. doi: 10.1002/cne.901970103
57. Villis T, Hepp K, Schwarz U, Henn V (1989) On the generation of vertical and torsional rapid eye movements in the monkey. Exp brain Res 77:1–11.
58. Moschovakis AK, Scudder CA, Highstein SM (1991) Structure of the primate oculomotor burst generator. I. Medium-lead burst neurons with upward on-directions. J Neurophysiol 65:203–17.
59. Moschovakis AK, Scudder CA, Highstein SM, Warren JD (1991) Structure of the primate oculomotor burst generator. II. Medium-lead burst neurons with downward on-directions. J Neurophysiol 65:218–29.
60. Suzuki Y, Büttner-Ennever JA, Straumann D, et al (1995) Deficits in torsional and vertical rapid eye movements and shift of Listing's plane after uni- and bilateral lesions of the rostral interstitial nucleus of the medial longitudinal fasciculus. Exp Brain Res 106:215–232. doi: 10.1007/BF00241117
61. Pierrot-Deseilligny CH, Chain F, Gray F, et al (1982) Parinaud's syndrome: electro-oculographic and anatomical analyses of six vascular cases with deductions about vertical gaze organization in the premotor structures. Brain 105 (Pt 4):667–96.
62. Doron KW, Funk CM, Glickstein M (2010) Fronto-cerebellar circuits and eye movement control: A diffusion imaging tractography study of human cortico-pontine projections. Brain Res 1307:63–71. doi: 10.1016/j.brainres.2009.10.029
63. Voogd J, Schraa-Tam CKL, van der Geest JN, De Zeeuw CI (2012) Visuomotor cerebellum in human and nonhuman primates. Cerebellum 11:392–410. doi: 10.1007/s12311-010-0204-7
64. Ohtsuka K, Noda H (1995) Discharge properties of Purkinje cells in the oculomotor vermis during visually guided saccades in the macaque monkey. J Neurophysiol 74:1828–40.
65. Yamada J, Noda H (1987) Afferent and efferent connections of the oculomotor cerebellar vermis

in the macaque monkey. J Comp Neurol 265:224–241. doi: 10.1002/cne.902650207
66. Thier P, Dicke PW, Haas R, Barash S (2000) Encoding of movement time by populations of cerebellar Purkinje cells. Nature 405:72–76. doi: 10.1038/35011062
67. Noda H, Sugita S, Ikeda Y (1990) Afferent and efferent connections of the oculomotor region of the fastigial nucleus in the macaque monkey. J Comp Neurol 302:330–48. doi: 10.1002/cne.903020211
68. Goffart L, Chen LL, Sparks DL (2003) Saccade Dysmetria during Functional Perturbation of the Caudal Fastigial Nucleus in the Monkey. Ann N Y Acad Sci 1004:220–228. doi: 10.1196/annals.1303.019
69. Straube A, Scheuerer W, Robinson FR, Eggert T (2009) Temporary lesions of the caudal deep cerebellar nucleus in nonhuman primates. Gain, offset, and ocular alignment. Ann N Y Acad Sci 1164:119–26. doi: 10.1111/j.1749-6632.2008.03734.x
70. Sato H, Noda H (1992) Saccadic dysmetria induced by transient functional decortication of the cerebellar vermis [corrected]. Exp brain Res 88:455–8.
71. Robinson FR, Straube A, Fuchs AF (1993) Role of the caudal fastigial nucleus in saccade generation. II. Effects of muscimol inactivation. J Neurophysiol 70:1741–58.
72. Takagi M, Zee DS, Tamargo RJ (1998) Effects of lesions of the oculomotor vermis on eye movements in primate: saccades. J Neurophysiol 80:1911–31.
73. Filippopulos F, Eggert T, Straube A (2013) Deficits of cortical oculomotor mechanisms in cerebellar atrophy patients. Exp brain Res 224:541–50. doi: 10.1007/s00221-012-3332-0
74. Robinson FR (2000) Role of the cerebellar posterior interpositus nucleus in saccades I. Effect of temporary lesions. J Neurophysiol 84:1289–302.
75. Thurtell MJ, Tomsak RL, Leigh RJ (2007) Disorders of saccades. Curr Neurol Neurosci Rep 7:407–16.
76. Bhidayasiri R, Plant GT, Leigh RJ (2000) A hypothetical scheme for the brainstem control of vertical gaze. Neurology 54:1985–93.
77. Pflugshaupt T, Nyffeler T, von Wartburg R, et al (2009) Loss of exploratory vertical saccades after unilateral frontal eye field damage. BMJ Case Rep. doi: 10.1136/bcr.08.2008.0687
78. Munoz DP, Everling S (2004) Look away: the anti-saccade task and the voluntary control of eye movement. Nat Rev Neurosci 5:218–228. doi: 10.1038/nrn1345
79. Yang Q, Kapoula Z (2006) The control of vertical saccades in aged subjects. Exp brain Res 171:67–77. doi: 10.1007/s00221-005-0249-x
80. Rottach KG, Riley DE, DiScenna AO, et al (1996) Dynamic properties of horizontal and vertical eye movements in parkinsonian syndromes. Ann Neurol 39:368–77. doi: 10.1002/ana.410390314
81. Mott FW, Schaefer EA (1890) On associated eye-movements produced by cortical faradization of the monkey's brain. Brain 13:165–173. doi: 10.1093/brain/13.2.165
82. Bender MB (1980) Brain control of conjugate horizontal and vertical eye movements: a survey of the structural and functional correlates. Brain 103:23–69.
83. Collewijn H, Erkelens CJ, Steinman RM (1988) Binocular co-ordination of human vertical saccadic eye movements. J Physiol 404:183–197. doi: 10.1113/jphysiol.1988.sp017285
84. Collewijn H, Erkelens CJ, Steinman RM (1988) Binocular co-ordination of human horizontal saccadic eye movements. J Physiol 404:157–182. doi: 10.1113/jphysiol.1988.sp017284
85. Goldring J, Fischer B (1997) Reaction times of vertical prosaccades and antisaccades in gap and overlap tasks. Exp brain Res 113:88–103.
86. Dafoe JM, Armstrong IT, Munoz DP (2007) The influence of stimulus direction and eccentricity on pro- and anti-saccades in humans. Exp brain Res 179:563–70. doi: 10.1007/s00221-006-0817-8
87. Bhidayasiri R, Riley DE, Somers JT, et al (2001) Pathophysiology of slow vertical saccades in progressive supranuclear palsy. Neurology 57:2070–7.
88. U. B, Büttner-Ennever JA, Henn V (1977) Vertical eye movement related unit activity in the rostral mesencephalic reticular formation of the alert monkey. Brain Res 130:239–252. doi: 10.1016/0006-8993(77)90273-6
89. Robinson DA, Fuchs AF (1969) Eye movements evoked by stimulation of frontal eye fields. J

Neurophysiol 32:637–48.
90. Dias EC, Segraves MA, Andersen RA, et al (1999) Muscimol-induced inactivation of monkey frontal eye field: effects on visually and memory-guided saccades. J Neurophysiol 81:2191–214. doi: 10.1002/cne.902320403
91. Blanke O, Seeck M (2003) Direction of saccadic and smooth eye movements induced by electrical stimulation of the human frontal eye field: effect of orbital position. Exp brain Res 150:174–83. doi: 10.1007/s00221-003-1395-7
92. Rasmussen T, Penfield W (1948) Movement of head and eyes from stimulation of human frontal cortex. Res Publ Assoc Res Nerv Ment Dis 27:346–61.
93. Bonnet C, Hanuška J, Rusz J, et al (2013) Horizontal and vertical eye movement metrics: what is important? Clin Neurophysiol 124:2216–29. doi: 10.1016/j.clinph.2013.05.002
94. Tzelepi A, Laskaris N, Amditis A, Kapoula Z (2010) Cortical activity preceding vertical saccades: a MEG study. Brain Res 1321:105–16. doi: 10.1016/j.brainres.2010.01.002
95. Tzelepi A, Yang Q, Kapoula Z (2005) The effect of transcranial magnetic stimulation on the latencies of vertical saccades. Exp brain Res 164:67–77. doi: 10.1007/s00221-005-2250-9
96. Honda H, Findlay JM (1992) Saccades to targets in three-dimensional space: dependence of saccadic latency on target location. Percept Psychophys 52:167–74.
97. Heywood S, Churcher J (2007) Structure of the visual array and saccadic latency: implications for oculomotor control. http://dx.doi.org/10.1080/14640748008401169
98. Previc FH (1990) Functional specialization in the lower and upper visual fields in humans: Its ecological origins and neurophysiological implications. Behav Brain Sci 13:519–542. doi: 10.1017/S0140525X00080018
99. Burkhalter A, Felleman DJ, Newsome WT, Van Essen DC (1986) Anatomical and physiological asymmetries related to visual areas V3 and VP in macaque extrastriate cortex. Vision Res 26:63–80. doi: 10.1016/0042-6989(86)90071-4
100. Abed F (1991) Cultural Influences on Visual Scanning Patterns. J Cross Cult Psychol 22:525–534. doi: 10.1177/0022022191224006
101. Viviani P, Berthoz A, Tracey D (1977) The curvature of oblique saccades. Vision Res 17:661–664. doi: 10.1016/0042-6989(77)90143-2
102. Van der Stigchel S, Theeuwes J (2008) Differences in distractor-induced deviation between horizontal and vertical saccade trajectories. Neuroreport 19:251–4. doi: 10.1097/WNR.0b013e3282f49b3f
103. Schneider KA, Kastner S (2005) Visual responses of the human superior colliculus: a high-resolution functional magnetic resonance imaging study. J Neurophysiol 94:2491–503. doi: 10.1152/jn.00288.2005
104. Laidlaw KEW, Kingstone A (2010) The time course of vertical, horizontal and oblique saccade trajectories: Evidence for greater distractor interference during vertical saccades. Vision Res 50:829–837. doi: 10.1016/j.visres.2010.02.009
105. Pitzalis S, Di Russo F (2001) Spatial anisotropy of saccadic latency in normal subjects and brain-damaged patients. Cortex 37:475–92.
106. Averbuch-Heller L, Stahl JS, Remler BF, Leigh RJ (1996) Bilateral ptosis and upgaze palsy with right hemispheric lesions. Ann Neurol 40:465–8. doi: 10.1002/ana.410400320
107. Jadhav AP, Zenonos G, Pless M, et al (2013) A Variant of the Anterior Opercular Syndrome With Supranuclear Gaze Palsy. JAMA Neurol 70:800. doi: 10.1001/jamaneurol.2013.1947
108. Colby CL, Goldberg ME (1999) Space and attention in parietal cortex. Annu Rev Neurosci 22:319–49. doi: 10.1146/annurev.neuro.22.1.319
109. Simó LS, Krisky CM, Sweeney JA (2005) Functional neuroanatomy of anticipatory behavior: dissociation between sensory-driven and memory-driven systems. Cereb Cortex 15:1982–91. doi: 10.1093/cercor/bhi073
110. Connolly JD, Goodale MA, Goltz HC, Munoz DP (2005) fMRI activation in the human frontal eye field is correlated with saccadic reaction time. J Neurophysiol 94:605–11. doi: 10.1152/jn.00830.2004
111. Ford KA, Goltz HC, Brown MRG, Everling S (2005) Neural processes associated with

antisaccade task performance investigated with event-related FMRI. J Neurophysiol 94:429–40. doi: 10.1152/jn.00471.2004
112. Neggers SFW, Raemaekers MAH, Lampmann EEL, et al (2005) Cortical and subcortical contributions to saccade latency in the human brain. Eur J Neurosci 21:2853–63. doi: 10.1111/j.1460-9568.2005.04129.x
113. Fischer B, Breitmeyer B (1987) Mechanisms of visual attention revealed by saccadic eye movements. Neuropsychologia 25:73–83.
114. Reuter-Lorenz PA, Hughes HC, Fendrich R (1991) The reduction of saccadic latency by prior offset of the fixation point: An analysis of the gap effect. Percept Psychophys 49:167–175. doi: 10.3758/BF03205036
115. Hutton SB, Ettinger U (2006) The antisaccade task as a research tool in psychopathology: A critical review. Psychophysiology 43:302–313. doi: 10.1111/j.1469-8986.2006.00403.x
116. Unsworth N, Schrock JC, Engle RW (2004) Working memory capacity and the antisaccade task: individual differences in voluntary saccade control. J Exp Psychol Learn Mem Cogn 30:1302–21. doi: 10.1037/0278-7393.30.6.1302
117. Mokler A, Fischer B (1999) The recognition and correction of involuntary prosaccades in an antisaccade task. Exp brain Res 125:511–6.
118. Olk B, Kingstone A (2003) Why are antisaccades slower than prosaccades? A novel finding using a new paradigm. Neuroreport 14:151–5. doi: 10.1097/01.wnr.0000053062.88427.d7
119. Everling S, Fischer B (1998) The antisaccade: a review of basic research and clinical studies. Neuropsychologia 36:885–899. doi: 10.1016/S0028-3932(98)00020-7
120. Ettinger U, Ffytche DH, Kumari V, et al (2008) Decomposing the neural correlates of antisaccade eye movements using event-related FMRI. Cereb Cortex 18:1148–59. doi: 10.1093/cercor/bhm147
121. Clementz BA, Brahmbhatt SB, McDowell JE, et al (2007) When does the brain inform the eyes whether and where to move? An EEG study in humans. Cereb Cortex 17:2634–43. doi: 10.1093/cercor/bhl171
122. Manoach DS, Thakkar KN, Cain MS, et al (2007) Neural activity is modulated by trial history: a functional magnetic resonance imaging study of the effects of a previous antisaccade. J Neurosci 27:1791–8. doi: 10.1523/JNEUROSCI.3662-06.2007
123. Everling S, Munoz DP (2000) Neuronal correlates for preparatory set associated with pro-saccades and anti-saccades in the primate frontal eye field. J Neurosci 20:387–400.
124. Massen C (2004) Parallel programming of exogenous and endogenous components in the antisaccade task. Q J Exp Psychol A 57:475–98. doi: 10.1080/02724980343000341
125. DeSouza JFX, Menon RS, Everling S (2003) Preparatory set associated with pro-saccades and anti-saccades in humans investigated with event-related FMRI. J Neurophysiol 89:1016–23. doi: 10.1152/jn.00562.2002
126. Blaukopf CL, DiGirolamo GJ (2006) Differential effects of reward and punishment on conscious and unconscious eye movements. Exp brain Res 174:786–92. doi: 10.1007/s00221-006-0685-2
127. Raichle ME (2015) The Brain's Default Mode Network. http://dx.doi.org/10.1146/annurev-neuro-071013-014030
128. Sweeney JA, Mintun MA, Kwee S, et al (1996) Positron emission tomography study of voluntary saccadic eye movements and spatial working memory. J Neurophysiol 75:454–68.
129. Polli FE, Barton JJS, Cain MS, et al (2005) Rostral and dorsal anterior cingulate cortex make dissociable contributions during antisaccade error commission. Proc Natl Acad Sci U S A 102:15700–5. doi: 10.1073/pnas.0503657102
130. Krebs RM, Woldorff MG, Tempelmann C, et al (2010) High-field FMRI reveals brain activation patterns underlying saccade execution in the human superior colliculus. PLoS One 5:e8691. doi: 10.1371/journal.pone.0008691
131. Antoniades C, Ettinger U, Gaymard B, et al (2013) An internationally standardised antisaccade protocol. Vision Res 84:1–5. doi: 10.1016/j.visres.2013.02.007
132. Logothetis NK (2008) What we can do and what we cannot do with fMRI. Nature 453:869–878. doi: 10.1038/nature06976

133. Logothetis NK, Wandell BA (2004) Interpreting the BOLD signal. Annu Rev Physiol 66:735–69. doi: 10.1146/annurev.physiol.66.082602.092845
134. Müri RM (2006) MRI and fMRI analysis of oculomotor function. Prog Brain Res 151:503–526. doi: 10.1016/S0079-6123(05)51016-1
135. Amaro E, Barker GJ (2006) Study design in fMRI: Basic principles. Brain Cogn 60:220–232. doi: 10.1016/j.bandc.2005.11.009
136. Logothetis NK, Pauls J, Augath M, et al (2001) Neurophysiological investigation of the basis of the fMRI signal. Nature 412:150–7. doi: 10.1038/35084005
137. Logothetis NK, Pfeuffer J (2004) On the nature of the BOLD fMRI contrast mechanism. Magn Reson Imaging 22:1517–1531. doi: 10.1016/j.mri.2004.10.018
138. Logothetis NK (2003) The underpinnings of the BOLD functional magnetic resonance imaging signal. J Neurosci 23:3963–71.
139. Moraschi M, DiNuzzo M, Giove F, et al (2012) On the origin of sustained negative BOLD response. J Neurophysiol 108:2339–42. doi: 10.1152/jn.01199.2011
140. Rosen BR, Buckner RL, Dale AM (1998) Event-related functional MRI: past, present, and future. Proc Natl Acad Sci U S A 95:773–80.
141. Zarahn E, Aguirre G, D'Esposito M (1997) A Trial-Based Experimental Design for fMRI. Neuroimage 6:122–138. doi: 10.1006/nimg.1997.0279
142. Friston KJ, Zarahn E, Josephs O, et al (1999) Stochastic Designs in Event-Related fMRI. Neuroimage 10:607–619. doi: 10.1006/nimg.1999.0498
143. Peck KK, Wierenga CE, Moore AB, et al (2004) Comparison of baseline conditions to investigate syntactic production using functional magnetic resonance imaging. Neuroimage 23:104–110. doi: 10.1016/j.neuroimage.2004.05.006
144. Stark CEL, Squire LR (2001) When zero is not zero: The problem of ambiguous baseline conditions in fMRI. Proc Natl Acad Sci 98:12760–12766. doi: 10.1073/pnas.221462998
145. Grosbras M-H, Laird AR, Paus T (2005) Cortical regions involved in eye movements, shifts of attention, and gaze perception. Hum Brain Mapp 25:140–54. doi: 10.1002/hbm.20145
146. Mort DJ, Perry RJ, Mannan SK, et al (2003) Differential cortical activation during voluntary and reflexive saccades in man. Neuroimage 18:231–46.
147. Müri RM, Heid O, Nirkko AC, et al (1998) Functional organisation of saccades and antisaccades in the frontal lobe in humans: a study with echo planar functional magnetic resonance imaging. J Neurol Neurosurg Psychiatry 65:374–7.
148. Kimmig H, Greenlee M, Gondan M, et al (2001) Relationship between saccadic eye movements and cortical activity as measured by fMRI: quantitative and qualitative aspects. Exp Brain Res 141:184–194. doi: 10.1007/s002210100844
149. Ozyurt J, Greenlee MW (2011) Neural correlates of inter- and intra-individual saccadic reaction time differences in the gap/overlap paradigm. J Neurophysiol 105:2438–47. doi: 10.1152/jn.00660.2009
150. Brown MRG, Vilis T, Everling S (2007) Frontoparietal activation with preparation for antisaccades. J Neurophysiol 98:1751–62. doi: 10.1152/jn.00460.2007
151. Neggers SFW, van Diepen RM, Zandbelt BB, et al (2012) A Functional and Structural Investigation of the Human Fronto-Basal Volitional Saccade Network. PLoS One 7:e29517. doi: 10.1371/journal.pone.0029517
152. Curtis CE, D'Esposito M (2006) Selection and maintenance of saccade goals in the human frontal eye fields. J Neurophysiol 95:3923–7. doi: 10.1152/jn.01120.2005
153. Kastner S, DeSimone K, Konen CS, et al (2007) Topographic maps in human frontal cortex revealed in memory-guided saccade and spatial working-memory tasks. J Neurophysiol 97:3494–507. doi: 10.1152/jn.00010.2007
154. Igor Kagan AIALRAA (2010) Space representation for eye movements is more contralateral in monkeys than in humans. Proc Natl Acad Sci U S A 107:7933.
155. de Weijer AD, Mandl RCW, Sommer IEC, et al (2010) Human fronto-tectal and fronto-striatal-tectal pathways activate differently during anti-saccades. Front Hum Neurosci 4:41. doi: 10.3389/fnhum.2010.00041

156. Leoné FTM, Toni I, Medendorp WP (2014) Two-dimensional spatial tuning for saccades in human parieto-frontal cortex. Neuroimage 87:476–489. doi: 10.1016/j.neuroimage.2013.09.067
157. Petit L, Zago L, Vigneau M, et al (2009) Functional Asymmetries Revealed in Visually Guided Saccades: An fMRI Study. J Neurophysiol 102:2994–3003. doi: 10.1152/jn.00280.2009
158. Corbetta M, Akbudak E, Conturo TE, et al (1998) A common network of functional areas for attention and eye movements. Neuron 21:761–73.
159. Schlag J, Schlag-Rey M (1987) Evidence for a supplementary eye field. J Neurophysiol 57:179–200.
160. Simon O, Mangin J-F, Cohen L, et al (2002) Topographical Layout of Hand, Eye, Calculation, and Language-Related Areas in the Human Parietal Lobe. Neuron 33:475–487. doi: 10.1016/S0896-6273(02)00575-5
161. Connolly JD, Goodale MA, Menon RS, Munoz DP (2002) Human fMRI evidence for the neural correlates of preparatory set. Nat Neurosci 5:1345–52. doi: 10.1038/nn969
162. Nyffeler T, Rivaud-Pechoux S, Pierrot-Deseilligny C, et al (2007) Visual vector inversion in the posterior parietal cortex. Neuroreport 18:917–20. doi: 10.1097/WNR.0b013e32811d6cf5
163. Medendorp WP, Goltz HC, Vilis T (2005) Remapping the remembered target location for anti-saccades in human posterior parietal cortex. J Neurophysiol 94:734–40. doi: 10.1152/jn.01331.2004
164. Sereno MI, Pitzalis S, Martinez A (2001) Mapping of contralateral space in retinotopic coordinates by a parietal cortical area in humans. Science 294:1350–4. doi: 10.1126/science.1063695
165. Schluppeck D, Curtis CE, Glimcher PW, Heeger DJ (2006) Sustained Activity in Topographic Areas of Human Posterior Parietal Cortex during Memory-Guided Saccades. J Neurosci 26:5098–5108. doi: 10.1523/JNEUROSCI.5330-05.2006
166. Domagalik A, Beldzik E, Fafrowicz M, et al (2012) Neural networks related to pro-saccades and anti-saccades revealed by independent component analysis. Neuroimage 62:1325–33. doi: 10.1016/j.neuroimage.2012.06.006
167. Swisher JD, Halko MA, Merabet LB, et al (2007) Visual Topography of Human Intraparietal Sulcus. J Neurosci 27:5326–5337. doi: 10.1523/JNEUROSCI.0991-07.2007
168. Amiez C, Petrides M (2009) Anatomical organization of the eye fields in the human and non-human primate frontal cortex. Prog Neurobiol 89:220–230. doi: 10.1016/j.pneurobio.2009.07.010
169. Botvinick MM, Cohen JD, Carter CS (2004) Conflict monitoring and anterior cingulate cortex: an update. Trends Cogn Sci 8:539–546. doi: 10.1016/j.tics.2004.10.003
170. Chan JL, Kucyi A, DeSouza JFX (2015) Stable Task Representations under Attentional Load Revealed with Multivariate Pattern Analysis of Human Brain Activity. J Cogn Neurosci 27:1789–800. doi: 10.1162/jocn_a_00819
171. Ettinger U, Antonova E, Crawford TJ, et al (2005) Structural neural correlates of prosaccade and antisaccade eye movements in healthy humans. Neuroimage 24:487–94. doi: 10.1016/j.neuroimage.2004.08.019
172. Funahashi S, Bruce CJ, Goldman-Rakic PS (1990) Visuospatial coding in primate prefrontal neurons revealed by oculomotor paradigms. J Neurophysiol 63:814–31.
173. Funahashi S, Bruce CJ, Goldman-Rakic PS (1991) Neuronal activity related to saccadic eye movements in the monkey's dorsolateral prefrontal cortex. J Neurophysiol 65:1464–83.
174. Cameron IGM, Coe BC, Watanabe M, et al (2009) Role of the basal ganglia in switching a planned response. Eur J Neurosci 29:2413–25. doi: 10.1111/j.1460-9568.2009.06776.x
175. Liu P, Basso MA (2008) Substantia nigra stimulation influences monkey superior colliculus neuronal activity bilaterally. J Neurophysiol 100:1098–112. doi: 10.1152/jn.01043.2007
176. Watanabe M, Munoz DP (2009) Neural correlates of conflict resolution between automatic and volitional actions by basal ganglia. Eur J Neurosci 30:2165–76. doi: 10.1111/j.1460-9568.2009.06998.x
177. Domagalik A, Beldzik E, Oginska H, et al (2014) Inconvenient correlation – RT–BOLD relationship for homogeneous and fast reactions. Neuroscience 278:211–221. doi: 10.1016/j.neuroscience.2014.08.012

178. Wurtz RH, McAlonan K, Cavanaugh J, et al (2011) Thalamic pathways for active vision. Trends Cogn Sci 15:177–84. doi: 10.1016/j.tics.2011.02.004
179. Dyckman KA, Camchong J, Clementz BA, McDowell JE (2007) An effect of context on saccade-related behavior and brain activity. Neuroimage 36:774–784. doi: 10.1016/j.neuroimage.2007.03.023
180. Matsuda T, Matsuura M, Ohkubo T, et al (2004) Functional MRI mapping of brain activation during visually guided saccades and antisaccades: cortical and subcortical networks. Psychiatry Res Neuroimaging 131:147–155. doi: 10.1016/j.pscychresns.2003.12.007
181. Sylvester R, Haynes J-D, Rees G, et al (2005) Saccades differentially modulate human LGN and V1 responses in the presence and absence of visual stimulation. Curr Biol 15:37–41. doi: 10.1016/j.cub.2004.12.061
182. Goldberg ME, Wurtz RH (1972) Activity of superior colliculus in behaving monkey. II. Effect of attention on neuronal responses. J Neurophysiol 35:560–74.
183. Linzenbold W, Lindig T, Himmelbach M (2011) Functional neuroimaging of the oculomotor brainstem network in humans. Neuroimage 57:1116–23. doi: 10.1016/j.neuroimage.2011.05.052
184. Dieterich M, Bucher SF, Seelos KC, Brandt T (2000) Cerebellar activation during optokinetic stimulation and saccades. Neurology 54:148–55.
185. Nitschke MF, Binkofski F, Buccino G, et al (2004) Activation of cerebellar hemispheres in spatial memorization of saccadic eye movements: an fMRI study. Hum Brain Mapp 22:155–64. doi: 10.1002/hbm.20025
186. Schraa-Tam CKL, van Broekhoven P, van der Geest JN, et al (2009) Cortical and cerebellar activation induced by reflexive and voluntary saccades. Exp brain Res 192:175–87. doi: 10.1007/s00221-008-1569-4
187. Liem EIML, Frens MA, Smits M, van der Geest JN (2013) Cerebellar activation related to saccadic inaccuracies. Cerebellum 12:224–35. doi: 10.1007/s12311-012-0417-z
188. Hayakawa Y, Nakajima T, Takagi M, et al Human cerebellar activation in relation to saccadic eye movements: a functional magnetic resonance imaging study. Ophthalmol J Int d'ophtalmologie Int J Ophthalmol Zeitschrift für Augenheilkd 216:399–405. doi: 67551
189. Bodis-Wollner I, Bucher SF, Seelos KC (1999) Cortical activation patterns during voluntary blinks and voluntary saccades. Neurology 53:1800–5.
190. Bodis-Wollner I, Bucher SF, Seelos KC, et al (1997) Functional MRI mapping of occipital and frontal cortical activity during voluntary and imagined saccades. Neurology 49:416–20.
191. Lees AJ, Hardy J, Revesz T (2009) Parkinson's disease. Lancet (London, England) 373:2055–66. doi: 10.1016/S0140-6736(09)60492-X
192. Braak H, Tredici K Del, Rüb U, et al (2003) Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 24:197–211. doi: 10.1016/S0197-4580(02)00065-9
193. Jellinger KA (1991) Pathology of Parkinson's disease. Changes other than the nigrostriatal pathway. Mol Chem Neuropathol 14:153–97.
194. Burke RE, Dauer WT, Vonsattel JPG (2008) A critical evaluation of the Braak staging scheme for Parkinson's disease. Ann Neurol 64:485–91. doi: 10.1002/ana.21541
195. DeJong JD, Jones GM (1971) Akinesia, hypokinesia, and bradykinesia in the oculomotor system of patients with Parkinson's disease. Exp Neurol 32:58–68.
196. Antoniades C, Kennard C (2014) Ocular motor abnormalities in neurodegenerative disorders. Eye. doi: 10.1038/eye.2014.276
197. Gorges M, Müller H-P, Lulé D, et al (2013) Functional connectivity within the default mode network is associated with saccadic accuracy in Parkinson's disease: a resting-state FMRI and videooculographic study. Brain Connect 3:265–72. doi: 10.1089/brain.2013.0146
198. Briand KA, Strallow D, Hening W, et al (1999) Control of voluntary and reflexive saccades in Parkinson's disease. Exp brain Res 129:38–48.
199. Armstrong IT, Chan F, Riopelle RJ, Munoz DP (2002) Control of saccades in Parkinson's disease. Brain Cogn 49:198–201.
200. Macaskill MR, Graham CF, Pitcher TL, et al (2012) The influence of motor and cognitive impairment upon visually-guided saccades in Parkinson's disease. Neuropsychologia 50:3338–

47. doi: 10.1016/j.neuropsychologia.2012.09.025
201. Bonnet C, Rusz J, Megrelishvili M, et al (2014) Eye movements in ephedrone-induced parkinsonism. PLoS One 9:e104784. doi: 10.1371/journal.pone.0104784
202. Chan F, Armstrong IT, Pari G, et al (2005) Deficits in saccadic eye-movement control in Parkinson's disease. Neuropsychologia 43:784–96. doi: 10.1016/j.neuropsychologia.2004.06.026
203. White OB, Saint-Cyr JA, Tomlinson RD, Sharpe JA (1983) Ocular motor deficits in Parkinson's disease. II. Control of the saccadic and smooth pursuit systems. Brain 106 (Pt 3):571–87.
204. Terao Y, Fukuda H, Ugawa Y, et al (2013) New perspectives on the pathophysiology of Parkinson's disease as assessed by saccade performance: A clinical review. Clin Neurophysiol 124:1491–1506. doi: 10.1016/j.clinph.2013.01.021
205. Terao Y, Fukuda H, Yugeta A, et al (2011) Initiation and inhibitory control of saccades with the progression of Parkinson's disease - changes in three major drives converging on the superior colliculus. Neuropsychologia 49:1794–806. doi: 10.1016/j.neuropsychologia.2011.03.002
206. Kimmig H, Haussmann K, Mergner T, Lücking CH (2002) What is pathological with gaze shift fragmentation in Parkinson's disease? J Neurol 249:683–92. doi: 10.1007/s00415-002-0691-7
207. Machner B, Klein C, Sprenger A, et al (2010) Eye movement disorders are different in Parkin-linked and idiopathic early-onset PD. Neurology 75:125–128. doi: 10.1212/WNL.0b013e3181e7ca6d
208. Galvan A, Devergnas A, Wichmann T (2015) Alterations in neuronal activity in basal ganglia-thalamocortical circuits in the parkinsonian state. Front Neuroanat 9:5. doi: 10.3389/fnana.2015.00005
209. Müri R., Rivaud S, Gaymard B, et al (1998) Role of the prefrontal cortex in the control of express saccades. a transcranial magnetic stimulation study. Neuropsychologia 37:199–206. doi: 10.1016/S0028-3932(98)00094-3
210. Alexander GE, DeLong MR, Strick PL (1986) Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu Rev Neurosci 9:357–81. doi: 10.1146/annurev.ne.09.030186.002041
211. Middleton FA, Strick PL (2002) Basal-ganglia "Projections" to the Prefrontal Cortex of the Primate. Cereb Cortex 12:926–935. doi: 10.1093/cercor/12.9.926
212. Rieger JW, Kim A, Argyelan M, et al (2008) Cortical functional anatomy of voluntary saccades in Parkinson disease. Clin EEG Neurosci 39:169–74.
213. Cameron IGM, Pari G, Alahyane N, et al (2012) Impaired executive function signals in motor brain regions in Parkinson's disease. Neuroimage 60:1156–70. doi: 10.1016/j.neuroimage.2012.01.057
214. Nakao S, Shiraishi Y, Li WB, Oikawa T (1990) Mono- and disynaptic excitatory inputs from the superior colliculus to vertical saccade-related neurons in the cat Forel's field H. Exp brain Res 82:222–6.
215. Liscic RM, Srulijes K, Gröger A, et al (2013) Differentiation of progressive supranuclear palsy: clinical, imaging and laboratory tools. Acta Neurol Scand 127:362–70. doi: 10.1111/ane.12067
216. Litvan I, Agid Y, Calne D, et al (1996) Clinical research criteria for the diagnosis of progressive supranuclear palsy (Steele-Richardson-Olszewski syndrome): report of the NINDS-SPSP international workshop. Neurology 47:1–9.
217. Terao Y, Fukuda H, Shirota Y, et al (2013) Deterioration of horizontal saccades in progressive supranuclear palsy. Clin Neurophysiol 124:354–63. doi: 10.1016/j.clinph.2012.07.008
218. Pinkhardt EH, Jürgens R, Becker W, et al (2008) Differential diagnostic value of eye movement recording in PSP-parkinsonism, Richardson's syndrome, and idiopathic Parkinson's disease. J Neurol 255:1916–25. doi: 10.1007/s00415-009-0027-y
219. Chen AL, Riley DE, King SA, et al (2010) The disturbance of gaze in progressive supranuclear palsy: implications for pathogenesis. Front Neurol 1:147. doi: 10.3389/fneur.2010.00147
220. Rivaud-Péchoux S, Vidailhet M, Gallouedec G, et al (2000) Longitudinal ocular motor study in corticobasal degeneration and progressive supranuclear palsy. Neurology 54:1029–32.
221. Rivaud-Péchoux S, Vidailhet M, Brandel JP, Gaymard B (2007) Mixing pro- and antisaccades in patients with parkinsonian syndromes. Brain 130:256–64. doi: 10.1093/brain/awl315

222. Pierrot-Deseilligny C, Rivaud S, Pillon B, et al (1989) Lateral Visually-Guided Saccades In Progressive Supranuclear Palsy. Brain 112:
223. Garbutt S, Matlin A, Hellmuth J, et al (2008) Oculomotor function in frontotemporal lobar degeneration, related disorders and Alzheimer's disease. Brain 131:1268–81. doi: 10.1093/brain/awn047
224. Amtage F, Maurer C, Hellwig S, et al (2014) Functional correlates of vertical gaze palsy and other ocular motor deficits in PSP: an FDG-PET study. Parkinsonism Relat Disord 20:898–906. doi: 10.1016/j.parkreldis.2014.05.013
225. Belliveau JW, Kennedy DN, McKinstry RC, et al (1991) Functional mapping of the human visual cortex by magnetic resonance imaging. Science 254:716–9.
226. Rupp J, Dzemidzic M, Blekher T, et al (2012) Comparison of vertical and horizontal saccade measures and their relation to gray matter changes in premanifest and manifest Huntington disease. J Neurol 259:267–76. doi: 10.1007/s00415-011-6172-0

Chapter II

Supranuclear and internuclear eye movement disorders

Abstract.

Purpose of review: This work reviews supranuclear ocular motor disorders, highlighting new data published during the past year.

Recent findings: Perceptional adaptative mechanisms may explain recent research concerning the discrepancy between objective measurement of saccade abnormalities and their putative functional visual impairment. Eye movement classes seem to be selectively disrupted by different neurodegenerative disorders. Deep brain stimulation in Parkinson´s disease patients may improve pursuit deficits, highlighting the role of basal ganglia in the control of smooth pursuit. Subcortical optokinetic pathways seem to play an important role in maintaining the monocular nasotemporal optokinetic asymmetry seen in patients with infantile esotropia. Vergence-vestibular interaction has been further delineated in patients with idiopathic bilateral vestibular failure. Pharmacological treatment of central vestibular disorders with 4-aminopyridine has been extended to patients with ataxia-telangectasia in whom seems to reduce slow phase velocity of nystagmus.

Summary: Recent data derived from anatomic and functional imaging studies is providing new insights into supranuclear ocular motor circuitry. Novel pharmacological and surgical therapies may have future implications in visual and vestibular rehabilitation of patients with supranuclear eye movement disorders.

Keywords.

Eye movements; Saccades; Smooth pursuit; Vestibular; Vergence; Optokinetic nystagmus; Ocular fixation

1. Introduction.

Different types of eye movement serve the purpose of keeping an object of interest in the fovea (**Fig. 1**) [1]. In a hierarchical fashion, supranuclear centers widely distributed in the cortex and brainstem control eye movements by exerting their influence on cranial nerves (CN) 3, 4 and 6. This review will cover each type of eye movement and its disorders, highlighting the latest research.

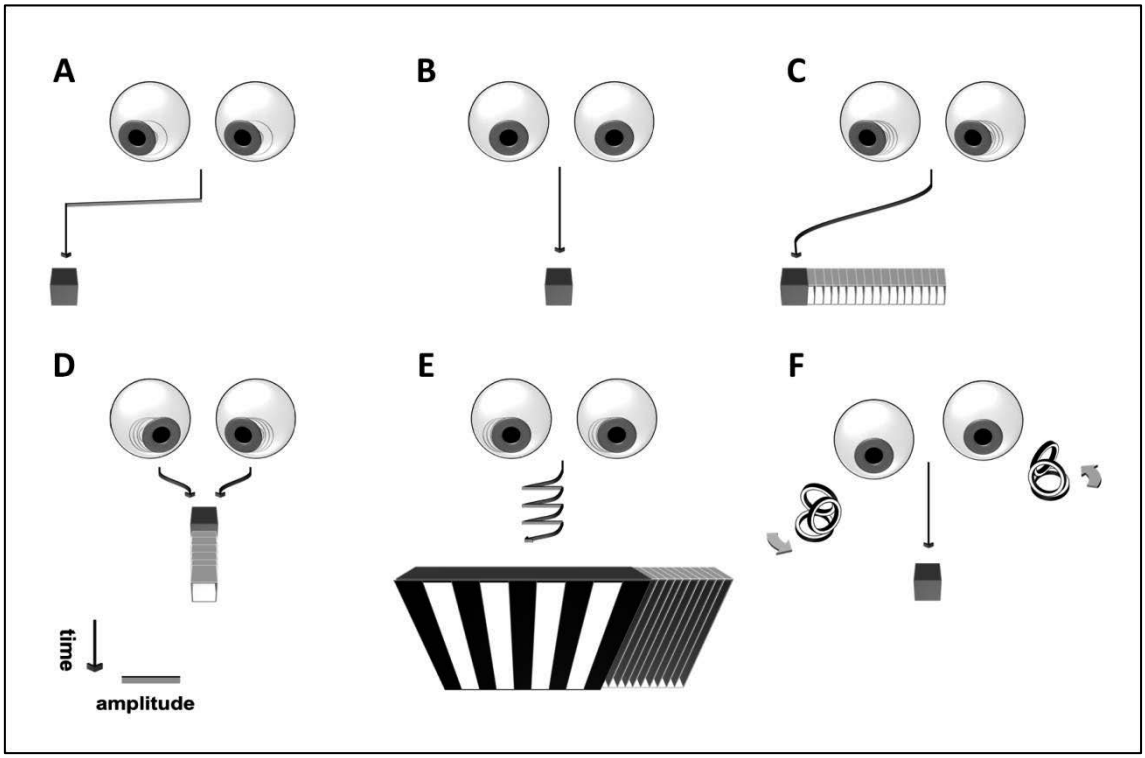

Figure 1. Human eye movements. A. Saccade is a rapid gaze shift that brings the image of an object of interest onto the fovea. B. Fixation holds the image of a stationary object on the fovea when the head is still. C. Smooth pursuit keeps the image of a small moving target on the fovea. D. Vergence moves the eyes in opposite directions to keep foveating an object of interest. E. Optokinetic nystagmus keeps the image of a large moving scene on the fovea. F. Vestibulo-ocular reflex holds the image of a stationary object on the fovea during brief head movements [1].

2. Saccadic disorders.

The supranuclear saccadic circuitry includes the cortex, basal ganglia, superior coliculus, pontine nuclei and the cerebellum (**Fig. 2**) [1]. Saccadic vertical gaze palsy consists of an impairment of upward and/or downward conjugate saccades, and is usually caused by midbrain lesions involving prenuclear oculomotor structures such as the rostral interstitial nucleus of the medial longitudinal fasciculus, interstitial nucleus of Cajal and/or posterior commissure. Variable deficits of smooth pursuit and vestibular eye movements are commonly associated [1]. Rarely, saccadic vertical gaze palsy

accompanies bilateral horizontal gaze palsy as a manifestation of bilateral middle cerebral artery infarcts that potentially disrupt input from the frontal and parietal eye fields to the midbrain and pons [2]. The presence of saccadic vertical gaze palsy helps in the differential diagnosis of neurological diseases such as neurodegenerative ataxia, and has now been described in one patient with autosomal recessive spastic ataxia of Charlevoix-Saguenay [3], warranting further ocular motor studies in a larger group sample.

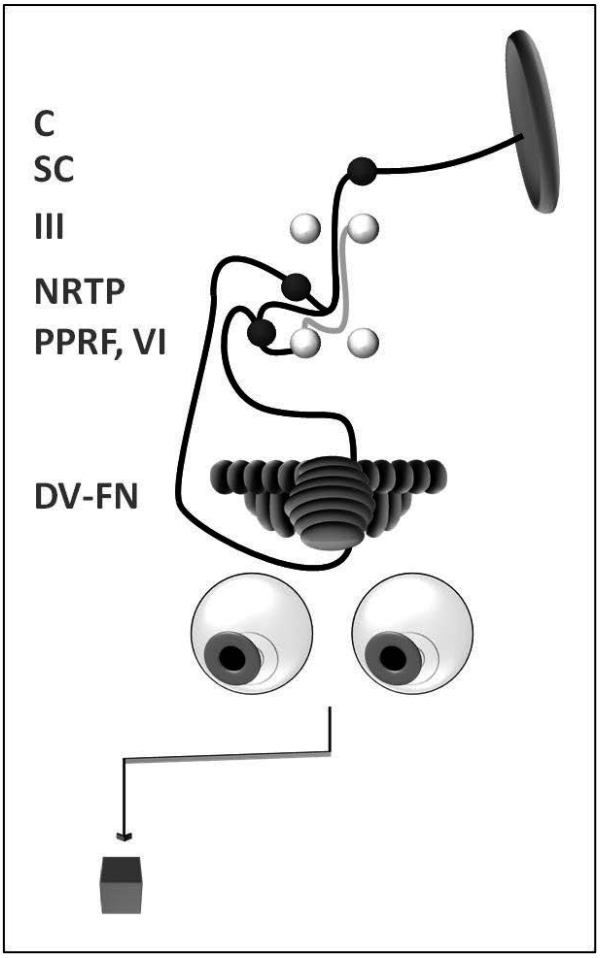

Figure 2. Cortical and subcortical anatomic circuitry of horizontal saccades (saccade to the right, in the example). Saccadic cortical fibers descend ipsilateraly to the superior coliculus (SC), subsequently crossing at the pontine level to reach paramedian pontine reticular formation (PPRF) and nucleus reticularis tegmentum pontis (NRTP). While PPRF projects to the ipsilateral sixth nucleus (VI), NRTP sends fibers to the contralateral cerebellum for continuous saccade monitoring by the dorsal vermis-fastigial nucleus complex (DV-FN). Sixth nucleus activates the contralateral third nucleus (III) via the medial longitudinal fasciculus (MLF) [1]. The omnipause neurons have been omitted for clarity.

Saccadic function has been extensively used to explore the cognitive control of behaviour, and processes involved in working memory and attention have been shown to influence saccade performance (**Fig. 3**) [4, 5]. Dong et al reported in a small study

comparing saccadic behaviour between stroke patients and controls, noting worse performance in the stroke group [6], especially in the antisaccade paradigm. Performance significantly improved during the recovery period, and appeared more sensitive than the clinical assessment scales in reflecting possible cognitive dysfunction. Further study with larger populations, controlling for potential confounders such as depression, stroke location, medication, and learning effect are required before saccadic assessment is accepted as a surrogate of cognition and stroke recovery [4, 7, 8].

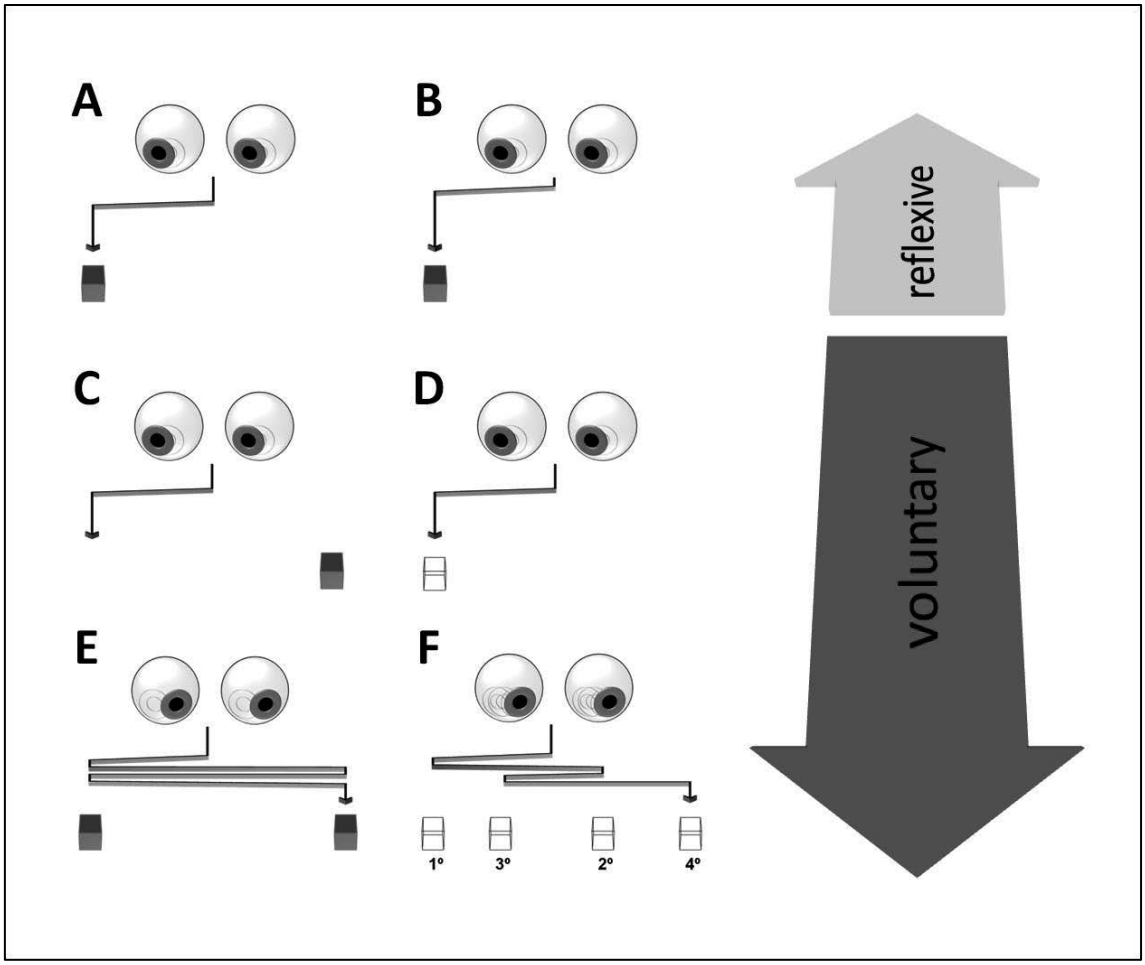

Figure 3. Saccadic behaviour. A. Reflexive saccade - a saccade directed towards an unexpected stimulus. B. Express saccade - a short-latency saccade that can be elicited in research paradigms by using a temporal gap between fixation target removal and novel stimulus appearance. C. Anti-saccade - a saccade directed to the opposite (mirrored) location of a sudden onset stimulus. D. Memory-guided saccade - a saccade directed towards a remembered stimulus after a ‘‘go signal”. E. Predictive saccades - saccades directed towards stimuli that alternate between two or more spatial locations and arise with a fixed temporal frequency. F. Sequence of saccades - memory-guided saccades directed towards stimuli that are presented sequentially at different locations. Reflexive saccadic behaviour (top row, light grey arrow) probably generated in posterior cortical areas such as the parietal eye field, contrasts with purposeful saccadic behaviour (middle and bottom row, grey arrow) believed to be generated in anterior cortical areas such as the frontal eye field, supplementary eye field and dorsolateral prefrontal cortex [5].

The growing expansion of the use of deep brain stimulation (DBS) for the treatment of Parkinson's disease (PD) patients allows an opportunity to help unravel the detailed role of the basal ganglia network concerning saccades. In 2013, Yugeta et al [9] recorded changes in DBS field potentials in the subthalamic nucleus (STN) of PD patients during saccade tasks; these potentials consisted of beta-band (15–30 Hertz) desynchronizations immediately before and during saccades, especially notable during more purposeful saccades. Thus, the STN probably exerts a dual role on saccadic motor output, inhibiting more reflexive behaviour (e.g., saccades directed toward novel stimuli) and facilitating more intended behaviour (e.g., antisaccades) [10]. Also in PD, an increased connectivity between posterior cingulated cortex and both medial temporal lobes was recently found to be correlated with saccadic hypometria, particularly in the vertical direction [11]. According to the authors, this finding may reflect a compensatory cerebral mechanism to maintain behavioural saccadic performance despite dopaminergic depletion. This study is an example of a recent trend in ocular motor research focusing on the study of the default mode network in patients, searching for patterns of disrupted connectivity between brain areas that usually show increased activation during wakeful rest in normal individuals.

Studies addressing the functional repercussion of saccadic abnormalities in daily life activities are scarce. Alexandre and co-workers studied the functional consequences of common saccadic abnormalities such as slow and/or hypometric saccades in 21 patients with degenerative ataxia and 20 controls [12]. Two important results came out from this study: 1. saccadic performance correlated with scanning tasks (i.e., high variability of saccade amplitude correlated with increased detection time of specific stimuli in a search task); 2. a questionnaire addressing the visual impact of ocular motor impairments showed poor correlation with any of the saccade parameters among patients. Whether this latter finding reflects insensitivity of the questionnaire or saccadic testing, or adaptative mechanisms effectively take over in order to reduce retinal slip perception remains to be elucidated in future research.

3. Fixation disorders.

Saccadic intrusions (SI) are involuntary conjugate saccades (fast eye movements) that interrupt fixation. Opsoclonus (OPS) is a multidirectional SI usually caused by paraneoplastic, post-infectious, toxic-metabolic or idiopathic phenomenon [1]. Matalia and co-workers reported a unique case of transient positional opsoclonus only in the vertical plane abated by cheek tapping in two 3-month old normal twins [13]. One can speculate that the attenuation of OPS after cheek tapping could represent excitation of a pontine tegmental region adjacent to burst neurons responsible for the OPS, resulting in a post-excitation refractory period of the latter [14]. Elicitation of OPS while supine is in line with animal research documenting augmentation of saccadic signals from the superior colliculus to saccadic burst neurons during head rotation [15]. Case reports describing a child and an adult with OPS associated with γ-aminobutyric acid-B

receptor (GABABR) antibodies have been published [16, 17]; this should now be included in the expanding phenotype of neurological autoimmune disorders. The high density of GABABR in the cerebellum favours the cerebellar hypothesis of OPS pathogenesis over the brainstem hypothesis, the former implicating a lack of inhibitory signals from the vermis-fastigial nucleus complex [18, 19].

Square wave jerks (SWJ) are saccadic intrusions consisting of small conjugate horizontal saccades with a normal inter-sacadic interval [1]. Gitchel et al [20] reported an increased number of SWJ and slowed saccades in patients with essential tremor (ET), compared to normal controls. These abnormalities have never been reported before in ET patients [21], and this will require replication with attention to medications and other possible confounding factors. Nonetheless, it is unlikely that such findings will help distinguish ET from PD patients, as increased SWJ have been reported in PD patients (among other groups) [22]. The authors suggest that transient saccadic decelerations in ET patients may reflect disruption of the "latch circuit" which normally inhibits pontine omnipause neurons in the brainstem, thus interrupting burst neurons discharge before the saccade is completed. Of note, patients with late-onset Tay-Sachs disease in whom this is the presumed mechanism for saccadic disturbance, show normal peak accelerations, suggesting burst neurons integrity [23].

Microsaccades (MiS) are one type of small amplitude eye movements (<1°) (**Fig. 4**) that probably form a continuum with SWJ (up to 5°) [24]. Microsaccades may aid in the differential and early diagnosis of neurodegenerative diseases. Detection of slow and small vertical MiS are more frequently present in patients with progressive supranuclear palsy while frequent horizontal MiS with normal amplitude characterize PD patients; the presence of oblique MiS may help differentiate Alzheimer's disease and mild cognitive impairment from controls [25, 26].

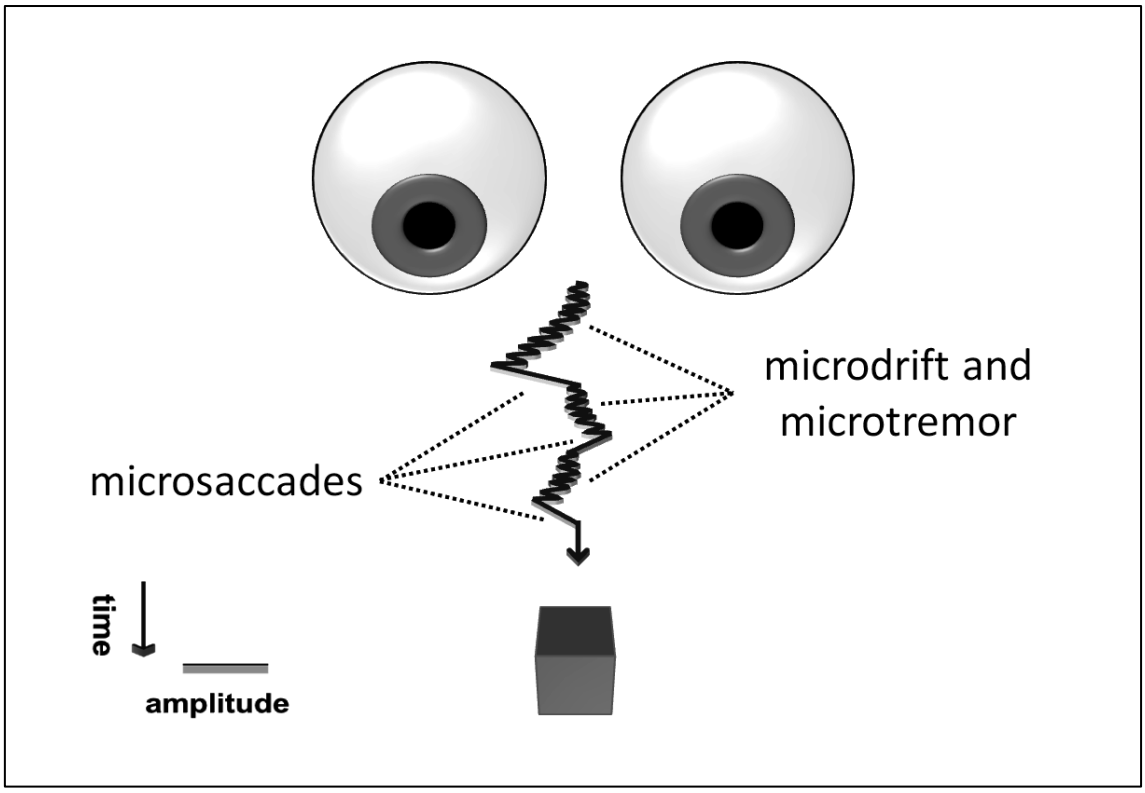

Figure 4. Fixational eye movements. Microtremor is an aperiodic, wave-like motion of the eyes; drifts consist of slow motions of the eyes, occurring between microsaccades and concomitantly with microtremor; microsaccades are small, jerk-like eye movements [24].

Memantine treatment (20 mg/daily for 6 months) of frequent SWJ in two sisters with an unrecognized form of degenerative ataxia resulted in only modest improvement in visual acuity and amplitude of SWJ, with no significant change in SWJ frequency [27]. Randomized control trials are needed to clarify the efficacy of memantine and other drugs in the treatment of saccadic intrusions.

4. Smooth Pursuit disorders.

Supranuclear smooth pursuit pathways involve an extended network that includes retina, lateral geniculate nucleus, striate cortex, secondary visual areas, pontine nucleus, brainstem reticular formation and cerebellum (**Fig. 5**) [1]. Specifically, the role of cerebellar flocculus-paraflocculus complex in the control of pursuit has been further defined in a recent case report [28] describing a patient with severe asymmetric loss of horizontal smooth pursuit but minimal vestibular signs after isolated right tonsilar infarction. These findings contrast with predominant vestibular features and subtle pursuit deficit described in another patient with isolated floccular and anterior tonsil infarction [29]. Together, these findings suggest that tonsil function may be more concerned with pursuit control while the flocculus specifically modulates VOR among other functions.

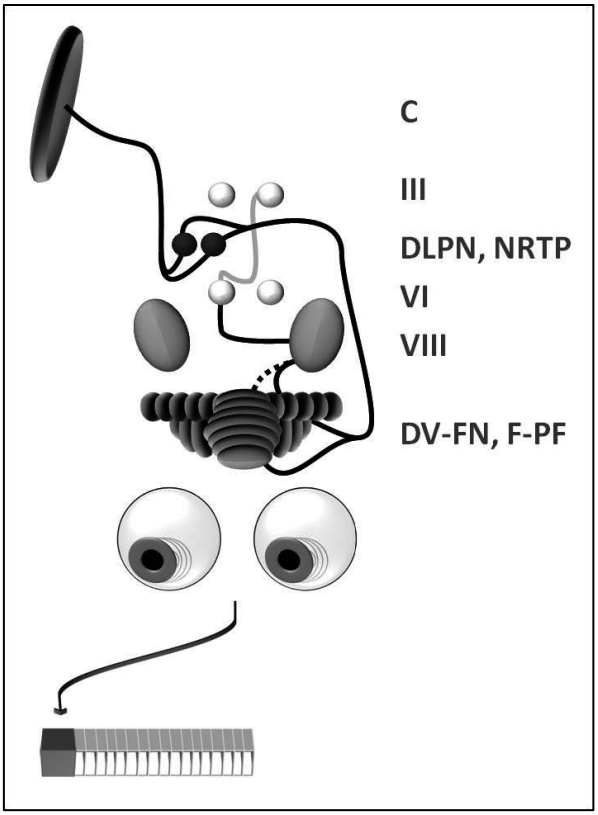

Figure 5. Cortical and subcortical anatomic circuitry of horizontal smooth pursuit (smooth pursuit to the right, in the example). Cortical (C) fibers originating in the medial superior temporal visual area and frontal eye field project to the ipsilateral dorsolateral pontine nuclei (DLPN)and nucleus reticularis tegmenti pontis (NRTP); fibers are sent from DLPN and NRTP to the contralateral dorsal vermis-fastigial nucleus complex (DV-FN) and flocculus-paraflocculus complex (F-PF, including tonsil), respectively (first decussation); F-PF then project to the ipsilateral medial vestibular nucleus (VIII), which in turn connects with the contralateral nucleus of the sixth nerve (VI) via the medial longitudinal fasciculus (MLF) (second decussation). Lastly, sixth nucleus interneurons will project to the contralateral nucleus of the third nerve via the MLF [1]. Pursuit efferent fibers from DV-FN have not yet been defined (dotted line).

Smooth pursuit may also be regulated by the basal ganglia thalamocortical pathways [30]. Nilsson et al studied the effects of STN stimulation from DBS) on ocular motor function in 9 patients with Parkinson's disease (PD) [31]. Improvement in smooth pursuit was striking when DBS was on, producing increased pursuit velocity gain and accuracy. However, the small sample size may have biased the data as previous research has failed to show a DBS effect on pursuit in PD patients, although with different baseline characteristics (e.g., disease duration), and medications. It is always possible that the DBS beneficial effect on pursuit may be mediated through improved attention rather than a direct effect on pursuit [32, 33]. Nevertheless, these are encouraging results suggesting that STN stimulation may be improve PD patient's performance in tasks that rely on smooth pursuit.

5. Optokinetic disorders.

Optokinetic nystagmus (OKN) is a reflex eye movement induced by motion of the entire visual surround, and consists of a slow tracking eye movement in the direction of environmental movement followed by a quick contraversive resetting saccade. Horizontal and vertical OKN responses should be symmetric in normal individuals, while a physiological monocular nasotemporal optokinetic asymmetry (MNTA) is evident transiently in infants while maturation of binocular cortical pursuit pathways is still underway [34]. If asymmetry is noted apart from this exception, further evaluation is required in search of an underlying disorder such as Parkinson's disease in adults or impaired binocular visual development in infancy [34, 35]. Patients with infantile esotropia are assumed to retain MNTA as a result of probable cortical pursuit deficit in the context of an abnormal cortical binocular vision development (**Fig. 6**). Brodsky and Klaehn [36] devised an optokinetic uncover test (a temporally directed optokinetic stimulation is presented to one uncovered eye, while the fellow eye is covered; subsequently, the fellow eye is uncovered and binocular optokinetic responses are compared). Infantile esotropia subjects showed improved optokinetic response once the occluded esodeviated eye was uncovered. Importantly, this effect persisted even in patients who showed no fixation shift immediately after uncovering the esodeviated eye, suggesting that peripheral retinal optokinetic input activates the still operational subcortical optokinetic pathways.

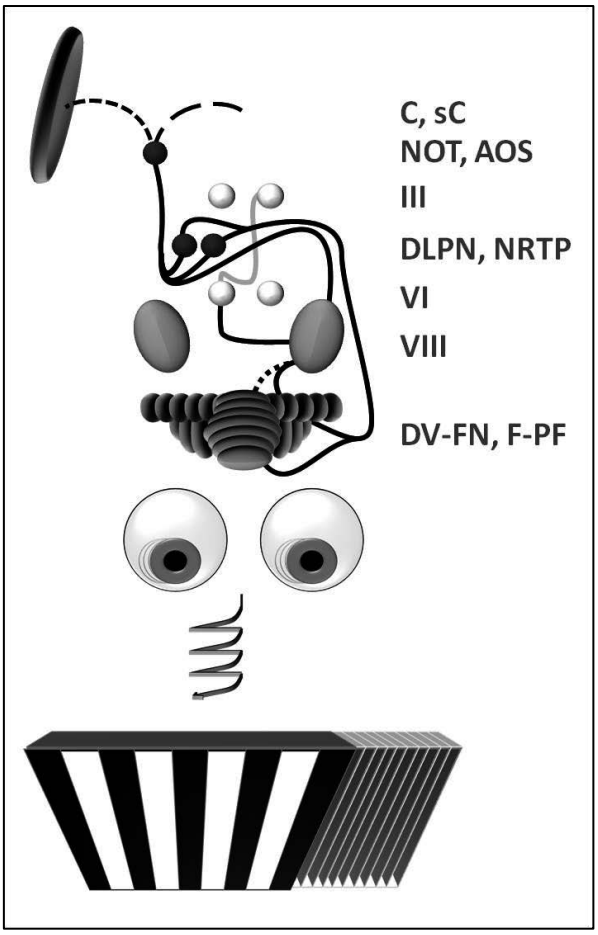

Figure 6. Cortical and subcortical anatomic circuitry of optokinetic response (optokinetic stimulus to the right, in the example). Two efferent pathways send optokinetic signals to the nucleus of the optic tract and accessory optic system (NOT and AOS): a direct subcortical (sC) pathway carrying retinal nasal signals from the contralateral eye (large dashed line) and an indirect cortical (C) pathway that carries temporal and nasal retinal signals from the ipsilateral and contralateral eye, respectively (small dashed line). From NOT and AOS, fibers mainly cross to reach the contralateral vestibular nucleus (VIII) [34]. (see **Fig. 5** legend). The inferior olive has been omitted for clarity.

In a recent literature review focusing on the clinical features of infantile-onset saccade initiation delay [37], Salman and Ikeda reported an impairment of the fast component of OKN response in 68.9% of the patients. This finding may be a direct consequence of saccadic initiation failure, not necessarily implying intrinsic damage of supranuclear pathways involved in the optokinetic response [38]. In a subsequent sub-analysis, these investigators noted this impairment was significantly more prevalent in patients with supra or infratentorial MRI abnormalities [39]. Unfortunately, no clear explanation for this finding was advanced nor was this impairment quantified. Due to the potential bias inherent in a conglomeration of heterogeneous studies included in the review, it is difficult to interpret this finding in isolation. Furthermore, as pointed out by the authors, most of these studies were evaluating pursuit using a hand-held optokinetic drum and not an optokinetic stimuli filling the entire field of vision [1].

6. Vergence disorders.

The vergence network is widely distributed in the central nervous system, including premotor neurons in the mesencephalic reticular formation [40]. Over the last decades, an interaction between the vergence and vestibular system has been hypothesized. An example of such interaction is the fact that translational VOR gain is known to depend on among other factors, viewing distance, and therefore may be modulated by vergence signals [41]. Consistent with this view, Kapoula et al recently argued that convergence deficits in patients with idiopathic bilateral vestibular failure (BVF) may be due to loss of vestibular input [42]. The authors were able to show significant hypometria, low mean velocity and increased amplitude of saccade intrusions during convergence eye movements in 11 BVF patients. Interestingly, BVF patients displayed deficient convergence, but they could still improve their postural stability by fixating a near target, implying at least partial integrity of putative mechanisms via which vergence can act on posture and the vestibular system.

7. Vestibular disorders.

A supranuclear vestibular network predominantly located in the temporo-insular and temporo-parietal cortex is believed to process and integrate the vestibular information provided from both labyrinths. Over the last two decades, fMRI (functional magnetic resonance imaging) and PET (positron emission tomography) studies in normal individuals using caloric or galvanic vestibular stimulation have shown that cortical activation of this network is more intense in the non-dominant hemisphere for subject's handedness and in the hemisphere ipsilateral to the stimulation and the slow phase of vestibular caloric nystagmus [43, 44]. Concomitant deactivation of visual areas while multisensory vestibular cortex is activated possibly reflects reciprocal inhibitory cortical interaction between these two systems [45].

Vestibular migraine (VM) is a migraine subtype clinically characterized by vestibular symptoms. It remains unclear if it is predominantly a central or a peripheral vestibulopathy, but ocular motor examination points to a central cause in about 50% of the cases [46]. Shin and colleagues reported for the first time the altered brain metabolism in 2 patients with VM, using ictal and inter-ictal 18F-fluorodeoxy glucose PET [47]. In line with the vestibular-visual reciprocal inhibition hypothesis, the authors were able to detect ictal increased metabolism of the temporo-parieto-insular cortex and bilateral thalami (vestibular areas), with concurrent occipitotemporal deactivation (visual areas). Additionally, the presence of ictal and inter-ictal cerebellar hypermetabolism was thought to reflect supranuclear inhibition of a hyperactive vestibular system in these patients. The central impairment of vestibular pathways in VM patients has been further disclosed by Russo and co-workers using fMRI during ear irrigation with cold water in 12 VM patients, 12 patients with migraine without aura and 12 controls [48]. Patients with VM displayed increased thalamic activation relative to both migraine without aura patients and controls.

In 2014, Yang and co-workers investigated the hypothesis that isolated vertical perceptual changes [i.e., subjective vertical visual tilt, (SVV) without accompanying ocular torsion or skew deviation] may indicate an impairment of an uncrossed supranuclear graviceptive pathway called the ipsilateral vestibulothalamic tract (IVTT) [49, 50]. Indeed, by applying voxel-based lesion-behavior mapping analyses in 82 stroke patients, lesions of the medial aspect of the medial lemniscus (IVTT) were associated with purely ipsiversive SVV without ocular torsion or skew deviation, corroborating the existence of a direct projection to the thalamus that bypasses the oculomotor nuclei (**Fig. 7**). Lesions above the brainstem affecting graviceptive pathways and causing SVV deviation have also been reported; these usually correspond to large infarctions of the middle cerebral artery territory affecting vestibular processing areas such as the insular cortex, superior temporal or inferior frontal gyrus [51]. Conversely, it was recently shown that isolated infarction in one of these areas (insular cortex) in 10 patients did not cause an abnormal SVV [52]. Thus, there may be a lesion volume threshold within the vestibular cortical network producing abnormal SVV tilt; small insular cortex strategic lesions might be compensated by neighbouring regions.

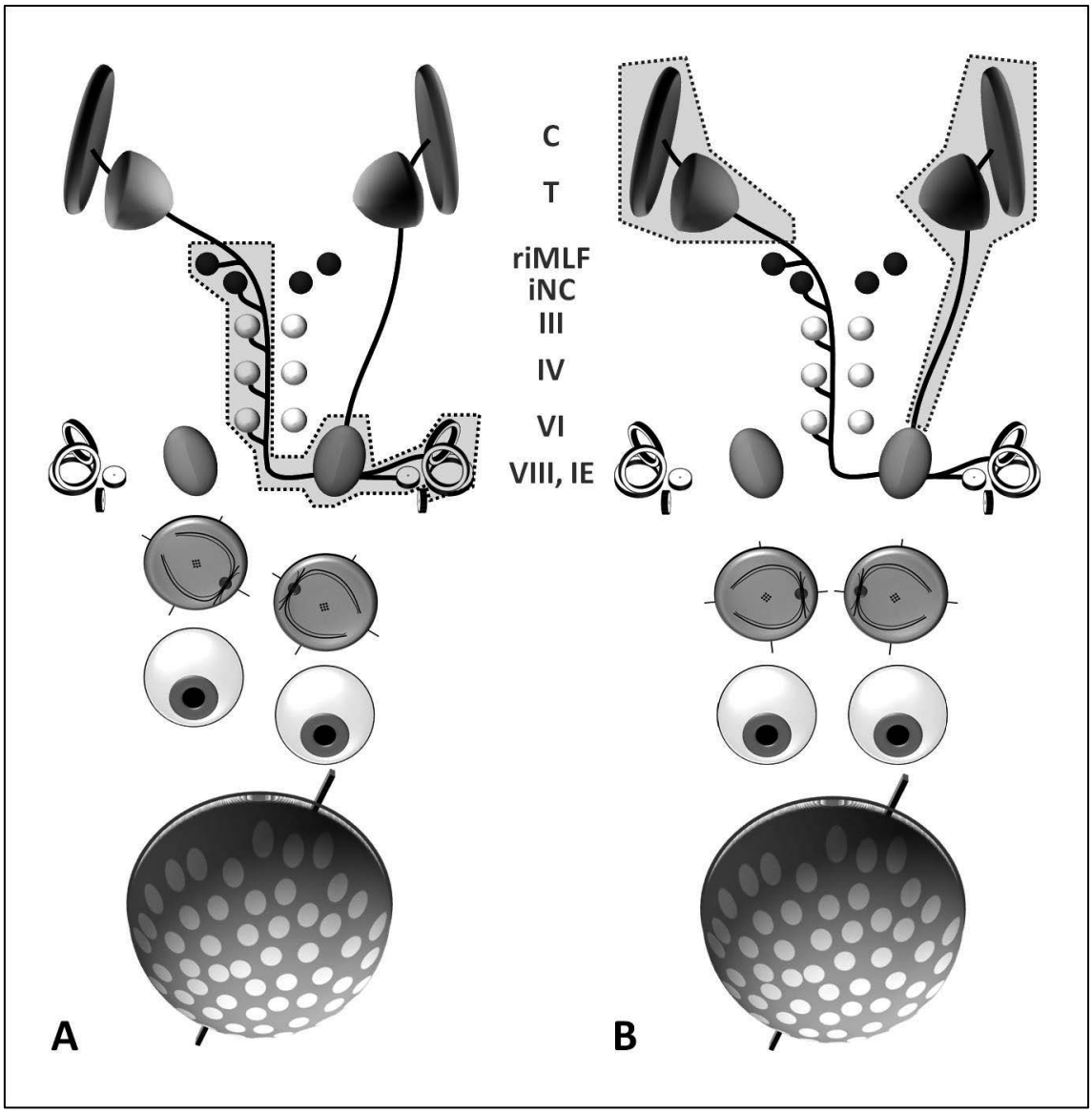

Figure 7. Graviceptive pathway disorders. Graviceptive fibers originating in the vertical canals and utricule of the inner ear (IE) project to the ipsilateral vestibular nucleus (VIII); from here, two putative supranuclear pathways ascend in the brainstem to reach the posterolateral thalamus (T) and multisensory vestibular cortex (C): a crossed tract via medial longitudinal fasciculus, sending fibers to several ocular motor structures (sixth nucleus, VI; fourth nucleus, IV; third nucleus, III; interstitial nucleus of Cajal, iNC; rostral interstitial nucleus of the medial longitudinal fasciculus, riMLF) and an uncrossed tract (ipsilateral vestibulothalamic tract), travelling in the medial side of the medial lemniscus, bypassing the oculomotor nuclei. A. A lesion affecting the IE, VIII or the crossed tract before reaching the thalamus (dotted area) will promote ocular torsion (OT), skew deviation (SD) and tilt of the subjective visual vertical (SVV). B. A lesion affecting the uncrossed tract or the crossed tract above the riMLF (dotted areas) will cause SVV tilt, but without accompanying OT or SD [49]. Ocular torsion, vertical misalignment and subjective visual vertical (from top to bottom) are represented in both lower halves of the scheme (A,B).

Pharmacological treatment of central vestibular disorders with 4-aminopyridine has shown promising results, especially in patients with downbeat nystagmus [53]. Restoring the inhibitory influence of Purkinje neurons on the vestibular nuclei is one of its putative mechanisms. Shaikh and co-workers demonstrated benefit of 4-aminopyridine in 4 patients with ataxia-telangectasia [54], reducing the slow phase velocity of horizontal, vertical and pendular alternating nystagmus. Further studies including other clinical outcome measures are warranted.

8. Conclusion.

In sum, there is an increasing body of evidence demonstrating altered brain resting-activity in several ocular motor supranuclear disorders, reflecting possible compensatory or disruptive mechanisms. This provides further anatomical and physiological refinement of the neuronal circuit responsible for the generation of eye movements. Specific fixation and saccadic abnormalities found in neurodegenerative disorders warrant their use as diagnostic and treatment response markers in future therapeutic trials. Anecdotal reports demonstrating improvement of saccadic intrusions and nystagmus with memantine and 4-aminopyridine in patients with neurodegenerative disorders highlights the need for randomized controlled trials.

Key points.

▪ Microsaccades seem to be distinctively impaired in neurodegenerative disorders such as progressive supranuclear palsy, Parkinson´s disease and Alzheimer´s disease.

▪ Resting-state activity functional magnetic resonance shows changes in brain activity of patients with supranuclear eye movement disorders that may reflect compensatory or disruptive mechanisms.

▪ Functional visual impairment caused by supranuclear eye movement disorders needs further investigation and standardized assessement.

▪ Pharmacological treatment of central saccadic and vestibular disorders has shown promising results with the use of memantine and 4-aminopyridine.

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None.

Conflicts of interest.

The authors have no conflicts of interest related to this article and subject.

References.

1. Leigh RJ, Zee DS. The neurology of eye movements. Oxford University Press; 2006.

2. Jadhav AP, Zenonos G, Pless M, Jovin TG, Wechsler L. A variant of the anterior opercular syndrome with supranuclear gaze palsy. JAMA Neurol. 2013 Jun;70(6):800-1.

3. Stevens JC, Murphy SM, Davagnanam I, Phadke R, Anderson G, Nethisinghe S, et al. The ARSACS phenotype can include supranuclear gaze palsy and skin lipofuscin deposits. J Neurol Neurosurg Psychiatry. 2013 Jan;84(1):114-6.

4. Gooding DC, Basso MA. The tell-tale tasks: a review of saccadic research in psychiatric patient populations. Brain Cogn. 2008 Dec;68(3):371-90.

5. Hutton SB. Cognitive control of saccadic eye movements. Brain Cogn. 2008 Dec;68(3):327-40.

6. Dong W, Yan B, Johnson BP, Millist L, Davis S, Fielding J, et al. Ischaemic stroke: the ocular motor system as a sensitive marker for motor and cognitive recovery. J Neurol Neurosurg Psychiatry. 2013 Mar;84(3):337-41.

7. Reilly JL, Lencer R, Bishop JR, Keedy S, Sweeney JA. Pharmacological treatment effects on eye movement control. Brain Cogn. 2008 Dec;68(3):415-35.

8. Ettinger U, Kumari V, Crawford TJ, Davis RE, Sharma T, Corr PJ. Reliability of smooth pursuit, fixation, and saccadic eye movements. Psychophysiology. 2003 Jul;40(4):620-8.

9. Yugeta A, Hutchison WD, Hamani C, Saha U, Lozano AM, Hodaie M, et al. Modulation of Beta oscillations in the subthalamic nucleus with prosaccades and antisaccades in Parkinson's disease. J Neurosci. 2013 Apr 17;33(16):6895-904.

10. Isoda M, Hikosaka O. Role for subthalamic nucleus neurons in switching from automatic to controlled eye movement. J Neurosci. 2008 Jul 9;28(28):7209-18.

11. Gorges M, Muller HP, Lule D, Ludolph AC, Pinkhardt EH, Kassubek J. Functional connectivity within the default mode network is associated with saccadic accuracy in Parkinson's disease: a resting-state FMRI and videooculographic study. Brain Connect. 2013;3(3):265-72.

12. Alexandre MF, Rivaud-Pechoux S, Challe G, Durr A, Gaymard B. Functional consequences of oculomotor disorders in hereditary cerebellar ataxias. Cerebellum. 2013 Jun;12(3):396-405.

13. Matalia J, G JC, Brodsky MC. Positional vertical opsoclonus: a transient phenomenon in normal healthy twins. J AAPOS. 2013 Oct;17(5):537-8.

14. Yonekawa T, Saito Y, Sakuma H, Sugai K, Shimizu Y, Inagaki M, et al. Augmented startle responses in opsoclonus-myoclonus syndrome. Brain Dev. 2011 Apr;33(4):335-8.

15. Kitama T, Ohki Y, Shimazu H, Tanaka M, Yoshida K. Site of interaction between saccade signals and vestibular signals induced by head rotation in the alert cat: functional properties and afferent organization of burster-driving neurons. J Neurophysiol. 1995 Jul;74(1):273-87.

16. DeFelipe-Mimbrera A, Masjuan J, Corral I, Villar LM, Graus F, Garcia-Barragan N. Opsoclonus-myoclonus syndrome and limbic encephalitis associated with GABAB receptor antibodies in CSF. J Neuroimmunol. 2014 Jul 15;272(1-2):91-3.

17. Kruer MC, Hoeftberger R, Lim KY, Coryell JC, Svoboda MD, Woltjer RL, et al. Aggressive Course in Encephalitis With Opsoclonus, Ataxia, Chorea, and Seizures: The First Pediatric Case of gamma-Aminobutyric Acid Type B Receptor Autoimmunity. JAMA Neurol. 2014 Mar 3.

18. Wong AM, Musallam S, Tomlinson RD, Shannon P, Sharpe JA. Opsoclonus in three dimensions: oculographic, neuropathologic and modelling correlates. J Neurol Sci. 2001 Aug 15;189(1-2):71-81.

19. Lujan R, Shigemoto R. Localization of metabotropic GABA receptor subunits GABAB1 and GABAB2 relative to synaptic sites in the rat developing cerebellum. Eur J Neurosci. 2006 Mar;23(6):1479-90.

20. Gitchel GT, Wetzel PA, Baron MS. Slowed saccades and increased square wave jerks in essential tremor. Tremor Other Hyperkinet Mov (N Y). 2013;3.

21. Helmchen C, Hagenow A, Miesner J, Sprenger A, Rambold H, Wenzelburger R, et al. Eye movement abnormalities in essential tremor may indicate cerebellar dysfunction. Brain. 2003 Jun;126(Pt 6):1319-32.

22. White OB, Saint-Cyr JA, Tomlinson RD, Sharpe JA. Ocular motor deficits in Parkinson's disease. II. Control of the saccadic and smooth pursuit systems. Brain. 1983 Sep;106 (Pt 3):571-87.

23. Rucker JC, Shapiro BE, Han YH, Kumar AN, Garbutt S, Keller EL, et al. Neuro-ophthalmology of late-onset Tay-Sachs disease (LOTS). Neurology. 2004 Nov 23;63(10):1918-26.

24. Martinez-Conde S, Macknik SL, Hubel DH. The role of fixational eye movements in visual perception. Nat Rev Neurosci. 2004 Mar;5(3):229-40.

25. Otero-Millan J, Schneider R, Leigh RJ, Macknik SL, Martinez-Conde S. Saccades during attempted fixation in parkinsonian disorders and recessive ataxia: from microsaccades to square-wave jerks. PLoS One. 2013;8(3):e58535.

26. Kapoula Z, Yang Q, Otero-Millan J, Xiao S, Macknik SL, Lang A, et al. Distinctive features of microsaccades in Alzheimer's disease and in mild cognitive impairment. Age (Dordr). 2014 Apr;36(2):535-43.

27. Rosini F, Federighi P, Pretegiani E, Piu P, Leigh RJ, Serra A, et al. Ocular-motor profile and effects of memantine in a familial form of adult cerebellar ataxia with slow saccades and square wave saccadic intrusions. PLoS One. 2013;8(7):e69522.

28. Lee SH, Park SH, Kim JS, Kim HJ, Yunusov F, Zee DS. Isolated unilateral infarction of the cerebellar tonsil: ocular motor findings. Ann Neurol. 2014 Mar;75(3):429-34.

29. Park HK, Kim JS, Strupp M, Zee DS. Isolated floccular infarction: impaired vestibular responses to horizontal head impulse. J Neurol. 2013 Jun;260(6):1576-82.

30. Yoshida A, Tanaka M. Neuronal activity in the primate globus pallidus during smooth pursuit eye movements. Neuroreport. 2009 Jan 28;20(2):121-5.

31. Nilsson MH, Patel M, Rehncrona S, Magnusson M, Fransson PA. Subthalamic deep brain stimulation improves smooth pursuit and saccade performance in patients with Parkinson's disease. J Neuroeng Rehabil. 2013;10:33.

32. Pinkhardt EH, Jurgens R, Lule D, Heimrath J, Ludolph AC, Becker W, et al. Eye movement impairments in Parkinson's disease: possible role of extradopaminergic mechanisms. BMC Neurol. 2012;12:5.

33. Bockova M, Chladek J, Jurak P, Halamek J, Balaz M, Rektor I. Involvement of the subthalamic nucleus and globus pallidus internus in attention. J Neural Transm. 2011 Aug;118(8):1235-45.

34. Knapp CM, Proudlock FA, Gottlob I. OKN asymmetry in human subjects: a literature review. Strabismus. 2013 Mar;21(1):37-49.

35. Knapp CM, Gottlob I, McLean RJ, Rajabally YA, Abbott RJ, Rafelt S, et al. Vertical optokinetic nystagmus in Parkinson's disease. Mov Disord. 2009 Jul 30;24(10):1533-8.

36. Brodsky MC, Klaehn L. The optokinetic uncover test: a new insight into infantile esotropia. JAMA Ophthalmol. 2013 Jun;131(6):759-65.

37. Salman MS, Ikeda KM. The syndrome of infantile-onset saccade initiation delay. Can J Neurol Sci. 2013 Mar;40(2):235-40.

38. Harris CM, Taylor DS, Vellodi A. Ocular motor abnormalities in Gaucher disease. Neuropediatrics. 1999 Dec;30(6):289-93.

39. Salman MS, Ikeda KM. Do the Clinical Features in Infantile-Onset Saccade Initiation Delay (Congenital Ocular Motor Apraxia) Correlate With Brain Magnetic Resonance Imaging Findings? J Neuroophthalmol. 2014 Mar 19.

40. Judge SJ, Cumming BG. Neurons in the monkey midbrain with activity related to vergence eye movement and accommodation. J Neurophysiol. 1986 May;55(5):915-30.

41. Ramat S, Zee DS. Ocular motor responses to abrupt interaural head translation in normal humans. J Neurophysiol. 2003 Aug;90(2):887-902.

42. Kapoula Z, Gaertner C, Yang Q, Denise P, Toupet M. Vergence and Standing Balance in Subjects with Idiopathic Bilateral Loss of Vestibular Function. PLoS One. 2013;8(6):e66652.

43. Dieterich M, Bense S, Lutz S, Drzezga A, Stephan T, Bartenstein P, et al. Dominance for vestibular cortical function in the non-dominant hemisphere. Cereb Cortex. 2003 Sep;13(9):994-1007.

44. Bense S, Bartenstein P, Lutz S, Stephan T, Schwaiger M, Brandt T, et al. Three Determinants of Vestibular Hemispheric Dominance during Caloric Stimulation: A Positron Emission Tomography Study. Annals of the New York Academy of Sciences. 2003;1004(1):440-5.

45. Bense S, Stephan T, Yousry TA, Brandt T, Dieterich M. Multisensory cortical signal increases and decreases during vestibular galvanic stimulation (fMRI). J Neurophysiol. 2001 Feb;85(2):886-99.

46. von Brevern M, Zeise D, Neuhauser H, Clarke AH, Lempert T. Acute migrainous vertigo: clinical and oculographic findings. Brain. 2005 Feb;128(Pt 2):365-74.

47. Shin JH, Kim YK, Kim HJ, Kim JS. Altered brain metabolism in vestibular migraine: comparison of interictal and ictal findings. Cephalalgia. 2014 Jan;34(1):58-67.

48. Russo A, Marcelli V, Esposito F, Corvino V, Marcuccio L, Giannone A, et al. Abnormal thalamic function in patients with vestibular migraine. Neurology. 2014 May 9.

49. Yang TH, Oh SY, Kwak K, Lee JM, Shin BS, Jeong SK. Topology of brainstem lesions associated with subjective visual vertical tilt. Neurology. 2014 Jun 3;82(22):1968-75.

50. Zwergal A, Buttner-Ennever J, Brandt T, Strupp M. An ipsilateral vestibulothalamic tract adjacent to the medial lemniscus in humans. Brain. 2008 Nov;131(Pt 11):2928-35.

51. Baier B, Suchan J, Karnath HO, Dieterich M. Neural correlates of disturbed perception of verticality. Neurology. 2012 Mar 6;78(10):728-35.

52. Baier B, Conrad J, Zu Eulenburg P, Best C, Muller-Forell W, Birklein F, et al. Insular strokes cause no vestibular deficits. Stroke. 2014 Sep;44(9):2604-6.

53. Claassen J, Spiegel R, Kalla R, Faldon M, Kennard C, Danchaivijitr C, et al. A randomised double-blind, cross-over trial of 4-aminopyridine for downbeat nystagmus--effects on slowphase eye velocity, postural stability, locomotion and symptoms. J Neurol Neurosurg Psychiatry. 2013 Dec;84(12):1392-9.

54. Shaikh AG, Marti S, Tarnutzer AA, Palla A, Crawford TO, Zee DS, et al. Effects of 4-aminopyridine on nystagmus and vestibulo-ocular reflex in ataxia-telangiectasia. J Neurol. 2013 Nov;260(11):2728-35

Chapter III

Saccadic intrusions: review and update

Abstract.

Purpose of review. The aim of this work is to review saccadic intrusions, focusing on recent developments in their pathophysiology and treatment.

Recent findings. Saccadic intrusions have been evidenced for the first time in ataxia with oculomotor apraxia type 2 and neuromyelitis optica. Additionally, novel fixation instabilities with a presumed pathological substrate have been identified, like the "staircase" square wave jerks or the pervasive ocular microtremor seen in patients with Parkinson's disease. The study of fixational eye movements, previously narrowed to normal individuals is now starting to focus on neurological patients, one such example being their reported instability in Parkinson´s disease and progressive supranuclear palsy, whereby the boundaries between fixational eye movements and saccadic intrusions are becoming less clear. While accumulating evidence confirms a wide network underlying the mechanism of pathological square wave jerks, involving cerebral hemispheres, subcortical structures, brainstem and cerebellum, the debate regarding the pathogenesis of ocular flutter and opsoclonus continues, wherein cerebellar and brainstem pathological contributions are the two most plausible hypotheses. The cerebellar hypothesis better correlates with recent functional imaging findings, like the intense hypermetabolism in the deep cerebellar nuclei recently shown in a 18F-fluoro-2-deoxyglucose positron emission tomography in a case of ocular flutter. The brainstem hypothesis, through a theoretical neuromimetic model which tries to incorporate anatomic and physiological data, provides possible explanations for some of the therapeutic responses observed as well as for the existence of accompanying clinical signs, like myoclonus, startle and tremor. In the largest prospective study of corticotropin-based immunotherapies in children with opsoclonus-myoclonus syndrome, treatment combinations (3- or 4-agent) were shown to be more effective than corticotropin alone and clinical response to this agent was greater than for corticosteroid-based therapy.

Summary. Saccadic intrusions recognition can help in the diagnosis of neurological disease. We are gaining new insights about their pathogenesis, with the help of theoretical models, functional imaging and genetic approaches. The first large prospective studies on the rare opsoclonus-myoclonus syndrome are now beginning.

Keywords.

Saccadic intrusions Saccadic oscillations, Square wave jerks, Square wave pulses, Macro square wave jerks, Macrosaccadic oscillations, Saccadic pulses, Ocular flutter, Opsoclonus

1. Introduction.

Saccadic Intrusions (SI) are involuntary conjugate saccades (fast eye movements) that interrupt fixation [1]. Having a larger amplitude (usually >0,5º), SI may be further differentiated from miniature fixational eye movements (microsaccades, tremors and drifts), the latter believed to help visual perception during fixation, preventing visual adaptation [2] and correcting fixation [3], although this differentiation is progressively becoming tenuous [3-6]. SI are called intrusions due to their sporadic character, but when their occurrence is continuous, they should be considered oscillations (SO) [7]. Although SI are usually found in healthy individuals, they are also present in certain neurological disorders (usually manifesting higher frequency and amplitude), the latter reflecting dysfunction of brainstem, cerebellum, superior colliculus, basal ganglia and/or cerebral hemispheres [1]. **Figure 1** illustrates each type of Saccadic Intrusion. Novel fixation instabilities have been reported [8-13] and recent attempts to tackle SI terminology and phenomenology have been made as well [4, 14]. We may distinguish two groups of SI by the presence or absence of an intersaccadic interval (ISI), a latent period that usually lasts 180 to 200-milisseconds between sequential saccades.

Below we review each type of Saccadic Intrusion, focusing on recent developments and subsequently we present an update to the treatment of these disorders.

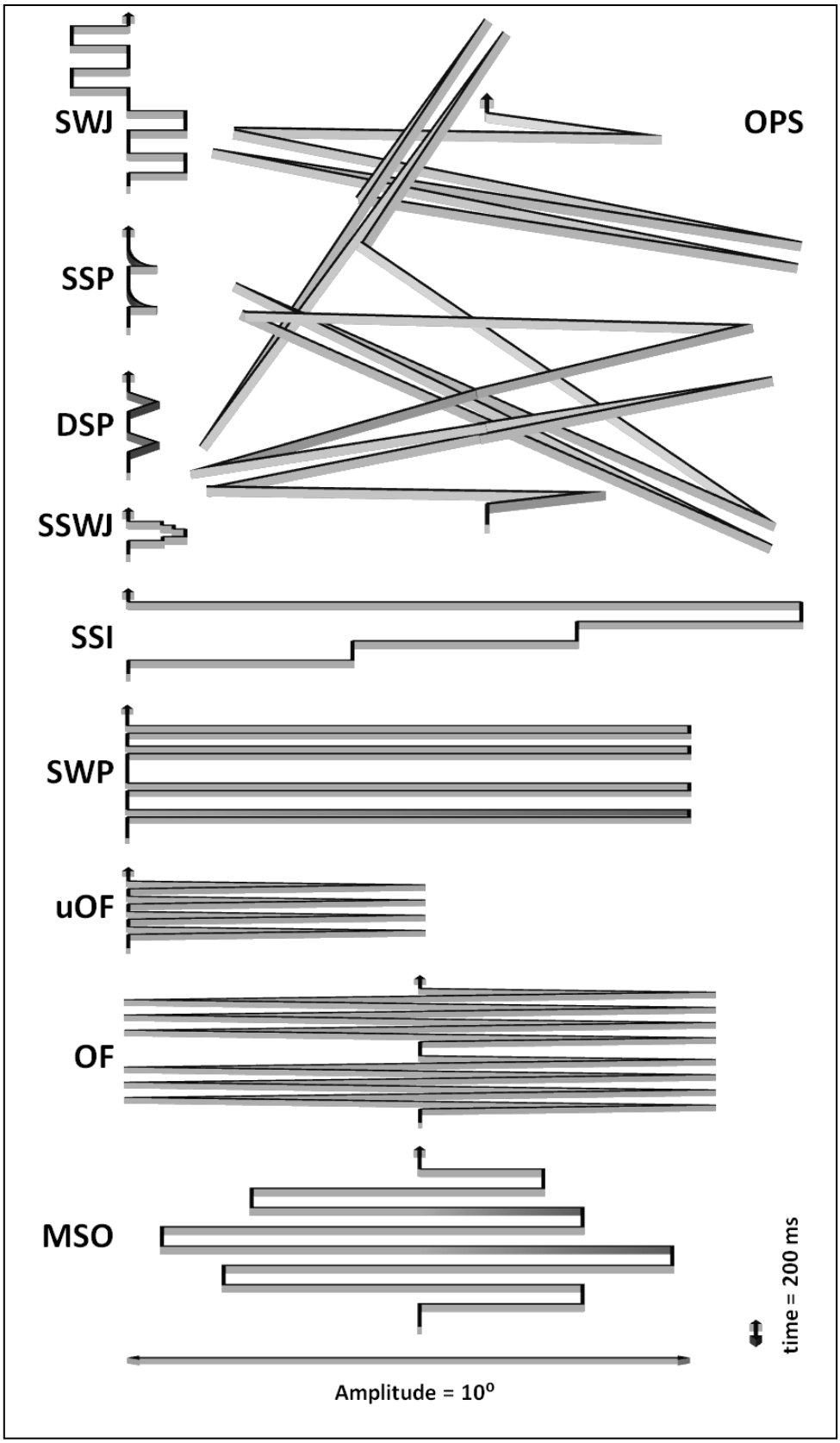

Figure 1. Schematic illustrations of saccadic intrusions: square wave jerk (SWJ), single saccadic pulse (SSP), double saccadic pulse (DSP), staircase square wave jerk (SSWJ), staircase saccadic intrusion (SSI), square wave pulse (SWP), unidirectional ocular flutter (uOF), ocular flutter (OF), macrosaccadic oscillations (MSO), opsoclonus (OPS).

2. Saccadic intrusions with normal intersaccadic intervals.

2.1. Square wave jerks (SWJ).

Square wave jerks are the most common type of SI [4] and consist in small conjugate couplets of horizontal back-to-back saccades ranging from 0.5^0 to 5^0 which take the eye from the fixation point and then return it after a period of about 200 msec (**Fig. 1**) [1]. Oscillopsia is not a common feature in SWJ [1]. Frequent SWJ, also called square wave oscillations (>9-16/min or >20/min in the dark) [15, 16], larger than 5^0, multiplanar and disconjugate [4] should alert the physician to the presence of a neurological disease, being a common finding in Huntington's disease [17, 18], Progressive supranuclear palsy (PSP) [3, 19], Friedereich's ataxia (FA) [20-22] and cerebral hemispheric disease [23, 24]. SWJ have recently been evidenced in a few other diseases, either for the first time [25-27] or in a more quantitative and thorough manner [20, 24].

Although some studies have suggested that the rate of SWJ increases in Parkinson's disease (PD) patients, compared to controls [19, 28-30], others disagree with this finding, claiming that in fact, one of the reasons for subjective deficits in ocular fixation of PD patients may be related to persistent ocular tremor, targeting this small-amplitude oscillation as a potential physiological biomarker for the diagnosis of PD [9]. Challenging the traditional view that a SWJ amplitude higher than 1^0 is characteristically more frequent in PSP and Multi-system atrophy (MSA) or Parkinson-plus syndrome than in PD, Shaikh and colleagues recently provided evidence of large SWJ (mean amplitude 2^0) in a small group of patients with early PD [8]. Besides replicating results from studies that show more frequent [29, 30] and larger [9, 30] SWJ in PSP, Otero-Millan and colleagues have elegantly shown that abnormally large microssacades lacking their vertical component were the best distinguishing feature between PSP patients and controls, reinforcing the role of miniature fixational eye movements as possible biomarkers [3].

The simultaneous occurrence of SWJ and downbeat nystagmus ("bow tie" nystagmus) in patients that share similar cerebellar cortical pathology, namely spinocerebellar ataxia type 6 (SCA 6) and familial cortical myoclonic tremor with epilepsy (FCMTE) [31], along with first time evidence showing an increased number of SWJ in patients with ataxia with ocular apraxia type 2 (AOA 2) [25] and the increase in the SWJ rate and amplitude in a case of Langerhans hystiocitosis [27], further stresses the role of cerebellum in the generation of SWJ.

According to Donaghy and colleagues, the larger the amplitude of SI, the more pronounced the impairment in measures of frontal lobe dysfunction in motor neuron disease patients supporting previous evidence that SI can also arise from the involvement of frontal-collicular pathways [24]. Similarly, in children and adolescents with Arnold Chiari type 2 malformation, the duration of SWJ correlated with the number of shunt revisions in those patients who underwent surgery for hydrocephalus [32].

The underlying mechanism of pathological SWJ is still unclear. Whether they represent a dysfunctional inhibitory system (basal ganglia, cerebellum, cerebral hemispheres or superior colliculus) which is no longer suppressing unwanted saccades by reinforcing omnipause neurons (OPN) inhibition [33], are a larger variant of fixational eye movements such as microssacades [3, 5, 6, 34-37]), or constitute a consequence of attentional shifts superimposing their influence on a normal saccadic system, possibly by raising neural activity on superior colliculus (SC) [6, 38, 39], these hypothesis are not mutually exclusive.

2.2. Square wave pulses (SWP).

Formerly known as macro square wave jerks [40], square wave pulses are similar to SWJ in their morphology and conjugacy, but they usually oscillate on one side of fixation [7] and have a distinctive shorter ISI (about 80 msec) (**Fig. 1**) [1]. Constituing a rare type SI, they may be seen in Multiple sclerosis (MS), where they might be associated with a specific visual handicap [40, 41], PSP [42] and MSA [43], and contrary to SWJ, they are not seen in healthy individuals. They probably reflect an anomalous input from SC or fastigial nucleus (FN) to OPN and/or a disorder of GABA-mediated synaptic inhibition from Substantia Nigra pars reticulate (SNpr) to SC [43, 44].

2.3. Macrosaccadic oscillations (MSO).

Macrosaccadic oscillations share a similar ISI duration with SWJ, but unlike these, they occur in bursts of conjugate and mainly horizontal saccades that increase and then damp in amplitude, oscillating around a fixation point (**Fig 1**) [1]. Midline cerebellar disease affecting FN is among the most frequent causes of MSO [45, 46]. Brainstem lesions can also promote MSO their possible mechanism being dysfunction of the afferent pathways to OPN, originated either in SC or FN [47, 48]. Can microssacades also trigger MSO, especially during fixation [2, 46], or in alternative could the disruption of cerebellar mossy fibers hypothesized in Spinocerebellar ataxia with saccadic intrusions (SCASI) patients reduce inihibition on the deep nuclei, causing MSO while fixating [49]? These are issues that remain unsolved.

2.4. Saccadic pulses (SP).

Saccadic pulses are brief saccadic intrusions that take the eye from the fixation point, being immediately followed by a slow glissadic drift that returns them to the previous position (**Fig. 1**) [1]. They may be single (SSP) or double (DSP), the former being in fact a pair of back-to-back saccades without an ISI [1]. SP may occur in runs or as doublets, usually in pathological states like MS [50, 51] and post-traumatic lesions [47].

SP might result from lack of eye position error feedback, damage to neural integrator structures and impaired supranuclear control of omnipause cells [50].

3. Saccadic intrusions without normal intersaccadic intervals.

3.1. Ocular flutter and opsoclonus (OF and OPS).

Ocular flutter consists of back-to-back horizontal conjugate saccades without an ISI, limited to one plane (usually horizontal), their amplitude ranging from 1^0 to 5^0 and their rate reaching 10-25 Hz (**Fig. 1**) [1]. Rarely it can be unidirectional [13] or positional [12]. Opsoclonus shares the same properties as OF, with the exception of having multi-directional saccades of varying amplitudes (**Fig. 1**), being more frequently continuous, and being usually accompanied by myoclonus (nonepileptic involuntary jerks of the limbs and trunk) - hence the term "opsoclonus-myoclonus syndrome" (OMS), ataxia and encephalopathy [1]. Contrary to the SI discussed so far, OF and OPS often cause oscillopsia and blurred vision [7, 33, 52-55]. OPS and/or OF have been reported for the first time in a presumed case of Neuromyelitis Optica [56], Hepatitis C infection [57], Krabbe's disease [12], Amyotrophic lateral sclerosis [58] and locked-in syndrome [59].

The differential diagnosis of OF and OPS includes parainfectious brainstem encephalitis, metabolic-toxic states, demyelinating diseases, inherited disorders, paraneoplastic conditions (neuroblastoma in children is the primary consideration whereas in adults, small cell lung carcinoma, breast carcinoma, or ovarian carcinoma is the most common primary consideration), and in many cases the cause remains unknown. In the last two clinical settings (paraneoplastic and idiopathic), OF and OPS are probably mediated by humoral heterogeneous immune mechanisms [60] and/or B and T-cell immune mechanisms [61]. Patients with paraneoplastic OMS are often seronegative for anti-neuronal antibodies, except when OMS is associated with anti-RI and anti-amphiphysin antibodies [62, 63]. Nevertheless, strengthening the autoimmune hypothesis for OMS, newly associations have been reported between OMS and anti–N-methyl-D-aspartate receptor (anti-NMDAR) antibodies [64], anti-ganglioside Q1b antibodies [65] and autoantibodies to glutamic acid decarboxylase (anti-GAD) [66], the latter association for the first time on an adult patient.

Regarding biological markers of OMS, in a prospective case-control study of 132 children, early in 2011, Pranzatelli et al. have observed that 35% of OMS patients had cerebrospinal fluid (CSF) oligoclonal bands (OCB), with higher frequency in severe cases (56%). However, the presence of OCB did not correlate with previous or subsequent relapses, OMS duration or neuroblastoma detection, reason why the authors concluded that OCB should not be a stand-alone biomarker in opsoclonus-myoclonus syndrome [67]. B cell-attracting CXCL13, an inflammatory chemokine, may as well became a biomarker of disease activity and treatment response, as recently shown in a prospective, case–control study enrolling 289 symptomatic OMS patients. CSF

CXCL13 concentration was 16.5-fold higher in untreated OMS than controls, relating directly to OMS severity and inversely to OMS duration [68].

The prognosis of children with OMS is poor, either with or without neuroblastoma, and about 80% of them are reported to have neurological sequelae [69, 70]. A recent retrospective study of 101 patients has shown that very young children at onset of the disease and with severe initial symptoms, seem to ultimately predict a chronic-relapsing disease course, and neurobehavioral sequelae [71]. Contrary to children, idiopathic OMS in adults is usually monophasic and patients usually make a good recovery. Meanwhile adults with paraneoplastic OMS are usually older than the idiopathic group, and display a worse outcome, especially if they don't receive antineoplastic therapy [63].

Saccadic oscillations pathophysiology has been a matter of dispute. There are two main theories: the brainstem and the cerebellar theory. The first theory states that saccadic oscillations are presumed to primarily arise from alterations in the membrane properties of saccadic burst neurons which makes them prone to excessive post-inhibitory rebound (PIR) excitation after sustained inhibition from OPN or alternatively from the malfunction of glycine receptors causing a reduction on efficacy of OPN inhibition [72]. Therefore, either an increase in neuronal excitability or a reduction of OPN inhibition can cause instability or oscillations [72-74]. This theory, with the help of a neuromimetic model, has recently become the basis to explain: (i) a rare, presumably genetic inherited disorder evidencing microsaccadic oscillations accompanying limb tremor (mSOLT) [53] (ii) patients with ataxia-telangectasia showing saccadic intrusions/oscillations and limb tremor [75] (iii) the presence of multi-directional micro-saccadic oscillations in a patient with Still 's disease [54] (iv) marked SO in a patient with a previous surgical resection of the fastigial nucleus [73] (v) pathologies featuring limb tremor like essential tremor [76] and cervical dystonia [77] (vi) the co-existence not only of myoclonus, but also of an accompanying exaggerated startle response in a reported patient with OMS [78]. One disappointing result however, was recently described by Takahiro Iizuka et al., where they tried to determine whether glycine receptor (GlyR) antibodies were found in patients with OPS and OF. None of the 13 patients had significant levels of (GlyR) antibodies, further suggesting that there must be other antibodies or immune factors involved in this condition [79].

The cerebellar theory relies on a different model based on dysfunctional cerebellar Purkinje cells no longer capable of exerting their inhibitory influence on the fastigial nucleus, this way reinforcing omnipause neurons inhibition by this nucleus, leaving saccadic burst neurons free to oscillate [80]. This has been corroborated by recent case reports of OPS showing evidence of dysfunctional cerebellar Purkinje cells by using single photon emission computed tomography [81] or functional magnetic resonance imaging [82]. Similarly, OF has recently been shown to correlate with hypermetabolism of deep cerebellar nuclei in a 18F-fluoro-2-deoxyglucose positron emission tomography [83]. Interestingly, in 2012, the first compound heterozygous missense mutation and a large deletion in the KCTD7 gene were reported in a patient with a clinical overlap of

OMS and progressive myoclonic epilepsy (PME), reinforcing the role of cerebellum in the pathophysiology of saccadic oscillations [80, 84].

4. Treatment of Saccadic intrusions (and oscillations).

SWJ are rarely symptomatic, usually not requiring further treatment. There are nonetheless single case reports claiming positive results with DBS [11], diazepam, clonazepam, phenobarbital or valproate [44, 85], by eventually restoring action of the GABAergic system tonic inhibitory system from SN to SC. In contrast, patients with MSO might benefit from treatment. Besides medications possibly acting on the already mentioned GABA system [44], a few other case reports have shown partially positive results with gabapentin [1] and memantine [49].

Symptomatic treatment of OPS and OF has also been reported so far as a single case reports, lacking prospective multicenter trials. Substances like propanolol, clonazepam, gabapentin, topiramate, levetiracetam and ethosuximide have been reported to abate OPS or OF, possibly by enhancing GABAergic transmission of Purkinje cells over the fastigial nucleus or by blocking the membranal T-type calcium channel on saccadic burst neurons [52, 59, 74, 86-89].

Specific etiological treatment depends largely on the cause and patient's age. In a recent prospective, exploratory, rater-blinded, active comparator-controlled study of corticotropin-based immunotherapies in 74 children with OMS, treatment combinations (3- or 4-agent) were shown to be more effective than corticotropin alone and response to this agent was greater than for oral steroid therapy alone. Importantly, a greater decline of disease severity was evidenced when corticotrophin was initiated earlier, underlining the importance of a timely treatment. However, serious adverse events were reported in 10% of the patients [90]. Rituximab has currently become one of the most promising agent in treating children OMS, after accumulating evidence demonstrating that as an adjunctive therapy, it depletes CSF B cells, lessens motor severity sustainedly and lowers the relapse rate [91, 92]. Ofatumumab, a second-generation fully humanized anti-CD20 biological antibody, might become a choice for rituximab-allergic children with severe OMS, as evidenced in a very recent report [93].

In adults with the idiopathic form, while steroids, Intravenous immunoglobulin (IVIG) and azathioprin seem to accelerate recovery [63], plasmapheresis has been just occasionally related with improvement [94]. In parainfectious cases immunotherapy may be added to the antibiotic treatment [95]. In the paraneoplastic form, contrary to children, treatment of the tumor seems to be the cornerstone for neurological recovery [63]. The use of immunotherapy might be considered, according to anecdotal case report results with protein A column immunoadsorption therapy [96], steroids [86, 97, 98] or IVIG [63].

5. Comments.

Ocular fixation assessment constitutes a very promising tool in neurology. Future studies on ocular fixation should use precise oculomotor testing, incorporating in their analysis all types of saccadic intrusions, fixational eye movements and parameters such as fixation periods and displacements.

Although radiological and pathological evidence favors a cerebellar origin of OPS and OF, the neuromimetic model proposed as an alternative, better endorses accompanying features such as myoclonus, startle or tremor. Furthermore, it would be of extreme importance to create similar models, which could be applied to other types of saccadic intrusions, integrating for example, basal ganglia neurophysiological properties in the suggested basal ganglia network controlling eye moments.

While CSF B cell, probably reflecting cellular immunity impairment, has become a biomarker of disease severity in children OMS, further studies in both children and adults are necessary to find specific antibodies and autoantigens that reflect disturbed humoral immunity. Prospective multi-centre trials are required in order to evaluate the efficacy of new treatments for opsoclonus-myoclonus syndrome. Studies on long term efficacy of rituximab, focusing on late neurological sequelae and the use of this agent as monotherapy are needed.

Key points.

▪ Saccadic intrusions can be found in normal asymptomatic individuals.

▪ Symptomatic saccadic intrusions almost always imply the presence of neurologic dysfunction.

▪ Saccadic intrusions remain an understudied but informative component of several ocular motility syndromes.

▪ The generation of common saccadic intrusions such as square wave jerks appears to involve widespread functional networks of neural tissue.

▪ Opsoclonus and ocular flutter may relate to brainstem-based, or cerebellar-based theories of dysfunction; each theory helps explain certain components of the observed clinical syndromes, and it is possible that both are applicable in individual circumstances.

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None.

Conflicts of interest.

The authors have no conflicts of interest related to this article and subject.

References.

1. Leigh, R.J. and D.S. Zee, The neurology of eye movements. Contemporary neurology series 70, 2006: p. x, 762 p.

2. Martinez-Conde, S., S.L. Macknik, and D.H. Hubel, The role of fixational eye movements in visual perception. Nat Rev Neurosci, 2004. 5(3): p. 229-40.

3. Otero-Millan, J., et al., Distinctive features of saccadic intrusions and microsaccades in progressive supranuclear palsy. J Neurosci, 2011. 31(12): p. 4379-87.

4. Abadi, R.V. and E. Gowen, Characteristics of saccadic intrusions. Vision Res, 2004. 44(23): p. 2675-90.

5. Feldon, S.E. and J.W. Langston, Square-wave jerks: a disorder of microsaccades? Neurology, 1977. 27(3): p. 278-81.

6. Gowen, E., et al., Modulation of saccadic intrusions by exogenous and endogenous attention. Brain Res, 2007. 1141: p. 154-67.

7. Sharpe, J.A. and W.A. Fletcher, Saccadic intrusions and oscillations. Can J Neurol Sci, 1984. 11(4): p. 426-33.

8. Shaikh, A.G., et al., 'Staircase' square-wave jerks in early Parkinson's disease. Br J Ophthalmol, 2011. 95(5): p. 705-9.

9. Gitchel, G.T., P.A. Wetzel, and M.S. Baron, Pervasive Ocular Tremor in Patients With Parkinson Disease. Arch Neurol, 2012.

10. Rucker, J.C., et al., "Staircase" saccadic intrusions plus transient yoking and neural integrator failure associated with cerebellar hypoplasia: a model simulation. Semin Ophthalmol, 2006. 21(4): p. 229-43.

11. Wark, H.A., et al., A case report on fixation instability in Parkinson's disease with bilateral deep brain stimulation implants. J Neurol Neurosurg Psychiatry, 2008. 79(4): p. 443-7.

12. Brodsky, M.C. and J.S. Hunter, Positional ocular flutter and thickened optic nerves as sentinel signs of Krabbe disease. J AAPOS, 2011. 15(6): p. 595-7.

13. Verhaeghe, S., et al., Unidirectional ocular flutter. J Neurol Neurosurg Psychiatry, 2007. 78(7): p. 764-6.

14. Canice McGivern, R. and J. Mark Gibson, Characterisation of ocular fixation in humans by analysis of saccadic intrusions and fixation periods: a pragmatic approach. Vision Res, 2006. 46(21): p. 3741-7.

15. Herishanu, Y.O. and J.A. Sharpe, Normal square wave jerks. Invest Ophthalmol Vis Sci, 1981. 20(2): p. 268-72.

16. Shallo-Hoffmann, J., J. Petersen, and H. Muhlendyck, How normal are "normal" square wave jerks? Invest Ophthalmol Vis Sci, 1989. 30(5): p. 1009-11.

17. Leigh, R.J., et al., Abnormal ocular motor control in Huntington's disease. Neurology, 1983. 33(10): p. 1268-75.

18. Blekher, T., et al., Saccades in presymptomatic and early stages of Huntington disease. Neurology, 2006. 67(3): p. 394-9.

19. Altiparmak, U.E., et al., The ratio of square wave jerk rates to blink rates distinguishes progressive supranuclear palsy from Parkinson disease. J Neuroophthalmol, 2006. 26(4): p. 257-9.

20. Fahey, M.C., et al., Vestibular, saccadic and fixation abnormalities in genetically confirmed Friedreich ataxia. Brain, 2008. 131(Pt 4): p. 1035-45.

21. Ribai, P., et al., Neurological, cardiological, and oculomotor progression in 104 patients with Friedreich ataxia during long-term follow-up. Arch Neurol, 2007. 64(4): p. 558-64.

22. Spieker, S., et al., Fixation instability and oculomotor abnormalities in Friedreich's ataxia. J Neurol, 1995. 242(8): p. 517-21.

23. Sharpe, J.A., Y.O. Herishanu, and O.B. White, Cerebral square wave jerks. Neurology, 1982. 32(1): p. 57-62.

24. Donaghy, C., et al., Ocular fixation instabilities in motor neurone disease. A marker of frontal lobe dysfunction? J Neurol, 2009. 256(3): p. 420-6.

25. Clausi, S., et al., Oculomotor deficits affect neuropsychological performance in oculomotor apraxia type 2. Cortex, 2012.

26. Gradstein L, D.A., Grafman J, Fitzgibbon EJ., Eye movements in chorea-acanthocytosis. Invest Ophthalmol Vis Sci., 2005. 46(6): p. 1979-87.

27. Anagnostou, E., et al., Square-wave jerks and smooth pursuit impairment as subtle early signs of brain involvement in Langerhans' cell histiocytosis. Clin Neurol Neurosurg, 2008. 110(3): p. 286-90.

28. White, O.B., et al., Ocular motor deficits in Parkinson's disease. II. Control of the saccadic and smooth pursuit systems. Brain, 1983. 106 (Pt 3): p. 571-87.

29. Rascol, O., et al., Square wave jerks in parkinsonian syndromes. J Neurol Neurosurg Psychiatry, 1991. 54(7): p. 599-602.

30. Pinnock, R.A., et al., An exploration of ocular fixation in Parkinson's disease, multiple system atrophy and progressive supranuclear palsy. J Neurol, 2010. 257(4): p. 533-9.

31. Bour, L.J., et al., Oculomotor abnormalities in myoclonic tremor: a comparison with spinocerebellar ataxia type 6. Brain, 2008. 131(Pt 9): p. 2295-303.

32. Salman, M.S., et al., Visual fixation in Chiari type II malformation. J Child Neurol, 2009. 24(2): p. 161-5.

33. Zee, D.S. and D.A. Robinson, A hypothetical explanation of saccadic oscillations. Ann Neurol, 1979. 5(5): p. 405-14.

34. Hafed, Z.M. and J.J. Clark, Microsaccades as an overt measure of covert attention shifts. Vision Res, 2002. 42(22): p. 2533-45.

35. Hafed, Z.M., Mechanisms for generating and compensating for the smallest possible saccades. Eur J Neurosci, 2011. 33(11): p. 2101-13.

36. Otero-Millan, J., et al., Triggering mechanisms in microsaccade and saccade generation: a novel proposal. Ann N Y Acad Sci, 2011. 1233: p. 107-16.

37. Rolfs, M., J. Laubrock, and R. Kliegl, Shortening and prolongation of saccade latencies following microsaccades. Exp Brain Res, 2006. 169(3): p. 369-76.

38. Gowen, E. and R.V. Abadi, Saccadic instabilities and voluntary saccadic behaviour. Exp Brain Res, 2005. 164(1): p. 29-40.

39. Shaffer, D.M., C.M. Krisky, and J.A. Sweeney, Frequency and metrics of square-wave jerks: influences of task-demand characteristics. Invest Ophthalmol Vis Sci, 2003. 44(3): p. 1082-7.

40. Dell'Osso, L.F., B.T. Troost, and R.B. Daroff, Macro square wave jerks. Neurology, 1975. 25(10): p. 975-9.

41. Tilikete, C., et al., Persistent ocular motor manifestations and related visual consequences in multiple sclerosis. Ann N Y Acad Sci, 2011. 1233: p. 327-34.

42. Tetrud, J.W., et al., Autopsy-proven progressive supranuclear palsy in two siblings. Neurology, 1996. 46(4): p. 931-4.

43. Klotz, L. and T. Klockgether, Multiple system atrophy with macrosquare-wave jerks. Mov Disord, 2005. 20(2): p. 253-4.

44. Fukazawa, T., et al., Multisystem degeneration: drugs and square wave jerks. Neurology, 1986. 36(9): p. 1230-3.

45. Selhorst, J.B., et al., Disorders in cerebellar ocular motor control. II. Macrosaccadic oscillation. An oculographic, control system and clinico-anatomical analysis. Brain, 1976. 99(3): p. 509-22.

46. Swartz, B.E., et al., Pathogenesis of clinical signs in recessive ataxia with saccadic intrusions. Ann Neurol, 2003. 54(6): p. 824-8.

47. Kim, J.S., et al., Double saccadic pulses and macrosaccadic oscillations from a focal brainstem lesion. J Neurol Sci, 2007. 263(1-2): p. 118-23.

48. Averbuch-Heller, L., et al., Dysfunction of pontine omnipause neurons causes impaired fixation: macrosaccadic oscillations with a unilateral pontine lesion. Neuroophthalmology, 1996. 16(2): p. 99-106.

49. Serra, A., et al., Suppression of saccadic intrusions in hereditary ataxia by memantine. Neurology, 2008. 70(10): p. 810-2.

50. Herishanu, Y.O. and J.A. Sharpe, Saccadic intrusions in internuclear ophthalmoplegia. Ann Neurol, 1983. 14(1): p. 67-72.

51. Doslak, M.J.D.o., L. F.; Daroff, R. B., Multiple double saccadic pulses occurring with other saccadic intrusions and oscillations. Neuro-Ophthalmology, 1983. Volume 3(2): p. 109-116.

52. Ashe, J., et al., Microsaccadic flutter. Brain, 1991. 114 (Pt 1B): p. 461-72.

53. Shaikh, A.G., et al., A new familial disease of saccadic oscillations and limb tremor provides clues to mechanisms of common tremor disorders. Brain, 2007. 130(Pt 11): p. 3020-31.

54. Shaikh, A.G., T.C. Hain, and D.S. Zee, Oculomotor disorders in adult-onset Still's disease. J Neurol, 2010. 257(1): p. 136-8.

55. Foroozan, R. and M.C. Brodsky, Microsaccadic opsoclonus: an idiopathic cause of oscillopsia and episodic blurred vision. Am J Ophthalmol, 2004. 138(6): p. 1053-4.

56. Hage, R., Jr., et al., Ocular oscillations in the neuromyelitis optica spectrum. J Neuroophthalmol, 2011. 31(3): p. 255-9.

57. Ertekin, V. and H. Tan, Opsoclonus-myoclonus syndrome attributable to hepatitis C infection. Pediatr Neurol, 2010. 42(6): p. 441-2.

58. Balaratnam, M.S., et al., Amyotrophic lateral sclerosis and ocular flutter. Amyotroph Lateral Scler, 2010. 11(3): p. 331-4.

59. Pistoia, F., M. Conson, and M. Sara, Opsoclonus-myoclonus syndrome in patients with locked-in syndrome: a therapeutic porthole with gabapentin. Mayo Clin Proc, 2010. 85(6): p. 527-31.

60. Bataller, L., et al., Autoantigen diversity in the opsoclonus-myoclonus syndrome. Ann Neurol, 2003. 53(3): p. 347-53.

61. Pranzatelli, M.R., et al., B- and T-cell markers in opsoclonus-myoclonus syndrome: immunophenotyping of CSF lymphocytes. Neurology, 2004. 62(9): p. 1526-32.

62. Ko, M.W., J. Dalmau, and S.L. Galetta, Neuro-ophthalmologic manifestations of paraneoplastic syndromes. J Neuroophthalmol, 2008. 28(1): p. 58-68.

63. Bataller, L., et al., Clinical outcome in adult onset idiopathic or paraneoplastic opsoclonus-myoclonus. Brain, 2001. 124(Pt 2): p. 437-43.

64. Kurian, M., et al., Opsoclonus-myoclonus syndrome in anti-N-methyl-D-aspartate receptor encephalitis. Arch Neurol, 2010. 67(1): p. 118-21.

65. Zaro-Weber, O., et al., Ocular flutter, generalized myoclonus, and trunk ataxia associated with anti-GQ1b antibodies. Arch Neurol, 2008. 65(5): p. 659-61.

66. Markakis, I., et al., Opsoclonus-myoclonus-ataxia syndrome with autoantibodies to glutamic acid decarboxylase. Clin Neurol Neurosurg, 2008. 110(6): p. 619-21.

67. Pranzatelli, M.R., et al., Cerebrospinal fluid oligoclonal bands in childhood opsoclonus-myoclonus. Pediatr Neurol, 2011. 45(1): p. 27-33.

68. Pranzatelli, M.R., et al., Key role of CXCL13/CXCR5 axis for cerebrospinal fluid B cell recruitment in pediatric OMS. J Neuroimmunol, 2012. 243(1-2): p. 81-8.

69. Gorman, M.P., Update on diagnosis, treatment, and prognosis in opsoclonus-myoclonus-ataxia syndrome. Curr Opin Pediatr, 2010. 22(6): p. 745-50.

70. De Grandis, E., et al., Long-term follow-up of neuroblastoma-associated opsoclonus-myoclonus-ataxia syndrome. Neuropediatrics, 2009. 40(3): p. 103-11.

71. Brunklaus, A., et al., Outcome and prognostic features in opsoclonus-myoclonus syndrome from infancy to adult life. Pediatrics, 2011. 128(2): p. e388-94.

72. Shaikh, A.G., et al., Saccadic burst cell membrane dysfunction is responsible for saccadic oscillations. J Neuroophthalmol, 2008. 28(4): p. 329-36.

73. Ramat, S., et al., Ocular oscillations generated by coupling of brainstem excitatory and inhibitory saccadic burst neurons. Exp Brain Res, 2005. 160(1): p. 89-106.

74. Ramat, S., et al., What clinical disorders tell us about the neural control of saccadic eye movements. Brain, 2007. 130(Pt 1): p. 10-35.

75. Shaikh, A.G., et al., Gaze fixation deficits and their implication in ataxia-telangiectasia. J Neurol Neurosurg Psychiatry, 2009. 80(8): p. 858-64.

76. Shaikh, A.G., et al., Hypothetical membrane mechanisms in essential tremor. J Transl Med, 2008. 6: p. 68.

77. Shaikh, A.G., et al., Irregularity distinguishes limb tremor in cervical dystonia from essential tremor. J Neurol Neurosurg Psychiatry, 2008. 79(2): p. 187-9.

78. Yonekawa, T., et al., Augmented startle responses in opsoclonus-myoclonus syndrome. Brain Dev, 2011. 33(4): p. 335-8.

79. Iizuka, T., et al., Glycine receptor antibodies are detected in progressive encephalomyelitis with rigidity and myoclonus (PERM) but not in saccadic oscillations. J Neurol, 2012.

80. Wong, A.M., et al., Opsoclonus in three dimensions: oculographic, neuropathologic and modelling correlates. J Neurol Sci, 2001. 189(1-2): p. 71-81.

81. van Toorn, R., H. Rabie, and J.M. Warwick, Opsoclonus-myoclonus in an HIV-infected child on antiretroviral therapy--possible immune reconstitution inflammatory syndrome. Eur J Paediatr Neurol, 2005. 9(6): p. 423-6.

82. Helmchen, C., et al., Cerebellar activation in opsoclonus: an fMRI study. Neurology, 2003. 61(3): p. 412-5.

83. Newey, C.R., A. Sarwal, and G. Wu, Radiological Correlate of Ocular Flutter in a Case with Paraneoplastic Encephalitis. J Neuroimaging, 2011.

84. Blumkin, L., et al., A compound heterozygous missense mutation and a large deletion in the KCTD7 gene presenting as an opsoclonus-myoclonus ataxia-like syndrome. J Neurol, 2012.

85. Traccis, S., et al., Square-wave jerks and square-wave oscillations: treatment with valproic acid. Neuro-ophthalmology, 1997. 18(2): p. 51-58.

86. Pranzatelli, M.R., The neurobiology of the opsoclonus-myoclonus syndrome. Clin Neuropharmacol, 1992. 15(3): p. 186-228.

87. Fernandes, T.D., et al., Topiramate effect in opsoclonus-myoclonus-ataxia syndrome. Arch Neurol, 2012. 69(1): p. 133.

88. Eggenberger, E. and V. Cherian, Levetiracetam Effect in Demyelinating Ocular Flutter with Myoclonus. Neuro-Ophthalmology, 2006. 30(4): p. 105-108.

89. Shaikh, A.G., et al., The effects of ion channel blockers validate the conductance-based model of saccadic oscillations. Ann N Y Acad Sci, 2011. 1233: p. 58-63.

90. Tate, E.D., et al., Active Comparator-Controlled, Rater-Blinded Study of Corticotropin-Based Immunotherapies for Opsoclonus-Myoclonus Syndrome. J Child Neurol, 2012.

91. Pranzatelli, M.R., et al., B cell depletion therapy for new-onset opsoclonus-myoclonus. Mov Disord, 2010. 25(2): p. 238-42.

92. Pranzatelli, M.R., et al., Rituximab (anti-CD20) adjunctive therapy for opsoclonus-myoclonus syndrome. J Pediatr Hematol Oncol, 2006. 28(9): p. 585-93.

93. Pranzatelli, M.R., et al., Ofatumumab for a rituximab-allergic child with chronic-relapsing paraneoplastic opsoclonus-myoclonus. Pediatr Blood Cancer, 2012. 58(6): p. 988-91.

94. Smith, J.H., et al., N-methyl-D-aspartate receptor autoimmune encephalitis presenting with opsoclonus-myoclonus: treatment response to plasmapheresis. Arch Neurol, 2011. 68(8): p. 1069-72.

95. Nunes, J.C., et al., Opsoclonus-myoclonus syndrome associated with Mycoplasma pneumoniae infection in an elderly patient. J Neurol Sci, 2011. 305(1-2): p. 147-8.

96. Cher, L.M., et al., Therapy for paraneoplastic neurologic syndromes in six patients with protein A column immunoadsorption. Cancer, 1995. 75(7): p. 1678-83.

97. Wirtz, P.W., et al., Anti-Ri antibody positive opsoclonus-myoclonus in a male patient with breast carcinoma. J Neurol, 2002. 249(12): p. 1710-2.

98. Groiss, S.J., M. Siebler, and A. Schnitzler, Full recovery of adult onset opsoclonus myoclonus syndrome after early immunotherapy: a case report. Mov Disord, 2011. 26(10): p. 1805-7.

Chapter IV

Distinct functional properties of the vertical and horizontal saccadic network in Health and Parkinson's Disease: an eye-tracking and fMRI study

Abstract.

Saccadic behaviour ranges from reflexive (e.g., prosaccade) to goal oriented voluntary movements (e.g., antisaccade). Behavioural asymmetries between vertical and horizontal saccades have been described both in normal individuals (greater delay of vertical prosaccades) and in disease states such as Parkinson's disease (prosaccades are short and antisaccades are delayed, especially in the vertical plane, possibly due to a frontostriatal deficit). Importantly, the cortical mechanisms for the generation of vertical saccades are largely unknown, both in health and disease, when compared with their horizontal counterpart. Moreover, studies exploring saccadic neural correlates and putative compensatory mechanisms at a functional level in PD are scarce. We investigated horizontal and vertical prosaccades and antisaccades in an eye tracking paradigm in 19 PD patients off medication and 22 healthy controls, followed by a block-design functional Magnetic Resonance Imaging (fMRI) study, consisting of two runs (prosaccade, antisaccade) of 6 blocks each (3 vertical, 3 horizontal). While saccade metrics were not significantly different between groups, PD showed left frontal underactivation during horizontal prosaccades and right parietal overactivation during horizontal and vertical prosaccades and horizontal antisaccades. Moreover, controls showed greater deactivation of the default-mode network (DMN) during antisaccades. Vertical prosaccades were associated with greater right frontal and cerebellar activity in controls, and cuneus hypoactivity in PD. Vertical antisaccades were associated with greater DMN deactivation in both groups and left parietal hypoactivity in PD. Putative functional compensatory changes in the right parietal cortex in PD patients may help to keep saccadic behaviour at the same level as the healthy controls. We provide first time evidence showing that functional cortical asymmetries between vertical and horizontal saccades occur distinctively in PD patients and healthy controls.

Keywords.

Parkinson’s disease; Eye movements; Saccades; Functional MRI; Basal ganglia

1. Introduction.

Saccades are rapid conjugate eye movements that are used to direct the gaze towards an object of interest [1]. Saccadic behaviour ranges from reflexive movements, e.g., a visually triggered saccade *toward* a novel stimulus (prosaccade) to explicit voluntary movements, e.g., a volitional saccade made in the mirror opposite direction to the stimulus (antisaccade). In Parkinson's disease (PD), a progressive bradykinetic disorder [2], hypometric prosaccades and delayed antisaccades *especially* in the vertical plane, and increased number of directional errors are among the most consistent findings [3–8] . Previous discrepancies in PD oculomotor studies could have been attributed to methodological differences (i.e., medication effects, cognitive and motor status, and age) [6–8]. These abnormalities have been explained by an excessive inhibition of superior colliculus (SC) neurons by the BG and/or decreased pre-oculomotor drive from frontal cortex through the BG to the SC [9]. Albeit saccadic amplitude and latency may be disproportionately affected along the vertical and horizontal planes in PD patients, and thus potentially reflecting asymmetric involvement of cortical and/or subcortical direction-specific neuronal populations, studies simultaneously investigating prosaccades and antisaccades in both planes in PD patients are surprisingly rare [4, 5, 10].

Functional Magnetic Resonance Imaging (fMRI) studies in normal individuals have consistently demonstrated that the execution of horizontal saccades reliably activates an oculomotor network comprising the frontal eye field (FEF), supplementary eye field (SEF) and intraparietal sulcus (parietal eye field, PEF), more so for antisaccades, in which additional recruitment in the dorsolateral prefrontal cortex (dlPFC) and anterior cingulate gyrus, and deactivation of the default-mode network (DMN) may be seen [11, 12]. Unfortunately, little or no attention has been given in the literature to the cortical control of vertical saccades. These seem to require bilateral cortical activation of the oculomotor network to be executed, while horizontal saccades are generated by a predominantly contralateral activation of the same underlying network [13]. To our best knowledge no fMRI study has specifically addressed cortical blood oxygenation-level dependent (BOLD) responses differences between horizontal and vertical saccades. Two fMRI studies investigated the cortical saccadic network in PD patients, both only concerning horizontal saccades. PD patients seem to demonstrated FEF, SEF and caudate nucleus hypoactivity and concomitant relative hyperactivity in parietal areas (e.g., inferior parietal lobule; precuneus) [14, 15].. These studies point to a probable frontostriatal executive failure in presetting the oculomotor network and the existence of a compensatory shift of activity to posterior areas.

In this study we investigated, in a behavioural and block-design fMRI study, the dynamic properties of reflexive (prosaccades) and voluntary (antisaccades) saccades separately for the vertical and horizontal planes in PD patients and controls, with the dual goal to understand the neural circuitry underlying vertical and horizontal oculomotor control in health and in PD. We expected BOLD frontal (e.g., FEF) hypoactivity (and/or compensatory parietal hyperactivity) during saccade performance

in PD when compared to controls, and further posited that the BOLD contrast in PD between vertical and horizontal saccades should echo previously reported asymmetries in behavioural data [4, 5, 14, 15]. We made no specific predictions for the latter contrast in controls, although some but not all behavioural studies in normal individuals have shown subtle asymmetries between saccadic planes (e.g, shorter latency of horizontal prosaccades) [16, 17] and one recent fMRI demonstrated that even in the absence of eye movements, orienting spatial attention along the vertical and horizontal meridian promotes distinctive BOLD patterns for each dimension [18].

2. Methods.

2.1. Participants.

Nineteen individuals with mild to moderate Parkinson's disease (Hoehn and Yahr stages 1–3); 7 females, mean age 64.9±6.3 S.D. years, range 54-74) were recruited from our movement disorders clinic at Coimbra University Hospital Center, from December 2012 to February 2014. Twenty two age-, education-, mood-, cognitive-matched controls (5 males, mean age 66.4±9.5 S.D. years, range 48-82) free of any neurological, psychiatric or visual disorder (other than refractive error) were recruited, comprising the spouses and/or carers of our patients, and hospital staff. Subjects in the latter group did not take any medication known to affect oculomotor behavior. The diagnosis of PD was made according to the UK Parkinson's Disease Society (UKPDS) Brain Bank criteria and was confirmed by a movement disorder specialist [19]. Participants in either group underwent an evaluation of cognitive status (Mini-Mental State Examination (MMSE)), and depression (30 item Geriatric Depression Scale (GDS)), and further evaluation of motor function (motor subscale III of the modified Unified Parkinson's Disease Rating Scale (UPDRS); modified Hoehn and Yahr Scale (H & Y) was performed in PD group. Exclusion criteria included other forms of parkinsonism, severe dementia (MMSE < 15), moderate to severe depression (GDS > 21), normal or corrected-to-normal visual acuity worse than 6/12 in the best eye, inability to perform the oculomotor task outside and inside fMRI, excessive head movement (>2mm) during fMRI scanning, and the presence of structural abnormalities affecting known saccade regions in MRI (i.e., FEF, SEF, PEF, BG, and dlPFC). PD patients were asked to interrupt their regular dopaminergic medication (levodopa, dopaminergic agonists and/or catechol O-methyltransferase inhibitors) for at least 12h before the experiment to avoid possible interference in saccade parameters [20, 21]. All subjects gave their signed and informed consent. The study was in agreement with the Declaration of Helsinki and accepted by the Ethics Committee of the University of Coimbra. Clinical data and participant demographics are shown in **Table 1**.

Table 1. Demographic and Clinical Data

	PD (n = 19)	CTL (n = 22)	P Value*
Median age (IQR), years	67 (12)	68 (14)	0.513
Gender male/female, no.	12/7	5/17	**0.009**
Median education (IQR), years	4 (0)	4 (0)	0.631
Median GDS score (IQR)	7 (6)	7.5 (12)	0.372
Median MMSE score (IQR)	29 (2)	29 (2)	0.968
Median UPDRS III score (IQR)	19 (19)	NA	NA
Median H&Y score (IQR)	1.5 (1)	NA	NA
Disease duration, years (IQR)	4 (8)	NA	NA

Significant values (P<0.05) are marked in bold. PD, Parkinson's disease; CTL, controls. IQR, interquartile range; GDS, Geriatric Depression Scale; MMSE, Mini Mental State Examination; UPDRS III, motor subscale III of the modified Unified Parkinson's Disease Rating Scale; H&Y, Hoehn & Yahr Scale; NA, not applicable.

*Statistical analysis was performed using Pearson's chi-square test and Mann–Whitney-U-tests.

2.2. Procedures.

Each participant first underwent a behavioral saccade task outside the scanner, which was then followed by a functional imaging experiment using a similar saccade task, during one morning stay of approximately 4 h duration (including all experimental sessions). To avoid excessive fatigue, participants were given a 40-min-break between tasks. One PD patient and three controls did not complete the fMRI task due to claustrophobia (3 controls) and discomfort (1 PD). Additional 5 subjects (2 controls; 3 PD) were eliminated due to excessive head movement during fMRI scanning.

2.2.1. Behavioral procedure.

The initial task consisted of an oculomotor behavior paradigm outside the scanner where subjects were asked to perform a 10-min block of reflexive saccades (prosaccades) followed by a ~5-min block of voluntary saccades (antisaccades), each comprising 64 trials, with a ~5-min break between blocks. A practice run of 20 trials was completed prior to each block, to demonstrate paradigm requirements. The experiment was run in a dark room. Subjects were seated in an armchair, with a head rest preventing head movements, facing the center of a computer screen monitor (Dell 22") used to display the visual stimuli, at a viewing distance of 70 cm. The screen covered a visual area of 30° horizontally by 20° vertically, with a resolution of 1680 x 1050 pixels and a refresh rate of 60 Hz. A remote, contact-free binocular eyetracking

setup with automatic eye and head tracking was used (RED500, SMI, Germany). Two dimensional movements of the left eye were recorded with iViewX™ at a 500Hz sampling rate with 0.03° of spatial resolution and and subsequently analyzed off-line. Each recording began with a 5-point system native calibration. Visual stimuli were programmed using Presentation software (Version 14.9; Neurobehavioral Systems Inc., CA) and presented against a grey background. These included a white fixation cross at the center of the screen and four blue targets located 10° left, 10° right, 10° above and 10° below the fixation cross, all subtending 0.8 x 0.8° of visual angle.

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2.2.1.1 Prosaccades.

In this block, each trial began with a white central fixation cross displayed for 1250, 1416, 1582 or 1750 ms (uniform distribution). Immediately after its disappearance, an eccentric blue target appeared in the screen for 0.5 seconds on one of four random directions (right, left, up, and down) (*no gap paradigm*). The trial was completed once the target was extinguished and replaced by a blank screen shown for 1 second. An interval of 1.5 seconds leaving a blank screen was interposed between trials. The subjects were instructed to look at the fixation cross and then to make a saccade toward the eccentric target as soon as it appeared, as fast and accurate as possible. Once the target disappeared, subjects were told to move their eyes back to the center and wait for the fixation cross to reappear. The fixation cross duration and target position were randomized and counterbalanced in order to prevent prediction by the participants. A complete block consisted of 64 trials, 32 vertical (16 down, 16 up) and 32 horizontal (16 right, 16 left).

2.2.1.2. Antisaccades.

The antissacade block comprised an identical sequence of events as the prosaccade experiment except that participants were asked to look in the *opposite* direction of the stimulus target, being specifically told to try to ‘mirror’ as precise as possible target position in the opposite field. All subjects accomplished both experimental tasks. Examples of the sequence of events for the prosaccade and antisaccade task are shown in **Fig. 1.a**.

2.2.2. Imaging procedure.

Functional imaging was then performed while each participant executed a–similar oculomotor paradigm over 2 consecutive runs in the MRI scanner.

2.2.2.1. fMRI data acquisition.

Imaging data were collected at the Institute of Nuclear Sciences Applied to Health, University of Coimbra using a 3.0 Tesla field strength Siemens Magnetom Trio scanner (Erlangen, Germany) fitted with a 12-channel receive-only head coil to measure blood-oxygen level–dependent signal changes related to neural activity [22]. We first collected a high resolution 3D T1 MPRAGE (magnetization prepared rapid gradient echo) anatomical sequence (TE=3 ms, TR=2530 ms, flip angle 9°; 176 partitions, 1x1x1 isotropic voxels, field of view 256 mm; matrix size 256 × 256) for coregistration with the fMRI data. Functional scans consisted of two dimensional gradient-echo echo-planar imaging (2D GRE EPI) sequence sensitive to BOLD contrast acquired in an interleaved fashion (43 slices, 3.0 mm slice thickness, TR=3000ms, TE=30 ms, matrix size 86×86, field of view 256x256mm, flip angle 90°, voxel size 3x3x3mm, 91 images) while subjects were engaged in eye movement tasks. Images were acquired during two block-design runs with a total scanning time per subject of 15 min and 25 s. Slices were aligned parallel to the anteroposterior commisural (AC-PC) line and covering the whole brain.

2.2.2.2. fMRI oculomotor task design.

Visual stimuli were generated using Presentation software (Version 14.9; Neurobehavioral Systems Inc., CA) on a personal computer. To ensure synchronization, the MRI sequences directly triggered the Presentation software using a trigger signal from the scanner. Images were back-projected onto an MRI-compatible high-contrast screen pad positioned at the rear of the magnet bore, using a SV-6011 Avotec LCD video projector with a resolution of 1024 x 768 pixels and a refresh rate of 60 Hz. Subjects viewed the images via a reflection mirror mounted on the head coil. Active screen dimensions were 20 cm horizontal and 15 cm vertical and viewing distance between participant eyes and the screen pad was 46.5 cm, subtending 23.2° x 17.8° of visual angle. Movements of the left eye were recorded using an MRI-compatible infrared oculographic pupil tracker (SMI SensoMotoric Instruments) positioned on the head coil, at a sampling rate of 60 Hz with 0.03° of spatial resolution. Further analysis of the eye movement data was performed off-line. A five-point calibration was performed before the first functional scan. First run consisted of a prosaccade task containing 3 blocks of horizontal prosaccades and 3 blocks of vertical prosaccades randomly interleaved and separated by 15 s of blank screen (rest condition). Each block comprised 6 trials and lasted 27s. Trials were 4500 ms (including intertrial interval) in length. The trials had identical stimulus characteristics to those displayed in the behavioral task outside MRI, except for shorter target amplitude (8°) to accommodate screen size (see above). Within each block, target location was randomized and counterbalanced (i.e., 3 right, 3 left in horizontal prosaccades; 3 up, 3 down in vertical prosaccades). The second run (antisaccades) consisted on the same stimulus but subjects were instructed to perform antisaccades (**Fig. 1.b**). Thus, while outside the scanner,

saccade trials were interleaved between four random directions (sequence example [target direction]: right, up, left, right, down), inside the scanner, blocks of 6 horizontal saccade trials were interleaved with blocks of 6 vertical saccade trials. Each run started with an additional period of fixation for 30 s and ended with a period of fixation for 16.5 s, to allow for the return of the hemodynamic response signal to the baseline level of activation [23]. During prosaccades run, participants had to make a saccade toward the eccentric target while during antisaccades run saccades had to be made in the opposite mirror direction. During the rest condition participants were instructed to actively keep their eyes centered on the screen. Each run was preceded with verbal information about the type of task. Participants were not informed however about the direction (vertical or horizontal) of saccade trials before each block, both in prosaccade and antisaccade runs. The entire session lasted 28 minutes.

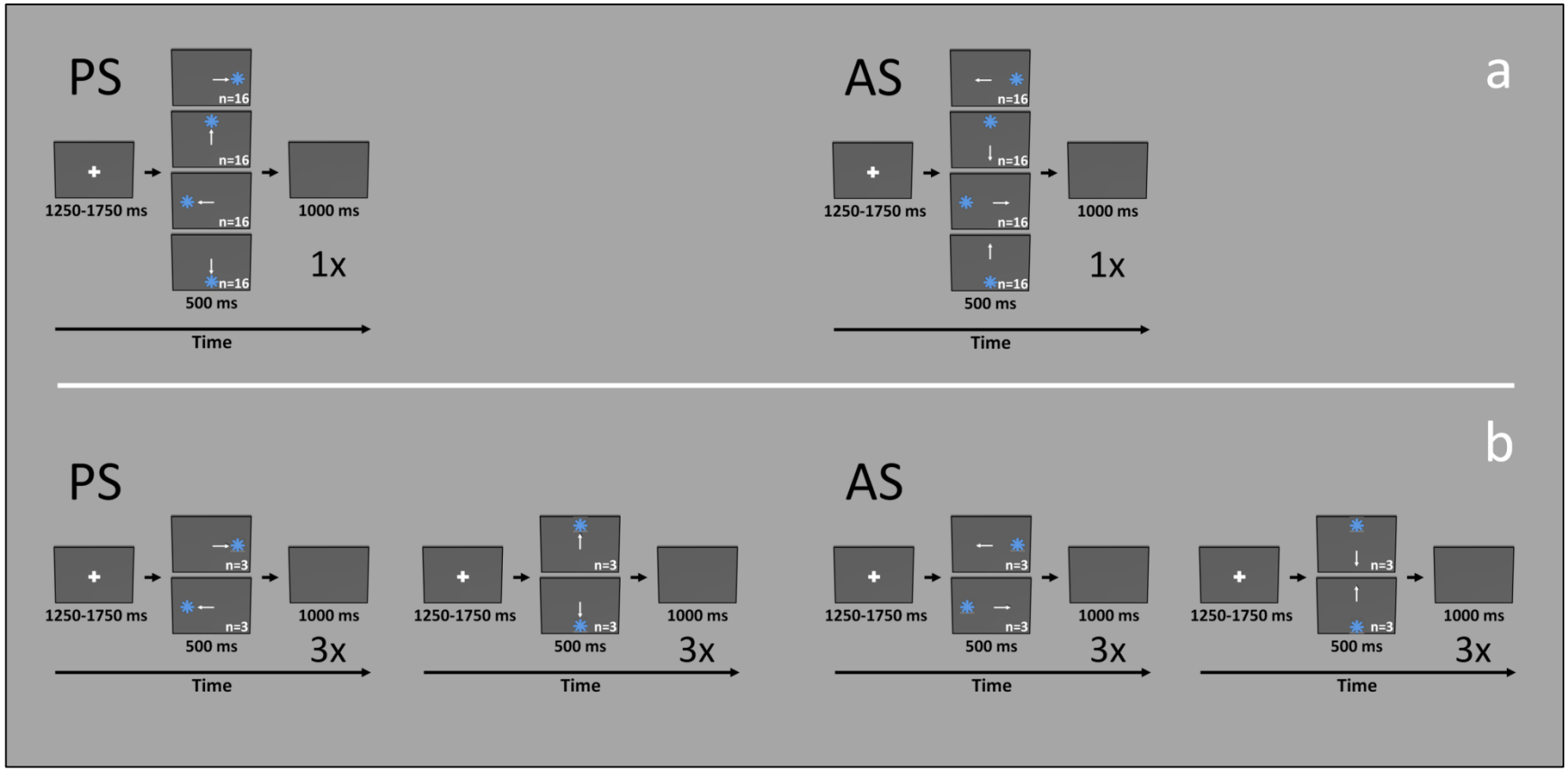

Figure 1. Schematic representation of stimuli and time course of the oculomotor tasks, outside (**a**) and inside fMRI (**b**). Participants were instructed to keep foveating a central fixation cross until its disappearance (total fixation time, 1250-1750 ms) (*left*, on each image). Immediately after, participants were required to make a saccade towards (prosaccade, PS) or to the opposite mirror location (antisaccade, AS) of an eccentric target, randomly appearing for 500 ms on one of four locations (10° up, 10° down, 10° left, or 10° right) (*center*, on each image). The screen was then cleared for 1000 ms (*right*, on each image) (intertrial interval is not shown). In both tasks (PS and AS), there was no gap between fixation point offset and target stimulus onset (no-gap paradigm). Note that the paradigms outside and inside the scanner exhibited slight differences. Thus, while outside fMRI, saccade trials were interleaved between four random directions (**a**), inside fMRI, blocks of 6 horizontal saccades were interleaved with blocks of 6 vertical saccades (**b**). (arrows in saccade trials represent instructed eye movement and not actual stimuli)

2.3. Data processing and analysis.

2.3.1 Behavioural data.

Eye movement data were analyzed off-line using the *SMI BeGaze 3.4™* software (SensoMotoric Instruments Inc, Teltow, Germany) and custom-written scripts in MATLAB 8.0 (The MathWorks Inc., Natick, MA, USA). For analysis, the following parameters were chosen from the left eye recordings in prosaccade and antisaccade tasks: latency, direction error rate of the primary saccade after target onset within each trial, peak velocity, and amplitude. Saccades were automatically detected on the basis of criteria of minimum duration (22 ms) and minimum velocity (40°/s) and subsequently verified by visual inspection of eye-position traces. The following definitions were applied: 1) latency as the time (ms) between target onset and saccade onset; 2) amplitude as the ratio of saccade amplitude (°) to target eccentricity (10°); peak velocity as the maximum velocity (°/s) occurring within the duration of the saccade; 4) direction errors as primary saccades directed away from the target in prosaccade task and towards the target in antisaccade task. The direction error rate was further calculated as the percentage of direction error trials over the total number of trials. Saccades were discarded from the analysis if: 1) latency <120 ms (anticipatory saccade) or >800 ms (delayed saccade); 2) preceded by a blink within 100 ms before target onset; 3) saccade starting point fell outside a >1.5° circle centered on fixation cross; 4) saccade landing point fell outside a 7° circle centered on the target in prosaccade trials (or on the mirror location of the target in antisaccade trials). The percentages of rejected trials from the analysis were 5.6% for PD group and 4.4% for control participants (p=0.433). Mean latency, peak velocity and gain were then calculated for each participant. Each parameter was extracted separately for horizontal and vertical direction. We were not able to carry out an offline analysis of the eye-movement data recorded inside the scanner due to technical limitations related to signal-to-noise in the eye-tracking data. However, participant´s eye movement performance was monitored with a video camera, demonstrating task compliance "on-line" in all subjects.

2.3.2 fMRI data.

Imaging data pre-processing and analysis was carried out using BrainVoyager Qx Software Package, version 2.60 (Maastricht, the Netherlands). Motion correction was achieved in the remaining EPI images by realigning each subject's time series to the first functional image, using a trilinear interpolation for motion detection and sinc interpolation for actual motion correction. Realignment parameters where checked to assure that maximum head movement never exceeded 2 mm during the scan run. Slice-scan time correction was then performed using a cubic-spline interpolation. Subsequently, images were smoothed using a full-width at a half maximum isotropic 4-mm Gaussian kernel. Finally, we applied a temporal high-pass filtering using general linear model with a fourier basis set with a window of 2 cycles plus temporal smoothing (0,001 data points). After inhomogeneity correction and transformation into a

coordinate system of Talairach space, anatomical data and functional images were then coregistered [24].

2.3.3 Statistical analysis.

For behavioral analysis, we first compared saccade latency, peak velocity, amplitude and direction error rate between groups using a Mann–Whitney-U-test, both for horizontal and vertical directions and for prosaccade and antisaccade tasks. For within-groups comparison between horizontal and vertical directions, the Wilcoxon test was used. Non parametric testing was used since the data were not normally distributed. Bonferroni correction according to the number of comparisons was further used. Spearman Rank Order tests were used to correlate saccadic performance with age, gender, education, GDS and MMSE scores and UPDRS and H & Y scores in each group. Due to the high number of correlations, p-value was adjusted to 0.00019. All statistical tests were two tailed with criterion for statistical significance set at $p<0.05$, unless otherwise stated. Statistical Package for the Social Sciences (SPSS) Version 20 (IBM Inc., Chicago, IL, USA) was used for statistical analysis. For first level fMRI analysis at (single subject) we applied a general linear model considering BOLD signal as the dependent variable, individual blocks as predictors (regressors) and a constant term related to overall mean signal. The 15 s of blank screen (baseline condition) was not modelled explicitly and thus served as the implicit baseline. Single-subject contrast maps were obtained for horizontal prosaccades > baseline, vertical prosaccades >baseline, vertical prosaccades > horizontal prosaccades, horizontal antisaccades > baseline, vertical antisaccades > baseline, and vertical antisaccades > horizontal antisaccades. In a whole-brain analysis, single-subject maps were then combined at the group level with a random effects GLM approach, separating subjects from predictors, followed by multiple comparison correction with a cluster-extent base thresholding (setting a primary $p <0.01$), to draw inferences about brain activation at the group level [25]. Between-groups analysis using t-tests was conducted on the comparisons of interest and followed by a within-group analysis using paired t-tests to examine differences in BOLD activation. Correlations between behavioral data and group fMRI contrast activations were also performed using Spearman Rank Order tests in SPSS. BOLD parameter estimates were extracted from 5-mm radius spheres around peak voxels reported for vertical>horizontal prosaccade and antisaccade contrasts using Talairach Coordinate to VOI Brainvoyager plug-in [26]. Due to the high number of correlations, p-value was adjusted to 0.00048.

3. Results.

3.1. Clinical and demographic data.

Except for gender (female gender predominance in controls contrasting with male predominance in PD groups, p=0.009), there were no significant differences between groups with respect to demographic and clinical data (see **Tables 1** and **2**).

Table 2. Clinical information for PD patients

Patients	Gender	Age (yrs)	GDS	MMSE	Education (yrs)	UPDRS	H&Y	Disease Duration (yrs)	Medication	LED (mg)
1	m	72	14	28	4	25	2	13	L	600
2	m	55	4	30	6	16	1	2	L; R	610
3	m	62	12	27	4	12	1	1	L	300
4	m	73	3	29	3	11	1	2	L	300
5	m	54	8	30	4	25	1	2	L	400
6	f	64	20	26	0	42	2,5	14	L; R	1420
7	f	66	9	25	4	19	1,5	5	L	675
8	m	72	5	27	3	17	1,5	3	L; R	540
9	f	56	17	28	4	7	1	1	L	300
10	m	67	8	29	12	4	1	2,5	L	550
11	f	67	7	28	3	38	1,5	6	L; T	475
12	f	70	6	30	4	11	1	3	L; R	790
13	m	57	1	30	11	4	1	4	L	300
14	m	63	4	30	4	27	2	10	L; R	780
15	m	58	1	28	4	14	3	3	L; R	560
16	f	68	8	30	4	24	1	10	L; R; T	990
17	f	69	3	30	4	30	1,5	10	L; R	1270
18	m	74	3	29	4	36	2,5	6	L; R	1265

19	m	67	9	28	4	30	2	17	L	1000

f, female; GDS, Geriatric Depression Scale; H & Y, Hoehn and Yahr Scale; L, Levodopa/carbidopa; LED, Levodopa Equivalent Dose; m, male; MMSE, Mini Mental State Examination; R, Ropinirole; T, Trihexyphenidyl; UPDRS, Unified Parkinson's Disease Rating Scale; Yrs, years

3.2. Behavioral data.

Fourty one subjects underwent a two-block saccadic paradigm outside the scanner (prosaccade block followed by antisaccade block), each block comprising 64 trials of saccades directed towards (during prosaccade block) or in the opposite direction (during antisaccade block) of a target placed on one of four random positions (10°right, 10°left, 10°up, 10°down) (see **Fig. 1.a**) (see **Methods** section for more detail). **Table 3** displays the results of the saccade behavioral data. Saccade latency, velocity, amplitude and direction error rate both for horizontal and vertical directions and for prosaccade and antisaccade tasks did not differ significantly between PD and controls except for a tendency for vertical antisaccades latency to be longer in PD than controls (503ms vs. 445ms, respectively; p=0.082 [effect size=0.69]). Consistently within each group, prosaccade and antisaccade vertical vs. horizontal plane comparisons demonstrated significantly or marginally-significantly longer latencies (prosaccades: PD, 293ms vs. 245ms, p=0.0002 [effect size=1.45]; controls, 280ms vs. 242ms, p<0.0001 [effect size=0.83]; antisaccades: PD, 503ms vs. 425ms, p=0.009 [effect size=0.54]; controls, 445ms vs. 400ms, p=0.0030 [effect size=0.78]) and slower velocities (only antisaccades: PD, 3448ms vs. 302ms, p=0.012 [effect size=1.51]; controls, 349ms vs. 301ms, p=0.009 [effect size=0.79]) for the vertical plane in both groups.

Table 3. Behavioral Data

	PD (n = 19)	CTL (n = 22)	P Value* between groups *effect size*	P value** within groups PD; CTL *effect size*
Horizontal Prosaccades				
Latency, ms	245 (46)	242 (43)	0.278 *0.06*	**<0.0001**; **0.0001** *1.45; 0.83*
Peak Velocity, °/s	354 (60)	322 (79)	0.290 *0.45*	0.042; 0.030 *0.50; 0.75*
Amplitude, °	9.9 (1.8)	9.6 (2.3)	1.000 *0.14*	0.074; 0.070 *0.34; 0.74*
Direction Error, %	2.2 (5.2)	3.1 (10)	0.564 *0.11*	0.310; 0.553 *0.15; 0.28*
Vertical Prosaccades				
Latency, ms	293 (56)	280 (63)	0.278 *0.21*	
Peak Velocity, °/s	322 (44)	302 (56)	0.296 *0.39*	
Amplitude, °	9.1 (1.6)	9.0 (1.5)	0.794 *0.06*	
Direction Error, %	1.5 (3.9)	3.5 (10)	0.298 *0.25*	
Horizontal Antisaccades				
Latency, ms	425 (117)	400 (85)	0.478 *0.24*	**0.003**; 0.009 *0.54; 0.78*
Peak Velocity, °/s	349 (50)	348 (90)	0.965 *0.01*	0.009; 0.012 *1.51; 0.69*
Amplitude, °	12.1 (4.3)	12.9 (5.4)	0.661 *0.16*	0.035; 0.048 *0.49; 0.56*
Direction Error, %	42.3 (27)	52.4 (31)	0.255 *0.34*	0.098; 0.355 *0.20; 0.75*

Vertical Antisaccades			
Latency, ms	503 (103)	445 (63)	0.082
			0.69
Peak Velocity, °/s	301 (80)	302 (71)	0.895
			0.01
Amplitude, °	10.3 (4.4)	11.3 (3.7)	0.373
			0.24
Direction Error, %	35.0 (23)	37.5 (33)	0.886
			0.08

Values are means (standard deviations).

*Statistical difference between groups (PD vs. CTL); Significant values, $p < 0.003$ (after Bonferroni correction) are marked in bold.

**Statistical difference within groups (horizontal vs. vertical PS, 1st to 4th row; horizontal vs. vertical AS, 8th to 12th row; PD, left column; CTL, right column); Significant values, $p < 0.006$ (after Bonferroni correction) are marked in bold; note that within-groups comparison was only performed between horizontal and vertical directions, and not between tasks, i.e., prosaccade vs. antisaccade.

PD, Parkinson´s disease; CTL, controls.

3.3. Correlations between behavioral data and demographic and clinical data.

We did not find any significant correlations between saccadic parameters and clinical and demographic variables for each group.

3.4. fMRI data.

From our original sample, 9 subjects did not complete the fMRI task or were subsequently eliminated by applying our exclusion criteria (see **Methods** section). Thus, the remaining 17 controls and 15 PD patients performed similar behavioural paradigms in the MRI scanner. Here however, changes were made to the original paradigm, to account for the dynamics of the BOLD response signal [23]. Thus, during the initial prosaccade run, blocks of horizontal prosaccades (each block comprising 6 trials) were randomly interleaved with blocks of vertical prosaccades (each block comprising 6 trials). The run was completed after 6 blocks. This was followed by an antisaccade run, consisting on the same stimulus but during which subjects were instructed to perform saccades in the *opposite* direction of the target (**Fig. 1.b**) (see **Methods** section for more detail). We conducted an initial analysis where we compared BOLD activity between groups to first replicate previous studies showing frontal hypoactivity (and/or parietal and temporal hyperactivity) in PD patients [14, 15]. We then proceeded to our main analysis where we hypothesized a distinct organization of vertical and horizontal saccadic circuits. We predicted that vertical vs. horizontal blocks contrast in PD patients should demonstrate BOLD differences putatively located in cortical areas known to modulate saccadic latency and amplitude (e.g., PEF and FEF), both of which seem to be predominantly impaired in the vertical direction in PD [4, 5]. Apart from the anatomical constraints which usually preclude the use of fMRI to study the brainstem [27], we did not expect to find BOLD differences in brainstem oculomotor structures, since these are relatively spared in PD patients [28]. To test whether brain activation was appropriately measured, the control and PD group results for prosaccades and antisaccades (compared to baseline) were verified ($P < 0.01$, corrected for multiple comparisons using a spatial extent correction method, i.e., cluster-based multiple comparisons correction [1000 iterations], setting as voxel-level primary threshold $p< 0.01$). **Figure 2** shows the t-statistical difference maps of brain activation in controls (**Fig. 2.a**) and PD group (**Fig. 2.b**) during prosaccades and antisaccades compared to baseline. In controls, as expected, the prosaccade contrast revealed selective bilateral activations of FEF, SEF, PEF/precuneus, dlPFC, basal ganglia (putamen; left thalamus), occipital lobe (middle occipital gyrus; inferior occipital gyrus), temporal lobe (fusiform gyrus), and cerebellum (dorsal vermis; cerebellar anterior lobe; cerebellar uvula/inferior semilunar lobule), and deactivations of areas overlapping the DMN (posterior cingulate gyrus; posterior insula). Antisaccade contrast analysis showed similar activations, with the exception of putaminal and left thalamic activations, now replaced by left caudate activation. Additional deactivations in the DMN were seen in the antisaccade contrast (i.e., anterior cingulated gyrus;

ventromedial and dorsomedial prefrontal cortex, vmPFC, dmPFC; paracentral lobule, in vicinity of posterior cingulate gyrus; middle temporal gyrus; left parahyppocampal gyrus). Similar results were observed using FDR approaches (data not shown). In PD patients, a similar activation pattern was seen, although dmPFC and vmPFC, middle temporal gyrus deactivations and right caudate activation during antisaccades were absent as well as left thalamus activation during prosaccades. Of note, in both groups, the activation of executive areas (i.e., dlPFC) classically associated with antisaccade task was also seen in the prosaccade task, and moreover, when comparing antisaccade to prosaccade activation in control and PD groups, BOLD differences were only seen in the DMN, demonstrating greater deactivation in antisaccade task. On the contrary, classical areas often denoting greater activation in antisaccade tasks (i.e., FEF, SEF and PEF) showed similar activity in both tasks. This was probably related to the nature of the experimental design (see **Supplemental Table 1**). Overall, saccade tasks activated an extensive network of cortical and subcortical areas known to participate in the execution of eye movements [11, 12].

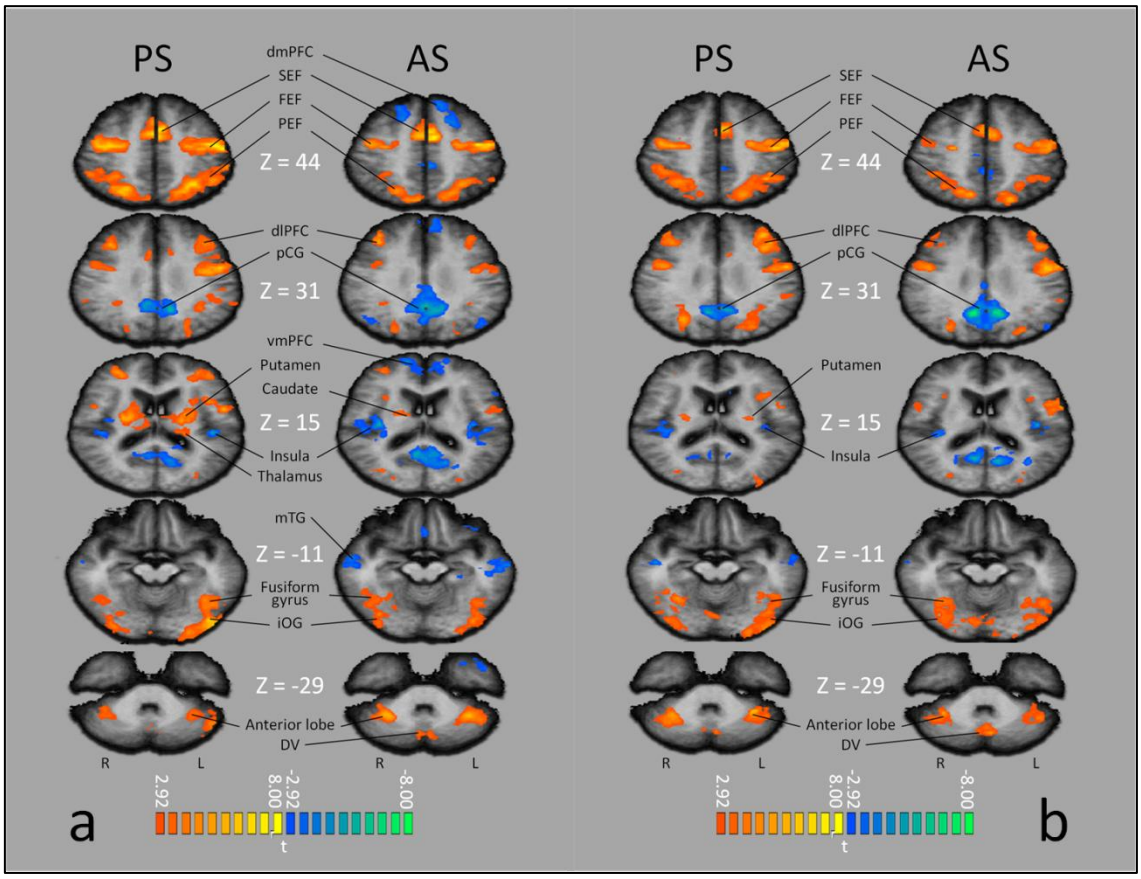

Figure 2. T-contrast maps of prosaccade (PS) and antisaccade (AS) blocks compared with baseline in control group (*n*=17; **a**) and PD group (n=15; **b**) ($p < 0.01$, corrected for multiple comparisons as estimated by Brain Voyager's Cluster-level Statistical Threshold Estimator with 1000 iterations). Yellow/red and blue/green regions represent greater and less saccade activation than baseline, respectively. L, left hemisphere; R, right hemisphere; dmPFC, dorsomedial prefrontal cortex; FEF, frontal eye field; SEF, supplementary eye field; PEF, parietal eye field; dlPFC, dorsolateral prefrontal cortex; pCG, posterior cingulate gyrus; vmPFC, ventromedial prefrontal cortex; mTG, middle temporal gyrus; iOG, inferior occipital gyrus; DV, dorsal vermis; PD, Parkinson's disease. (see **Results**, section 2.4 for details)

3.4.1. Prosaccades.

For completeness, horizontal and vertical prosaccades > baseline contrasts within groups can be found in **Supplemental Fig. 1** and **2**. Concerning between-group analysis, controls showed significantly greater BOLD signal than PD during prosaccades performance in the left FEF while exhibiting less BOLD activity in the right PEF, precuneus and cerebellum. This held true both for horizontal (FEF and PEF) and vertical prosaccades (only PEF). The right cuneus showed less activity in the control group only during horizontal prosaccades. Talairach locations of the peak activations for all key regions in between-groups analysis for the horizontal and vertical prosaccade tasks are presented in **Table 4**. Corrected T-statistical activation maps comparing horizontal prosaccade and vertical prosaccade contrasts between the two groups are displayed in **Fig. 3**.

Table 4. Talairach coordinates (x,y,z) and number of voxels (v) of peak activations in GLM contrast maps for horizontal prosaccades (PS) and vertical PS contrasts between controls and PD patients*

				Horizontal PS					Vertical PS			
				Local maxima peak coordinates (TLS)								
Anatomical Region or Functional Label	Side	BA	Dir	x	y	z	v	Dir	x	y	z	v
CTL > PD												
FEF	L	6	(+)	-31	-2	27	374					
PCu/PEF	R	39	(-)	29	-62	27	374					
	R	7						(-)	26	-59	27	413
Cuneus	R	18	(-)	2	-77	15	423					
Cerebellar aL/tonsil	R		(-)	35	-53	-33	517					
Cerebellar iSL	L		(-)	-16	-56	-36	503	(-)	-13	-59	-43	213

CTL, controls; PD, Parkinson's disease; FEF, frontal eye field; PCu, precuneus; PEF, parietal eye field; aL, (cerebellar) anterior lobe; iSL, (cerebellar) inferior semilunar lobe; TLS, Talairach standard; BA, Brodmann area

"Peak" refers to location of voxel with most significant activation

"Side" refers to the location of the activation: R, right hemisphere; L, left hemisphere.

"Dir" refers to the direction of contrast: greater (+) or less (-) saccade activation compared to baseline

"/" between two anatomical regions and/or functional labels indicates clusters involving two contiguous areas

* Random effects analysis, $p<0.01$, corrected for multiple comparisons at the cluster level

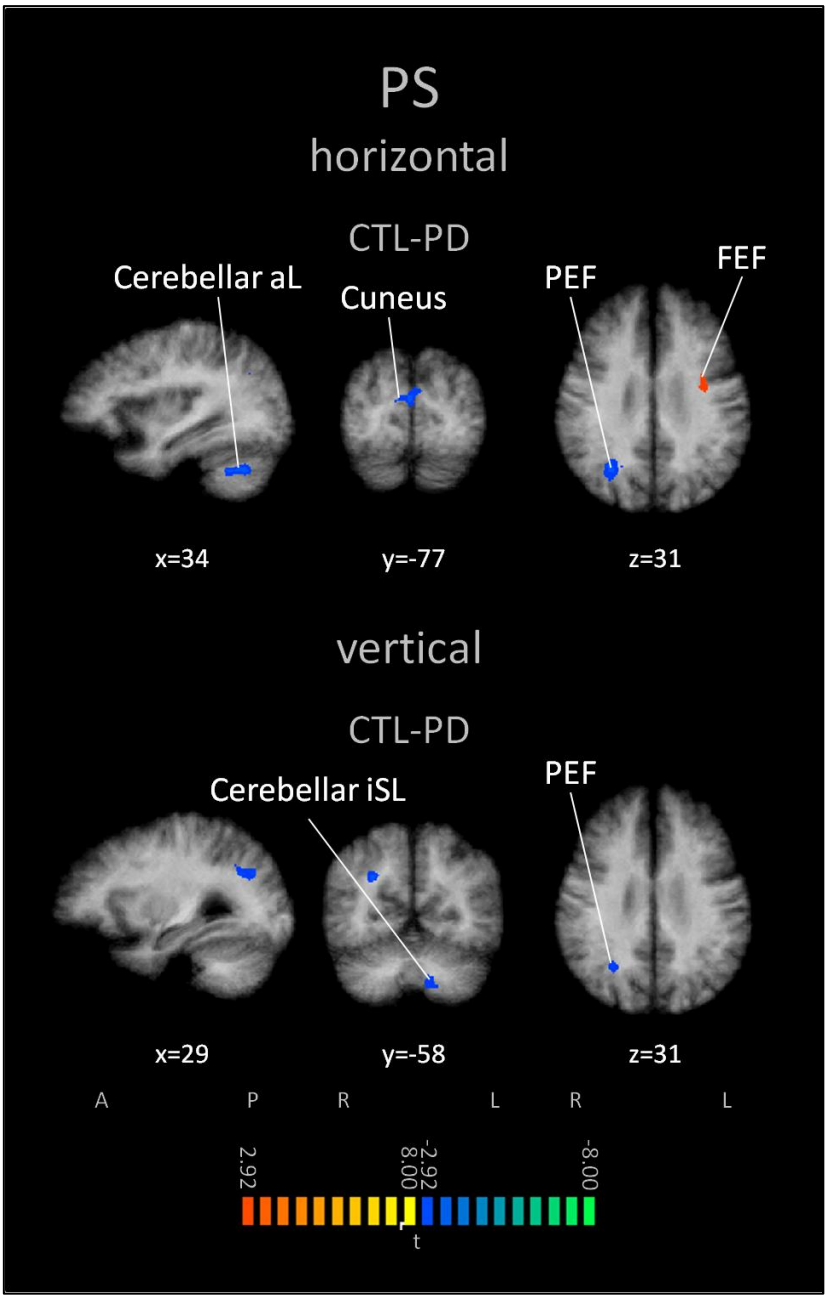

Figure 3. Group t-statistical activation maps comparing horizontal prosaccades (PS) and vertical PS contrasts between CTL (n=17) and PD (n=15) groups ($p < 0.01$, corrected for multiple comparisons as estimated by Brain Voyager's Cluster-level Statistical Threshold Estimator with 1000 iterations). Brain areas showing significant activation are displayed on standard T1 image. Yellow/red regions represent greater saccade activation for CTL than PD group. Blue/green regions represent less saccade activation for CTL than PD group. A and P denote anterior and posterior. L and R denote left and right. Coordinate values of planes in Talairach space are indicated. CTL, controls; PD, Parkinson's disease; aL, (cerebellar) anterior lobe; FEF, frontal eye field; PEF, parietal eye field; iSL, (cerebellar) inferior semilunar lobule

3.4.2. Antisaccades.

For completeness, horizontal and vertical antisaccades > baseline contrasts within groups can be found in **Supplemental Fig. 3** and **4**. Concerning between-group analysis, controls again exhibited less BOLD activity than PD group in right PEF while showing significantly stronger deactivations in the DMN (i.e., dmPFC, vmPFC, middle temporal gyrus). Extrastriate visual cortex (e.g., right middle occipital gyrus) on the other hand showed higher activations in controls. Of note, some differential activation patterns were only present in the horizontal (right middle occipital gyrus and FEF) or vertical (dmPFC) direction. Talairach locations of the peak activations for all key regions in between-groups analysis for the horizontal and vertical antisaccade tasks are presented in **Table 5**. Corrected T-statistical activation maps comparing horizontal antisaccade and vertical antisaccade contrasts between the two groups are displayed in **Fig. 4**.

Table 5. Talairach coordinates (x,y,z) and number of voxels (v) of peak activations in GLM contrast maps for horizontal AS and vertical AS contrasts between controls and PD patients*

			Horizontal AS					Vertical AS				
			Local maxima peak coordinates (TLS)									
Anatomical Region or Functional Label	Side	BA	Dir	x	y	z	v	Dir	x	y	z	v
CTL > PD												
PEF	R	32	(-)	32	-59	30	226					
dmPFC	L	8	(-)	-25	19	48	497	(-)	-19	29	51	400
vmPFC	R	10						(-)	14	58	15	286
iTG/mTG	L	20						(-)	-46	-23	-12	466
mTG	L	21	(-)	-61	-26	-12	513					
Paracentral lobule	R	5	(-)	11	-44	60	194					
mOG	R	18	(+)	29	-89	3	681					
mTG/mOG	R	39	(+)	44	-71	15	313					

CTL, controls; PD, Parkinson's disease; dmPFC, dorsomedial prefrontal cortex; iTG, inferior temporal gyrus; mTG, middle temporal gyrus; mOG, middle occipital gyrus; TLS, Talairach standard; BA, Brodmann area

"Peak" refers to location of voxel with most significant activation

"Side" refers to the location of the activation: R, right hemisphere; L, left hemisphere

"Dir" refers to the direction of contrast: greater (+) or less (-) saccade activation compared to baseline

"/" between two anatomical regions and/or functional labels indicates clusters involving two contiguous areas

* Random effects analysis, $p<0.01$, corrected for multiple comparisons at the cluster level

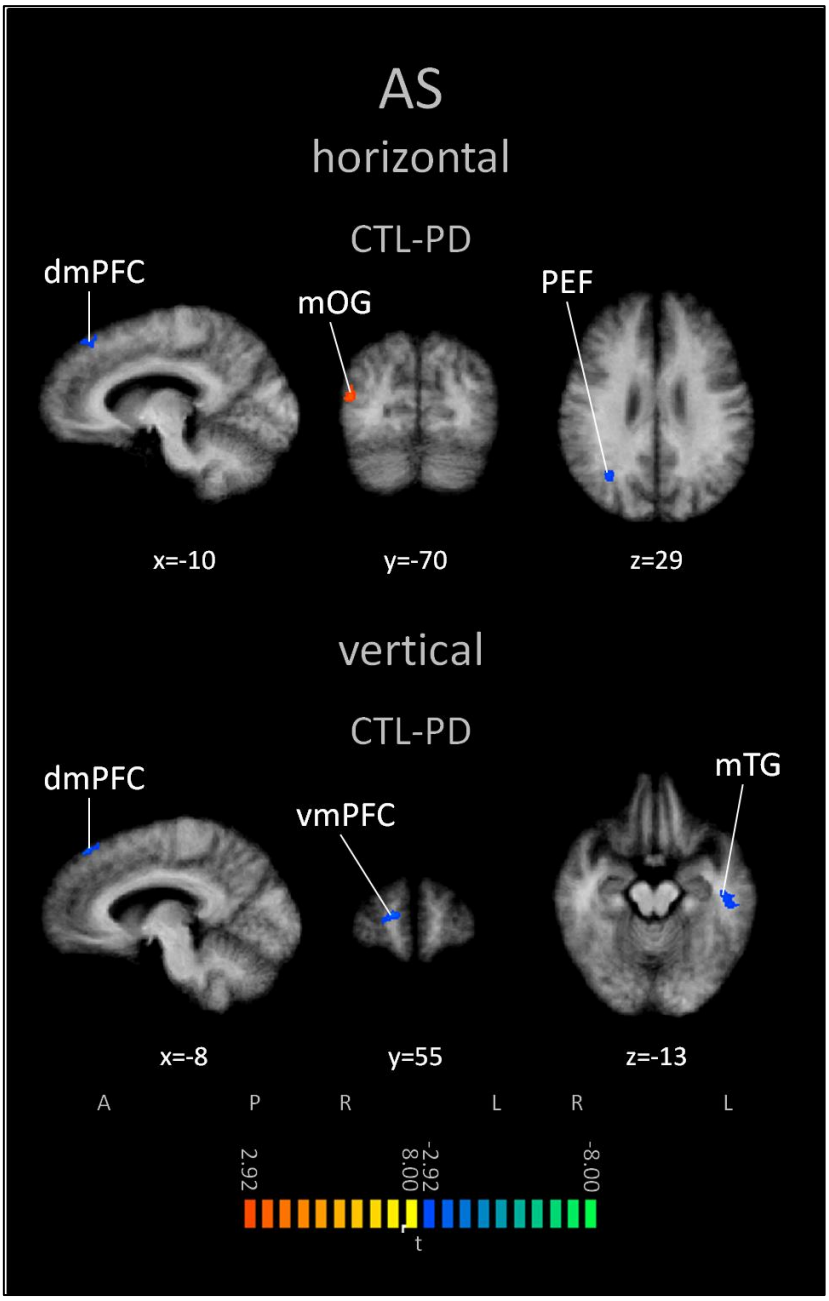

Figure 4. Group t-statistical activation maps comparing horizontal antisaccades (AS) and vertical AS contrasts between CTL (n=17) and PD (n=15) groups ($p < 0.01$, corrected for multiple comparisons as estimated by Brain Voyager's Cluster-level Statistical Threshold Estimator with 1000 iterations). Brain areas showing significant activation are displayed on standard T1 image. Yellow/red regions represent greater saccade activation for CTL than PD group. Blue/green regions represent less saccade activation for CTL than PD group. A and P denote anterior and posterior. L and R denote left and right. Coordinate values of planes in Talairach space are indicated. CTL, controls; PD, Parkinson's disease; dmPFC, dorsomedial prefrontal cortex; mOG, middle occipital gyrus; PEF, parietal eye field; mTG, middle temporal gyrus; vmPFC, ventromedial prefrontal cortex

3.4.3. Vertical > horizontal contrast analysis.

We subsequently proceeded to our main contrast analysis. The contrast of vertical prosaccade blocks minus horizontal prosaccade blocks revealed a positive BOLD effect in the right FEF, cerebellar posterior lobe and superior temporal gyrus in the control group. The PD group on its turn showed less BOLD activity in both cuneus during vertical prosaccades. The vertical antisaccade-horizontal antisaccade contrast evidenced greater deactivations during vertical antisaccades in the DMN (e.g., vmPFC) in both controls and PD. Both groups exhibited additional negative BOLD effect during vertical antisaccades in visual processing areas (controls: right cuneus and lingual gyrus; PD: lingual gyrus). One additional negative cluster in left PEF was found only in PD during vertical antisaccades. Talairach locations of the peak activations for all key regions in within-groups analysis for the horizontal > vertical prosaccade and horizontal > vertical antisaccade contrasts are presented in **Table 6**. Corrected T-statistical activation maps comparing vertical prosaccade to horizontal prosaccade and vertical antisaccade to horizontal antisaccade for both groups are displayed in **Fig. 5** and **Fig. 6**, respectively.

Table 6. Talairach coordinates (x,y,z) and number of voxels (v) of peak activations in GLM contrast maps for vertical PS-horizontal PS and vertical AS-horizontal AS contrasts in controls and Parkinson's disease patients*

			Vertical PS > Horizontal PS					Vertical AS > Horizontal AS				
				Local maxima peak coordinates (TLS)								
Anatomical Region or Functional label	Side	BA	Dir	x	y	z	v	Dir	x	y	z	v
CTL												
FEF	R	4	(+)	41	-17	36	307					
sTG	R	39	(+)	44	-50	15	243					
Cerebellar pL	R		(+)	29	-62	-30	299					
vmPFC	L	9						(-)	-10	55	27	3866
	R	9						(-)	4	55	15	1904
aCG	R	24						(-)	8	31	12	393
pCG	L	31						(-)	-16	-65	15	610
Cuneus	R	19						(-)	2	-81	33	677
LG	R	18						(-)	14	-77	3	1442
PD												
Cuneus	R	30	(-)	11	-68	9	465					
	L	23	(-)	-13	-74	9	595					
PEF	L	40						(-)	-43	-41	39	314
Pcu	R	7						(-)	2	-62	45	299
LG	R	18						(-)	11	-65	0	388
	L	18						(-)	-13	-77	-3	211
PCu/pCG	L	7						(-)	-10	-59	39	2928
vmPFC	R	10						(-)	8	55	0	221

CTL, controls; PD, Parkinson's disease; FEF, frontal eye field; LG, lingual gyrus; vmPFC, ventromedial prefrontal cortex; aCG, anterior cingulate gyrus; PCu, precuneus; PEF, parietal eye field; FuG, fusiform gyrus; pCG, posterior cingulate gyrus; TLS, Talairach standard; BA, Brodmann area

"Peak" refers to location of voxel with most significant activation

"Side" refers to the location of the activation: R, right hemisphere; L, left hemisphere.

"Dir" refers to the direction of contrast: greater (+) or less (-) saccade activation compared to baseline

"/" between two anatomical and/or functional labels indicates clusters involving two contiguous areas

* Random effects analysis, $p<0.01$, corrected for multiple comparisons at the cluster level

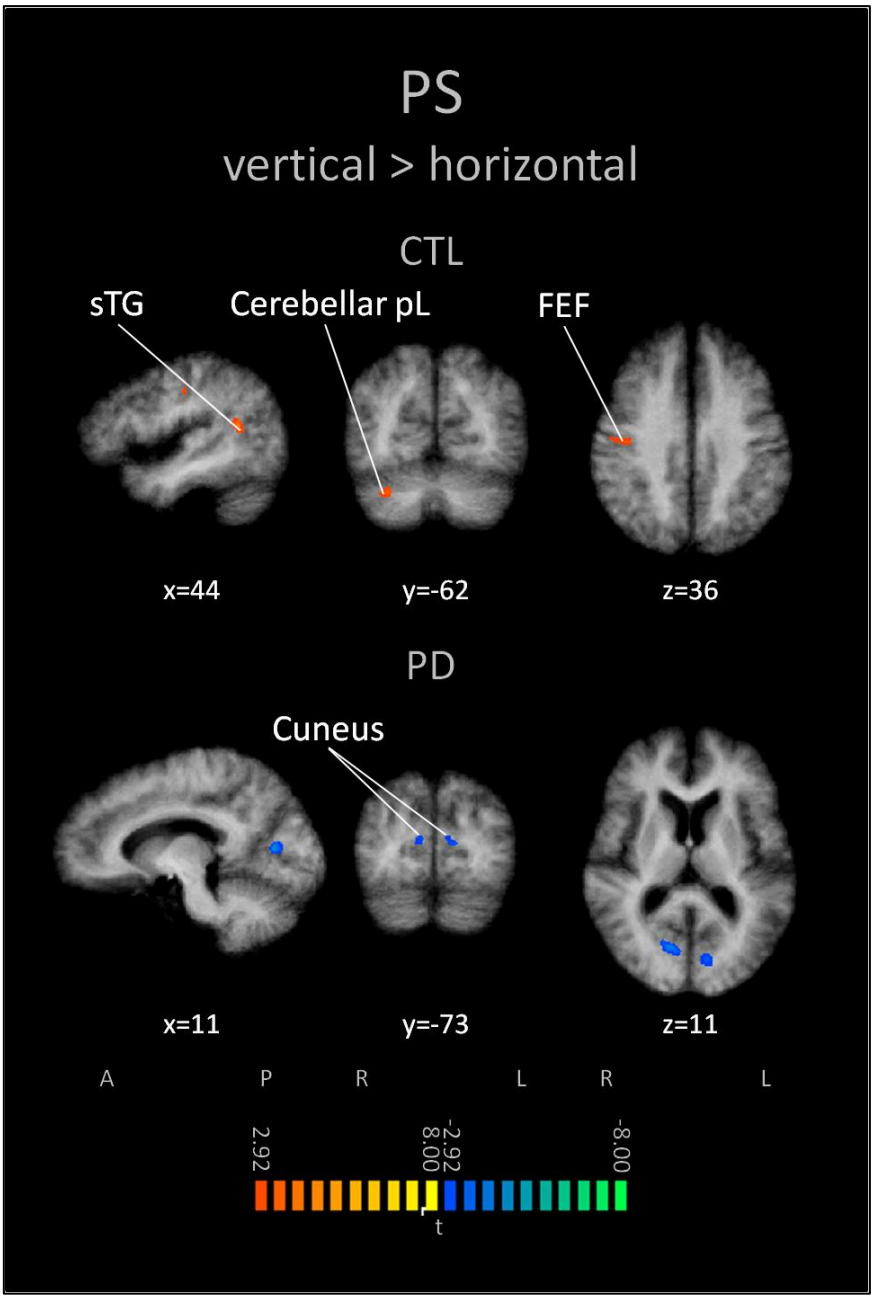

Figure 5. Group t-statistical activation maps comparing vertical prosaccades (PS) to horizontal PS in CTL and PD group ($p < 0.01$, corrected for multiple comparisons as estimated by Brain Voyager's Cluster-level Statistical Threshold Estimator with 1000 iterations). Brain areas showing significant activation in the control group (CTL, n= 17, upper panel) and PD group (PD, n=15, lower panel) are displayed on standard T1 image. Yellow/red regions represent greater activation for vertical PS than horizontal PS. Blue/green regions represent less activation for vertical PS than horizontal PS. A and P denote anterior and posterior. L and R denote left and right. Coordinate values of planes in Talairach space are indicated. CTL, controls; PD, Parkinson's disease; sTG, superior temporal gyrus; pL, (cerebellar) posterior lobe; FEF, frontal eye field

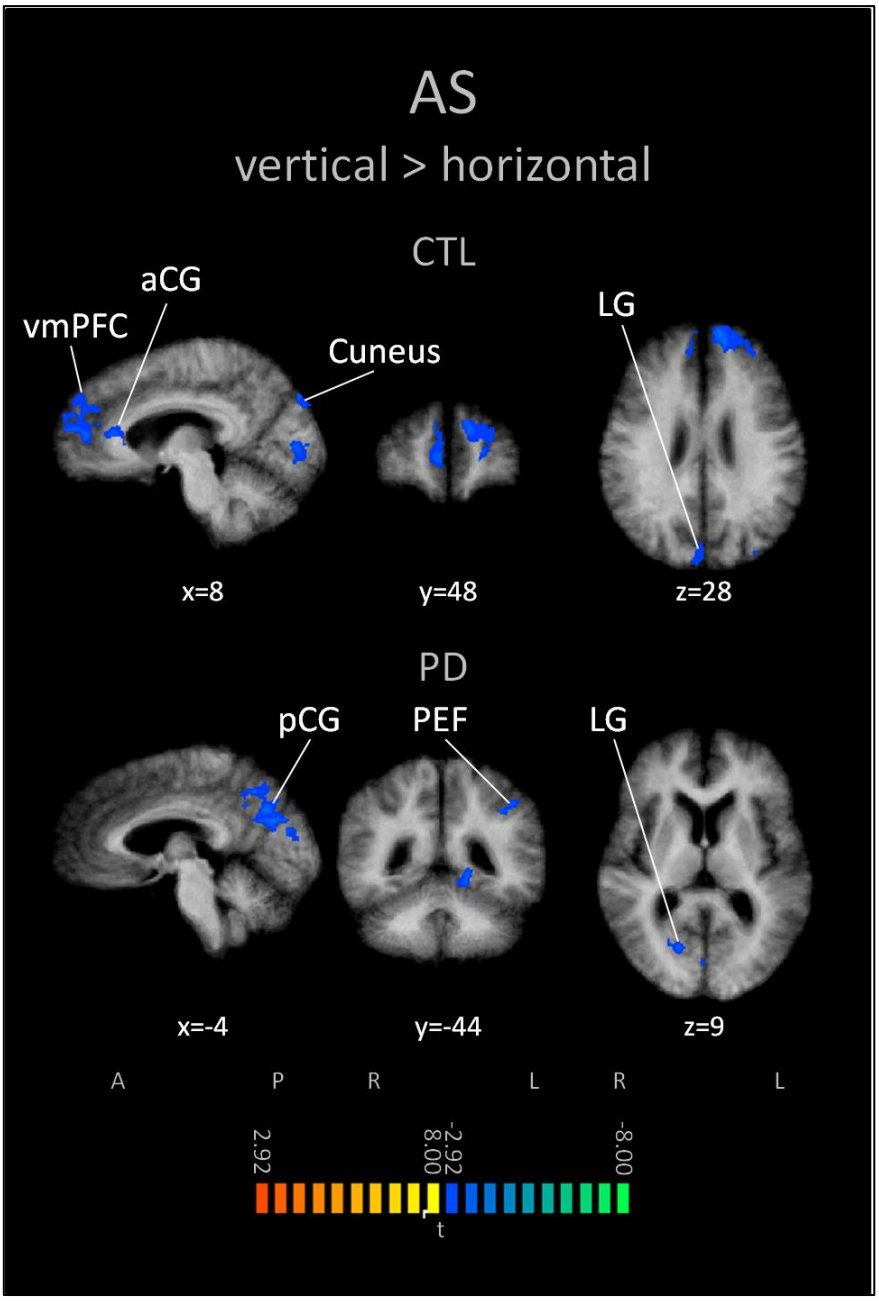

Figure 6. Group t-statistical activation maps comparing vertical antisaccades (AS) to horizontal AS in CTL and PD group ($p < 0.01$, corrected for multiple comparisons as estimated by Brain Voyager's Cluster-level Statistical Threshold Estimator with 1000 iterations). Brain areas showing significant activation in control group (CTL, n= 17, upper panel) and PD group (PD, n=15, lower panel) are displayed on standard T1 image. Yellow/red regions represent greater activation for vertical AS than horizontal AS. Blue/green regions represent less activation for vertical AS than horizontal AS. A and P denote anterior and posterior. L and R denote left and right. Coordinate values of planes in Talairach space are indicated. CTL, controls; PD, Parkinson's disease; vmPFC, ventromedial prefrontal cortex; aCG, anterior cingulate gyrus; LG, lingual gyrus; pCG, posterior cingulate gyrus; PEF, parietal eye field

3.5. Correlations between behavioral data and functional data.

We did not find any significant correlations between saccadic performance measured outside the scanner in the same day and fMRI BOLD activity for each group or when collapsing data across groups.

4. Discussion.

In this report, we have contributed to the understanding of the neural organization of the horizontal and vertical saccade systems in PD patients as well as in age-matched controls. Our study had a twofold purpose: (1) to investigate whether cortical and subcortical BOLD activity patterns reflect a distinct neural organization of horizontal and vertical saccade systems, as inspired by behavioural data suggesting asymmetries between saccade planes both in PD and healthy individuals [4, 5, 16]; (2) to attempt to identify compensatory BOLD patterns during saccade performance in PD patients, in whom specific saccadic behaviour is occasionally matched to those of controls [15, 29]. Indeed, in our behavioural study, saccade performance was almost identical between PD and control groups, with the exception of vertical antisaccades latency, which tended to be longer in PD patients, and thus highlighting the potential utility of adding vertical antisaccades to common saccade paradigms. Strikingly contrasting with behavioural data, albeit not for all directions or tasks, PD patients showed left FEF hypoactivity and right PEF hyperactivity when compared to controls, suggesting that the a frontal deficit can be compensated by the posterior areas in patients. PD patients were also less able to deactivate the default mode network than controls, particularly during vertical antisaccades, which further reinforces the idea that saccade-related cortical impairment in PD spreads beyond the frontal oculomotor network. Within controls, there was significantly greater right FEF and cerebellum BOLD activity during vertical prosaccades when compared to horizontal prosaccades. Vertical antisaccades were associated with greater deactivation of the default mode network than their horizontal counterpart in the same group. Within PD patients, cuneus was less active during prosaccades executed along the vertical plane. The same group showed less left FEF BOLD activity and greater DMN deactivation when antisaccades were executed along the vertical plane. These findings support the differential functional segregation of vertical and horizontal saccade cortical networks in health and disease.

4.1. The influence of demographic and clinical data on oculomotor behaviour.

Despite our intention of having both groups as homogeneous as possible, we were not able to match them for gender. Yet, gender did not seem to influence saccade metrics in a recent large study involving 145 healthy subjects [30]. Concerning PD motor status, although an effort was made to record PD patients' eye movements in the practically defined "off" condition [31], their median UPDRS III score (19) was in fact lower than

that reported in other oculomotor studies including PD patients that *kept* their medication during the study [15, 20, 21], suggesting that DP therapy 12 hour-withdrawal in our sample perhaps was not sufficient to achieve a true medication-off state. Alternatively, since our PD sample was only mildly affected (median H & Y, 1.5), patients in our sample may not be strongly medication-dependent.

4.2. Behavioural data.

We found no significant differences in reflexive and voluntary saccades between controls and PD patients, contrasting to a certain degree with others' findings [3–5, 9, 10, 32–35]. Previously reported abnormalities in PD seem to fall into three major findings: (1) saccade hypometria (antisaccades and prosaccades); (2) increased saccade latency (antisaccades, rarely in prosaccades); (3) and increased number of directional errors (antisaccades). While both hypometria and long latency seem to be caused by an excessive tonic supression of SC by substantia nigra *pars reticulata* (SNpr) probably heightened by the existence of a weak frontostriatal (FEF) pre-oculomotor drive, increased directional errors (automatic saccades) in the antisaccade task are thought to arise from dLPFC impairment and consequent loss of its inhibitory action on FEF and SC [7, 9, 33, 36]. Prosaccade amplitude and latency deficits in PD are usually subtle in early stages, the latter deficit being associated with cognitive decline [7]. This may explain the lack of differences in prosaccade performance between PD and controls in our sample. Prosaccade velocity on the other hand, especially in the vertical direction, seems to be relatively preserved in PD, possibly reflecting sparing of the midbrain vertical saccade generator in this disease. This is in sharp contrast with another parkinsonian disorder, i.e., progressive supranuclear palsy (PSP) in which the characteristic slowness of vertical saccades in these patients is associated with pronounced degeneration of the vertical saccade generator in the midbrain [37]. Antisaccades were indeed shorter and more delayed in PD patients, but only latency and exclusively for the vertical plane, reached a trend level ($p=0.082$, effect size=0.69) in between-groups comparison. The utility of vertical antisaccades latency measurement has been recently highlighted in another BG disorder (Huntington's disease), showing a striking correlation with atrophy in SEF, left inferior parietal lobule and caudate nuclei [38]. Since these areas also seem to be affected in PD (see above), vertical antisaccades latency may hold promise for future research as a marker of disease progression. Interestingly, within-groups analysis revealed a significant or near-significant delay of vertical saccades in both groups, which is consistent with earlier research in healthy individuals [16, 39]. Such directional bias appears to be the result of the following non-mutually exclusive reasons: (1) anatomical underrepresentation of the vertical meridian in brain areas involved in visual sensory processing (e.g., SC, visual cortex) [40–42]; (2) automatic orienting to salient quotidian objects, which tend to be horizontally distributed (bottom-up saliency) [17]; (3) top-down influence of cortical and subcortical areas involved in saccade processing such as FEF and PEF, reflecting a behavioural strategy influenced by societal/cultural experiences (e.g., reading habits) with

consequent behavioural advantage of the horizontal meridian [43, 44]; (4) additional neuronal degeneration of the same areas in senescence [45]. While it is tempting to postulate that neuronal degeneration may play a greater role in vertical vs. horizontal latency asymmetry in PD patients, our behavioural paradigm was not designed to specifically address the relative contribution of these factors. Correlation between saccadic performance and PD clinical data including the UPDRS III score did not herald significant results. We concur with Pinkhardt and colleagues' view that this lack of correlation may be due to the impact of extradopaminergic mechanisms (e.g., frontostriatal impairment) on PD eye movement disturbance [46]. Finally, it is worth mentioning that antisaccade error rate in our sample was higher than that usually described in the literature (<30%), which may have influenced BOLD signal responses (see below) [47]. Interestingly however, more contemporary work in normal individuals, also using vertical saccades, has provided similar antisaccade error rates, reaching up to 80% in advanced age [30]. We believe that having added a second dimension (vertical antisaccades) to our paradigm, especially in a interleaved fashion, may have increased task difficulty, thus making subjects more prone to antisaccade directional errors.

4.3. Imaging data.

In the present work it is striking how differences expressed by functional data contrasted with behavioural performance in group comparisons. During horizontal prosaccades, our study showed that PD patients exhibited less left FEF BOLD activity than controls. FEF activation during prosaccades is a consistent finding in the literature, possibly reflecting its role in saccade initiation [12]. This is particularly true for the left FEF, which may be related to a general motor dominance of the left hemisphere [48]. We think that decreased left FEF activity in PD patients is mostly due to a reduced nigro-thalamo-cortical output [14, 15, 49, 50]. The right PEF on the other hand showed *less* BOLD activity in controls than PD patients during the same task. Rieger et al. also found that BOLD decrease in PEF in PD patients was not as marked as in FEF, albeit in their case, both FEF and PEF activity was lower in PD than controls [14]. Taken together, we believe that the decreased activation in FEF relative to PEF in PD patients possibly represents a cortical reorganizational mechanism in response to FEF hypoactivation [14]. Within that perspective, Pflugshaupt et al. proposed a similar mechanism to explain the loss of exploratory saccades with apparent sparing of visually guided saccades in a patient recovering from a FEF lesion [51]. Importantly, the relative hyperactivity of PEF in PD patients may have helped maintain a normal saccadic performance in our sample [7, 9, 12]. The right cuneus was also more active in PD patients during horizontal prosaccades, which may have either represented a similar compensatory mechanism in visual cortex or an altered/unbalanced FEF and/or PEF top-down control on stimulus evaluation [52, 53]. Lastly, greater cerebellar BOLD signal (i.e., right anterior lobe and tonsil, and left inferior semilunar lobule) in PD during horizontal prosaccades may have also reflected a similar process [54]. Although

the inferior cerebellar lobule has been implicated in saccadic accuracy, the cerebellum does not seem to be directly involved in the etiopathogenesis of saccadic impairment in PD [55, 56]. In vertical prosaccades comparison between groups, results were distinct, considering that only PEF and inferior cerebellar lobule clusters were identified while the other clusters were absent. These group differences possibly reflect selective impairment of cortical subregions in PD that may drive saccades independently for the vertical and horizontal dimension (see below). Groups also differed concerning antisaccades functional activity. Here, areas that exhibited negative BOLD activity in prosaccade and antisaccade contrasts versus baseline and thought to correspond to the DMN (i.e., dmPFC and middle temporal gyrus), showed greater deactivation for controls than patients in both saccadic planes. This is consistent with previous PD research showing failure to deactivate parts of DMN during a facial emotion recognition task [57]. The presence of non-motor symptoms during the off-state may limit the availability of attentional resources and interfere in DMN deactivation [58]. The right middle occipital gyrus exhibited greater activity in controls during horizontal antisaccades, which could reflect impaired visual processing in PD patients [59]. We again saw greater PEF recruitment, putatively reflecting compensatory activity during horizontal antisaccades in the PD group.

Our last and main goal of contrast analyses dealt with functional differences between saccade planes in health and disease. To our knowledge, BOLD differences between vertical and horizontal saccades have not been investigated yet. However, in the clinic, vertical and horizontal saccadic amplitude and/or latency may be distinctively impaired in diseases such as PD, raising the possibility of disproportionate impairment of neuronal populations coding for vertical vs. horizontal saccades at a cortical and/or subcortical level [4, 60]. While fMRI spatial and temporal resolution may be insufficient to resolve topographical proximity and dynamic interplay of neural populations with vertical and horizontal response fields [61], we nevertheless hypothesized that pathological aging (i.e., PD) (or healthy aging per se, i.e., controls) could exacerbate vertical vs. horizontal saccade asymmetries to the point of making them recognizable at a functional cortical/subcortical level. Indeed, controls showed greater BOLD activity in the right FEF, cerebellar posterior lobe and superior temporal gyrus during vertical prosaccades, favouring the existence of an at least partial functional segregation for the vertical and horizontal cortical saccade motor networks. The finding concerning the right FEF is most interesting, since up to date the only reported strictly *unilateral* cortical lesion promoting loss of exploratory *vertical* saccades was located in the right FEF [51]. Similarly to horizontal saccades, there may be a right functional asymmetry of the saccadic system during the execution of vertical saccades [48]. Alternatively, instead of representing motor activity, the clusters found here could simply reflect greater recruitment of attentional resources during vertical saccades, since most of the salient information in everyday life is distributed along the horizontal dimension [17, 18, 62]. One additional factor that could have influenced FEF BOLD signal during vertical prosaccades is saccade latency, since previous data has shown a positive correlation between these variables [63, 64], and prosaccades latency

was indeed significantly greater for the vertical plane in our study. However, no correlation was seen between any of the saccade metrics and BOLD activity in any of the clusters. Vertical vs. horizontal prosaccades contrast in PD was sharply different. Here only negative BOLD activity was seen (implying larger activity for horizontal prosaccades), specifically in cuneus bilaterally (putatively, areas V2/3) [65]. The presence of negative cuneus activity in PD may indicate that, unlike healthy participants, fewer resources are used to guide attention along the vertical relative to the horizontal dimension. In fact, weakened spatio-attentional processing in PD has been shown before within the vertical and horizontal dimension, namely for the left and superior visual fields [66], but we are not aware of a previous comparison *between* planes. Alternatively, relative hypoactivity in the cuneus during vertical prosaccades in PD could represent a reduced top-down control from FEF and/or PEF on stimulus direction-specific modulation by cuneus [50, 53, 67], or an underrepresentation of the vertical meridian in visual cortex [40]. Contrasts in antisaccades were equally revealing. Vertical antisaccades were associated with stronger deactivation of areas that overlap with the DMN (e.g., vmPFC, posterior cingulate gyrus) in both groups, albeit additional anterior cingulate gyrus and dmPFC deactivations were only observed in controls. This suggests greater attentional and/or working memory demands for vertical than horizontal antisaccades [68, 69]. Recently, Georges et al. demonstrated that altered DMN connectivity was closely correlated with vertical prosaccades amplitude in PD, highlighting the importance of DMN integrity in the generation of eye movements [10, 70]. The lingual gyrus showed less activation during vertical antisaccades in both groups, which could also be related to the underrepresentation of the vertical meridian in visual cortex or may reflect biased spatial attention along the horizontal meridian [18, 40]. Finally, left PEF BOLD signal was decreased in vertical antisaccades only in PD, which may suggest that the compensatory shift from FEF to PEF previously discussed, is probably not without a cost [9, 14]. Just as it has been shown that PEF and FEF seem to exhibit *directional* preferences during the execution of vertical saccades (e.g., upward bias for cue-related activity in left FEF vs. downward bias for saccade-related activity in left PEF) [71], we hypothesize that these areas may also exhibit saccade *plane* preferences (e.g. horizontal bias for left PEF). Consequently, the compensatory contribution of left PEF in PD patients, which was underactive during vertical antisaccades, may come with the additional cost of enhancing a normal bias between vertical and horizontal saccades, potentiating performance differences between saccade planes in PD.

4.4. Study limitations.

Our study has several limitations. Because we used a block design, it is not possible to investigate at the event level if the observed BOLD activation differences were attributable to stimulus-related activity or saccade-related activity, or a combination of these. Moreover, we were not able to dissect the possible effect of the antisaccade direction errors in BOLD responses [72] . Thus, caution is necessary in overinterpreting

antisaccades fMRI findings. It is known that areas including the presupplementary motor area, SEF, dlPFC, anterior cingulate gyrus, superior frontal sulcus, FEF and PEF seem to be distinctively activated by correct antisaccades and antisaccade errors [72, 73]. Since antisaccade error rate outside fMRI was equally high in PD patients and controls, it is therefore difficult to ascertain the relative contribution of antisaccade errors versus corrected antisaccades on the BOLD differences found between vertical and horizontal antisaccades. Nevertheless, antisaccade errors outside fMRI did not significantly differ between the horizontal and vertical plane, which suggests that the BOLD differences found between antisaccade planes cannot be accounted by the amount of antisaccade errors. We were also not able to carry an off-line analysis of the eye movement data collected inside the MRI scanner, which limits correlational analysis to the same data acquired outside the scanner in the same day [74]. It is also possible that fatigue and order effects might have influenced the antisaccade task. We did not conduct retinotopic mapping, which would have provided more insight on BOLD activity location during saccade performance [65, 75]. Finally, we used Mini Mental State Examination score to evaluate cognition in PD. Currently there are other screening tests such as Montreal Cognitive Assessment (MoCA), which demonstrate greater diagnostic accuracy for screening all levels of cognition in PD [76].

4.5. Conclusions.

In sum, we have demonstrated frontal hypoactivity and possible compensatory parietal overactivity in PD patients during saccade execution in the absence of significant behavioral saccadic deficits. Moreover, we found BOLD differences between horizontal and vertical planes both in PD patients and healthy controls, suggesting that normal physiological cortical asymmetries do exist during the execution of saccades at an attentional and/or motor level, and can be further modified in disease. Therefore, altered and/or compensatory brain activity may predate and/or mask saccade abnormalities in PD. Our results may also help to explain previous inconsistencies in PD oculomotor research and serve as a basis for the development of future studies addressing cortical control of saccades in disorders where there is a disproportionate impairment between saccade planes.

Competing interests.

The authors declare that they have no conflicts of interest related to this manuscript.

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References.

1. Leigh RJ, Zee DS (2015) The neurology of eye movements, 5th ed. Oxford University Press, New York
2. Lees AJ, Hardy J, Revesz T (2009) Parkinson's disease. Lancet (London, England) 373:2055–66. doi: 10.1016/S0140-6736(09)60492-X
3. Briand KA, Strallow D, Hening W, et al (1999) Control of voluntary and reflexive saccades in Parkinson's disease. Exp brain Res 129:38–48.
4. Rottach KG, Riley DE, DiScenna AO, et al (1996) Dynamic properties of horizontal and vertical eye movements in parkinsonian syndromes. Ann Neurol 39:368–77. doi: 10.1002/ana.410390314
5. Bonnet C, Rusz J, Megrelishvili M, et al (2014) Eye movements in ephedrone-induced parkinsonism. PLoS One 9:e104784. doi: 10.1371/journal.pone.0104784
6. Antoniades C, Kennard C (2014) Ocular motor abnormalities in neurodegenerative disorders. Eye. doi: 10.1038/eye.2014.276
7. Macaskill MR, Graham CF, Pitcher TL, et al (2012) The influence of motor and cognitive impairment upon visually-guided saccades in Parkinson's disease. Neuropsychologia 50:3338–47. doi: 10.1016/j.neuropsychologia.2012.09.025
8. MacAskill MR, Anderson TJ (2016) Eye movements in neurodegenerative diseases. Curr Opin Neurol 29:61–8. doi: 10.1097/WCO.0000000000000274
9. Terao Y, Fukuda H, Yugeta A, et al (2011) Initiation and inhibitory control of saccades with the progression of Parkinson's disease - changes in three major drives converging on the superior colliculus. Neuropsychologia 49:1794–806. doi: 10.1016/j.neuropsychologia.2011.03.002
10. Gorges M, Müller H-P, Lulé D, et al (2013) Functional connectivity within the default mode network is associated with saccadic accuracy in Parkinson's disease: a resting-state FMRI and videooculographic study. Brain Connect 3:265–72. doi: 10.1089/brain.2013.0146
11. Domagalik A, Beldzik E, Fafrowicz M, et al (2012) Neural networks related to pro-saccades and anti-saccades revealed by independent component analysis. Neuroimage 62:1325–33. doi: 10.1016/j.neuroimage.2012.06.006
12. McDowell JE, Dyckman KA, Austin BP, Clementz BA (2008) Neurophysiology and neuroanatomy of reflexive and volitional saccades: evidence from studies of humans. Brain Cogn 68:255–70. doi: 10.1016/j.bandc.2008.08.016
13. Bender MB (1960) Comments on the physiology and pathology of eye movements in the vertical plane. J Nerv Ment Dis 130:156–66.
14. Rieger JW, Kim A, Argyelan M, et al (2008) Cortical functional anatomy of voluntary saccades in Parkinson disease. Clin EEG Neurosci 39:169–74.
15. Cameron IGM, Pari G, Alahyane N, et al (2012) Impaired executive function signals in motor brain regions in Parkinson's disease. Neuroimage 60:1156–70. doi: 10.1016/j.neuroimage.2012.01.057
16. Dafoe JM, Armstrong IT, Munoz DP (2007) The influence of stimulus direction and eccentricity on pro- and anti-saccades in humans. Exp brain Res 179:563–70. doi: 10.1007/s00221-006-0817-8
17. Foulsham T, Kingstone A, Underwood G (2008) Turning the world around: patterns in saccade direction vary with picture orientation. Vision Res 48:1777–90. doi: 10.1016/j.visres.2008.05.018
18. Mao L, Zhou B, Zhou W, Han S (2007) Neural correlates of covert orienting of visual spatial attention along vertical and horizontal dimensions. Brain Res 1136:142–53. doi: 10.1016/j.brainres.2006.12.031
19. Gibb WR, Lees AJ (1988) The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 51:745–52.
20. Crevits L, Versijpt J, Hanse M, De Ridder K (2000) Antisaccadic effects of a dopamine agonist as add-on therapy in advanced Parkinson's patients. Neuropsychobiology 42:202–6. doi: 26694
21. Hood AJ, Amador SC, Cain AE, et al (2007) Levodopa slows prosaccades and improves

antisaccades: an eye movement study in Parkinson's disease. J Neurol Neurosurg Psychiatry 78:565–70. doi: 10.1136/jnnp.2006.099754
22. Ogawa S, Lee TM, Kay AR, Tank DW (1990) Brain magnetic resonance imaging with contrast dependent on blood oxygenation. Proc Natl Acad Sci U S A 87:9868–72.
23. Bandettini PA, Cox RW (2000) Event-related fMRI contrast when using constant interstimulus interval: theory and experiment. Magn Reson Med 43:540–8.
24. Talairach J (Jean), Tournoux P (1988) Co-planar stereotaxic atlas of the human brain : 3-dimensional proportional system : an approach to cerebral imaging. G. Thieme
25. Forman SD, Cohen JD, Fitzgerald M, et al (1995) Improved assessment of significant activation in functional magnetic resonance imaging (fMRI): use of a cluster-size threshold. Magn Reson Med 33:636–47.
26. Aichert DS, Williams SCR, Möller H-J, et al (2012) Functional neural correlates of psychometric schizotypy: an fMRI study of antisaccades. Psychophysiology 49:345–56. doi: 10.1111/j.1469-8986.2011.01306.x
27. Linzenbold W, Lindig T, Himmelbach M (2011) Functional neuroimaging of the oculomotor brainstem network in humans. Neuroimage 57:1116–23. doi: 10.1016/j.neuroimage.2011.05.052
28. Jellinger KA (1991) Pathology of Parkinson's disease. Changes other than the nigrostriatal pathway. Mol Chem Neuropathol 14:153–97.
29. Lueck CJ, Tanyeri S, Crawford TJ, et al (1990) Antisaccades and remembered saccades in Parkinson's disease. J Neurol Neurosurg Psychiatry 53:284–8.
30. Bonnet C, Hanuška J, Rusz J, et al (2013) Horizontal and vertical eye movement metrics: what is important? Clin Neurophysiol 124:2216–29. doi: 10.1016/j.clinph.2013.05.002
31. Langston JW, Widner H, Goetz CG, et al (1992) Core assessment program for intracerebral transplantations (CAPIT). Mov Disord 7:2–13. doi: 10.1002/mds.870070103
32. Armstrong IT, Chan F, Riopelle RJ, Munoz DP (2002) Control of saccades in Parkinson's disease. Brain Cogn 49:198–201.
33. Chan F, Armstrong IT, Pari G, et al (2005) Deficits in saccadic eye-movement control in Parkinson's disease. Neuropsychologia 43:784–96. doi: 10.1016/j.neuropsychologia.2004.06.026
34. DeJong JD, Jones GM (1971) Akinesia, hypokinesia, and bradykinesia in the oculomotor system of patients with Parkinson's disease. Exp Neurol 32:58–68.
35. White OB, Saint-Cyr JA, Tomlinson RD, Sharpe JA (1983) Ocular motor deficits in Parkinson's disease. II. Control of the saccadic and smooth pursuit systems. Brain 106 (Pt 3):571–87.
36. Kimmig H, Haussmann K, Mergner T, Lücking CH (2002) What is pathological with gaze shift fragmentation in Parkinson's disease? J Neurol 249:683–92. doi: 10.1007/s00415-002-0691-7
37. Chen AL, Riley DE, King SA, et al (2010) The disturbance of gaze in progressive supranuclear palsy: implications for pathogenesis. Front Neurol 1:147. doi: 10.3389/fneur.2010.00147
38. Rupp J, Dzemidzic M, Blekher T, et al (2012) Comparison of vertical and horizontal saccade measures and their relation to gray matter changes in premanifest and manifest Huntington disease. J Neurol 259:267–76. doi: 10.1007/s00415-011-6172-0
39. Goldring J, Fischer B (1997) Reaction times of vertical prosaccades and antisaccades in gap and overlap tasks. Exp brain Res 113:88–103.
40. Janik J, Ropella K, DeYoe E (2003) Distortions of human retinotopy obtained with temporal phase mapped fMRI. In: Soc Neurosci Abstr. p 29:658
41. Schneider KA, Richter MC, Kastner S (2004) Retinotopic organization and functional subdivisions of the human lateral geniculate nucleus: a high-resolution functional magnetic resonance imaging study. J Neurosci 24:8975–85. doi: 10.1523/JNEUROSCI.2413-04.2004
42. Schneider KA, Kastner S (2005) Visual responses of the human superior colliculus: a high-resolution functional magnetic resonance imaging study. J Neurophysiol 94:2491–503. doi: 10.1152/jn.00288.2005
43. Abed F (1991) Cultural Influences on Visual Scanning Patterns. J Cross Cult Psychol 22:525–534. doi: 10.1177/0022022191224006
44. Bell AH, Everling S, Munoz DP (2000) Influence of stimulus eccentricity and direction on characteristics of pro- and antisaccades in non-human primates. J Neurophysiol 84:2595–604.

45. Yang Q, Kapoula Z (2006) The control of vertical saccades in aged subjects. Exp brain Res 171:67–77. doi: 10.1007/s00221-005-0249-x
46. Pinkhardt EH, Jürgens R, Lulé D, et al (2012) Eye movement impairments in Parkinson's disease: possible role of extradopaminergic mechanisms. BMC Neurol 12:5. doi: 10.1186/1471-2377-12-5
47. Hutton SB, Ettinger U (2006) The antisaccade task as a research tool in psychopathology: A critical review. Psychophysiology 43:302–313. doi: 10.1111/j.1469-8986.2006.00403.x
48. Petit L, Zago L, Vigneau M, et al (2009) Functional Asymmetries Revealed in Visually Guided Saccades: An fMRI Study. J Neurophysiol 102:2994–3003. doi: 10.1152/jn.00280.2009
49. Alexander GE, DeLong MR, Strick PL (1986) Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu Rev Neurosci 9:357–81. doi: 10.1146/annurev.ne.09.030186.002041
50. Logothetis NK, Wandell BA (2004) Interpreting the BOLD signal. Annu Rev Physiol 66:735–69. doi: 10.1146/annurev.physiol.66.082602.092845
51. Pflugshaupt T, Nyffeler T, von Wartburg R, et al (2009) Loss of exploratory vertical saccades after unilateral frontal eye field damage. BMJ Case Rep. doi: 10.1136/bcr.08.2008.0687
52. Bressler SL, Tang W, Sylvester CM, et al (2008) Top-down control of human visual cortex by frontal and parietal cortex in anticipatory visual spatial attention. J Neurosci 28:10056–61. doi: 10.1523/JNEUROSCI.1776-08.2008
53. McDowell JE, Kissler JM, Berg P, et al (2005) Electroencephalography/magnetoencephalography study of cortical activities preceding prosaccades and antisaccades. Neuroreport 16:663–8.
54. Wu T, Hallett M (2013) The cerebellum in Parkinson's disease. Brain 136:696–709. doi: 10.1093/brain/aws360
55. Ettinger U, Antonova E, Crawford TJ, et al (2005) Structural neural correlates of prosaccade and antisaccade eye movements in healthy humans. Neuroimage 24:487–94. doi: 10.1016/j.neuroimage.2004.08.019
56. MacAskill MR, Anderson TJ, Jones RD (2002) Adaptive modification of saccade amplitude in Parkinson's disease. Brain 125:1570–82.
57. Delaveau P, Salgado-Pineda P, Fossati P, et al (2010) Dopaminergic modulation of the default mode network in Parkinson's disease. Eur Neuropsychopharmacol 20:784–92. doi: 10.1016/j.euroneuro.2010.07.001
58. Apkarian AV, Bushnell MC, Treede R-D, Zubieta J-K (2005) Human brain mechanisms of pain perception and regulation in health and disease. Eur J Pain 9:463–84. doi: 10.1016/j.ejpain.2004.11.001
59. Watanabe H, Senda J, Kato S, et al (2013) Cortical and subcortical brain atrophy in Parkinson's disease with visual hallucination. Mov Disord 28:1732–6. doi: 10.1002/mds.25641
60. Bender MB (1980) Brain control of conjugate horizontal and vertical eye movements: a survey of the structural and functional correlates. Brain 103:23–69.
61. Everling S, Munoz DP (2000) Neuronal correlates for preparatory set associated with pro-saccades and anti-saccades in the primate frontal eye field. J Neurosci 20:387–400.
62. Grosbras M-H, Laird AR, Paus T (2005) Cortical regions involved in eye movements, shifts of attention, and gaze perception. Hum Brain Mapp 25:140–54. doi: 10.1002/hbm.20145
63. Neggers SFW, Raemaekers MAH, Lampmann EEL, et al (2005) Cortical and subcortical contributions to saccade latency in the human brain. Eur J Neurosci 21:2853–63. doi: 10.1111/j.1460-9568.2005.04129.x
64. Ozyurt J, Greenlee MW (2011) Neural correlates of inter- and intra-individual saccadic reaction time differences in the gap/overlap paradigm. J Neurophysiol 105:2438–47. doi: 10.1152/jn.00660.2009
65. Dougherty RF, Koch VM, Brewer AA, et al (2003) Visual field representations and locations of visual areas V1/2/3 in human visual cortex. J Vis 3:586–98. doi: 10.1167/3.10.1
66. Nys GMS, Santens P, Vingerhoets G (2010) Horizontal and vertical attentional orienting in Parkinson's disease. Brain Cogn 74:179–85. doi: 10.1016/j.bandc.2010.07.004
67. Simpson G V, Weber DL, Dale CL, et al (2011) Dynamic activation of frontal, parietal, and sensory regions underlying anticipatory visual spatial attention. J Neurosci 31:13880–9. doi:

10.1523/JNEUROSCI.1519-10.2011
68. Mayer JS, Roebroeck A, Maurer K, Linden DEJ (2010) Specialization in the default mode: Task-induced brain deactivations dissociate between visual working memory and attention. Hum Brain Mapp 31:126–39. doi: 10.1002/hbm.20850
69. Persson J, Lustig C, Nelson JK, Reuter-Lorenz PA (2007) Age differences in deactivation: a link to cognitive control? J Cogn Neurosci 19:1021–32. doi: 10.1162/jocn.2007.19.6.1021
70. Gorges M, Müller H-P, Lulé D, et al (2015) The association between alterations of eye movement control and cerebral intrinsic functional connectivity in Parkinson's disease. Brain Imaging Behav 10:79–91. doi: 10.1007/s11682-015-9367-7
71. Tzelepi A, Laskaris N, Amditis A, Kapoula Z (2010) Cortical activity preceding vertical saccades: a MEG study. Brain Res 1321:105–16. doi: 10.1016/j.brainres.2010.01.002
72. Ford KA, Goltz HC, Brown MRG, Everling S (2005) Neural processes associated with antisaccade task performance investigated with event-related FMRI. J Neurophysiol 94:429–40. doi: 10.1152/jn.00471.2004
73. Curtis CE, D'Esposito M (2003) Success and failure suppressing reflexive behavior. J Cogn Neurosci 15:409–18. doi: 10.1162/089892903321593126
74. Müri RM, Heid O, Nirkko AC, et al (1998) Functional organisation of saccades and antisaccades in the frontal lobe in humans: a study with echo planar functional magnetic resonance imaging. J Neurol Neurosurg Psychiatry 65:374–7.
75. Rieger JW, Schoenfeld MA, Heinze H-J, Bodis-Wollner I (2008) Different spatial organizations of saccade related BOLD-activation in parietal and striate cortex. Brain Res 1233:89–97. doi: 10.1016/j.brainres.2008.07.108
76. Dalrymple-Alford JC, MacAskill MR, Nakas CT, et al (2010) The MoCA: well-suited screen for cognitive impairment in Parkinson disease. Neurology 75:1717–25. doi: 10.1212/WNL.0b013e3181fc29c9

Chapter V

Cortical control of vertical and horizontal saccades in progressive supranuclear palsy: an exploratory fMRI study

Abstract.

Progressive Supranuclear Palsy (PSP) is a neurodegenerative disorder showing predominant brainstem involvement, thought to promote marked slowing of rapid eye movements (saccades), particularly along the vertical plane. While the contribution of the brainstem damage for the saccadic disturbance in PSP has been extensively studied, much less is known about its cortical and subcortical pathomechanisms. We measured reflexive (prosaccades) and voluntary (antisaccades) saccades in the vertical and horizontal plane in PSP patients (n=8) and controls (n=10) in an eye tracking study, followed by the measurement of blood oxygenation-level dependent (BOLD) activation (PSP, n=6; controls, n=10) during similar saccade paradigms. Behaviorally, PSP patients evidenced slower and shorter prosaccades (horizontal and vertical) and shorter antisaccades (vertical) than controls. Functionally, patients showed decreased frontostriatal BOLD activation during prosaccades (horizontal and vertical) and antisaccades (vertical), relative to controls. Additionally, PSP patients showed less default mode network (DMN) deactivation than controls for all types of saccades. Within groups, controls showed no BOLD differences between horizontal and vertical prosacades while PSP patients demonstrated greater DMN deactivation during vertical prosaccades. Both groups evidenced greater DMN deactivation during vertical antisaccades when compared to their horizontal counterpart and patients further showed relative frontostriatal BOLD hypoactivity during vertical antisaccades. We found fMRI evidence of frontostriatal hipoactivity in PSP patients relative to controls, especially during vertical saccades. These new findings highlight the impact of cortical impairment in saccadic disturbance of PSP.

Keywords.

Progressive supranuclear palsy; Saccades; Functional MRI; Basal ganglia; Eye movement measurements

1. Introduction.

Saccadic behaviour ranges from reflexive movements directed towards a stimulus (prosaccade) to voluntary movements directed away from the target (antisaccade) [1]. Functional Magnetic Resonance Imaging (fMRI) studies have demonstrated that horizontal saccades reliably activate a cortical oculomotor network comprising the frontal eye field (FEF), supplementary eye field (SEF) and parietal eye field (PEF) and an executive control network including the dorsolateral prefrontal cortex (dlPFC), usually at a greater extent for antisaccades, in which additional default-mode network (DMN) deactivation may be seen [2–5]. Concerning vertical saccades, previous research has widely focused on their brainstem control mechanisms, but little or no attention has been given to their cortical control [6–8]. Previous electrophysiological data support the view that horizontal saccades are generated predominantly by the contralateral cortex while vertical saccades require simultaneous bilateral cortical activation of the same neural pathways [9]. However, we did not find in the literature fMRI studies that specifically contrasted blood-oxygen-level dependent (BOLD) responses between horizontal and vertical prosaccades or antisaccades.

Also in the clinic, evaluation of saccades both in the horizontal and vertical plane is of paramount importance, since certain disorders can present with isolated impairment of vertical saccades early in the course of disease. A classical example is progressive supranuclear palsy (PSP), a severe neurodegenerative disorder characterized by symmetric akinetic-rigidity, postural instability, frontal behavioural dysfunction and slow velocity and hypometric vertical prosaccades [10, 11] . The latter finding is considered to be a cardinal feature of the disease and is usually followed by similar impairment of horizontal prosaccades later on the disease. Sequential involvement of midbrain and pons in the brainstem is believed to account for the oculomotor findings [12, 13]. Prosaccade latency data on the other hand has given inconsistent results. Horizontal prosaccades latency has been described as normal [14, 15], increased [16] or highly variable [17], while vertical prosaccades latency seems to be increased [18]. This possibly reflects variable involvement of the cortical decision network including areas such as the dlPFC and PEF [15, 17]. Concerning antisaccades, PSP patients characteristically show increased number of direction errors and prolonged latency along the horizontal plane, suggesting additional involvement of the dlPFC and/or superior colliculus [14, 15]. To the authors' best knowledge, no study has addressed vertical antisaccades to evaluate PSP patients. Taken together, while saccadic disturbance in PSP has been largely attributed to the extensive brainstem damage, less is known about the contribution of cortical and subcortical involvement to oculomotor deficits. Recently, Amtage et al. in a fluorodeoxyglucose positron emission tomography (FDG-PET) study with PSP patients found a correlation between prosaccades velocity and amplitude, and metabolic activity in the cerebellar vermis and right anterior cingulate gyrus, highlighting the growing importance of addressing cortical function in PSP oculomotor studies [19].

In this study we first investigated the dynamic properties of horizontal and vertical saccades in two separate blocks (prosaccades; antisaccades) in an eye-tracking behavioural experiment. This was followed by an fMRI block-design experiment using a similar oculomotor paradigm. Apart from the expected deficits at a behavioral level in PSP patients, we hypothesized that PSP patients should evidence an overall decrease of BOLD activity in FEF, dlPFC and/or PEF, where the major cortical pathological burden of the disease is thought to occur [18, 20]. We further conjectured that patients should demonstrate cortical and/or subcortical BOLD signal differences between vertical and horizontal saccades, taking into account the previously observed latency and/or amplitude bias between saccade planes in PSP [18]. Regarding controls, we made no specific predictions for the BOLD contrast between saccade planes, although subtle behavioural asymmetries (e.g, shorter latency of horizontal prosaccades) have been described, possibly reflecting the relevance of the horizontal meridian in our quotidian [21, 22]. We did not expect to find brainstem BOLD differences between saccade planes, due to the anatomical constraints which usually preclude the use of fMRI in this area [23]. We believe that this study offers new insights into the cortical and subcortical correlates of saccade execution in PSP patients.

2. Methods.

2.1. Participants.

Eight individuals with probable PSP (6 males, mean age 74.3±7.3 S.D. years, range 64-84) were recruited from our movement disorders clinic at Coimbra University Hospital Center, from April 2013 to May 2015, along with 10 age-, gender- and mood-matched controls (3 males, mean age 71.5±6.5 S.D. years, range 63-82) free of any neurological, psychiatric or visual disorder (other than refractive error). Subjects in the latter group did not take any medication known to affect oculomotor behaviour. The diagnosis of PSP was made according to the NINDS-SPSP criteria and was confirmed by a movement disorder specialist [10]. Importantly, by using these criteria we excluded PSP subtypes other than Richardson syndrome, since many of the these subtypes either do not show a clear eye movement disturbance or need more data to better delineate its ocular motor impairment [11, 16, 24]. Participants in either group underwent an evaluation of cognitive status (Mini-Mental State Examination, MMSE), and depression (30 item Geriatric Depression Scale, GDS). Exclusion criteria included other forms of parkinsonism, severe dementia (MMSE < 15), moderate to severe depression (GDS > 21), normal or corrected-to-normal visual acuity worse than 6/12 in the best eye, excessive head movement (>2mm) during fMRI scanning, inability to perform the oculomotor task outside and inside fMRI and the presence of structural abnormalities affecting known saccade regions in MRI (i.e., FEF, SEF, PEF, basal ganglia, and dlPFC). All subjects gave their signed and informed consent. The study was in agreement with the Declaration of Helsinki and accepted by the Ethics Committee of

the University of Coimbra. Clinical data and participant demographics are shown in **Table 1**.

Table 1. Demographic and Clinical Data

	PSP (n=8)	CTL (n = 10)	P Value*
Median age (IQR), years	75 (14)	70 (10)	0.397
Gender male/female, no.	6/2	3/7	0.153
Median GDS score (IQR)	12 (5)	7.5 (11)	0.305
Median MMSE score (IQR)	26 (6)	29.5 (3)	**0.019**
Median disease duration, years (IQR)	4.2 (3)	NA	

Significant values (P<0.05) are marked in bold. PSP, progressive supranuclear palsy; CTL, controls. IQR, interquartile range; GDS, Geriatric Depression Scale; MMSE, Mini Mental State Examination; NA, not applicable.

*CTL-PSP; statistical analysis was performed using Fisher's exact test and the Mann–Whitney-U-test

2.2. Procedures.

Each participant first underwent a behavioral saccade task session outside MRI, which was then followed by an fMRI experiment in the same day using a similar saccade task. Altogether these sessions, plus the breaks lasted approximately 3 hours. Two PSP patients were excluded from the fMRI study due to excessive head movement during fMRI.

2.2.1. Behavioral procedure.

The initial experiment consisted of an oculomotor behavior paradigm outside MRI where subjects performed a ~5-min block of prosaccades followed by a ~5-min block of antisaccades, each block comprising 64 trials. A practice run of 20 trials was completed prior to each block, to demonstrate paradigm requirements. Subjects were seated in a dark room facing the center of a computer screen monitor, at a viewing distance of 70 cm. A remote, contact-free binocular eyetracking setup with automatic eye and head tracking was used (RED500, SMI, Germany). Two dimensional movements of the left eye were recorded with iViewX™ at a 500Hz sampling rate. Visual stimuli were programmed using Presentation software (Version 14.9; Neurobehavioral Systems Inc., CA) and comprised a white fixation cross at the center of the screen and four blue targets located 10° left, 10° right, 10° above and 10° below the fixation cross. In the prosaccade block, each trial set off with a white fixation cross at the center of the

screen. An eccentric blue target appeared in the screen on one of four random directions (right, left, up, and down) at the time of the extinction of the central fixation cross (no gap paradigm). The subjects were instructed to look at the fixation cross and then to make a saccade toward the eccentric target as soon as it appeared, as fast and accurate as possible. The fixation cross duration and target position were randomized and counterbalanced. The antisaccade block comprised an identical sequence of events as the prosaccade experiment except that participants were instructed to look at the mirror location opposite to the location of the visually presented stimulus target. Examples of the sequence of events for the prosaccade and antisaccade task are shown in **Fig. 1.a**.

2.2.2. Imaging procedure.

Functional images were then acquired while each participant executed similar oculomotor experiments over 2 consecutive runs in the MRI scanner. Scans were performed at the Institute of Nuclear Sciences Applied to Health, Coimbra using a 3.0-tesla magnetic field strength Siemens Magnetom Trio scanner (Erlangen, Germany). High-resolution structural images were acquired using a three-dimensional T1 MPRAGE (magnetization prepared rapid gradient echo) anatomical sequence for coregistration with fMRI data. Functional scans were collected using a two-dimensional GRE EPI (gradient-echo echo-planar imaging) sequence sensitive to BOLD contrast. Images were acquired during two block-design runs with a total scanning time per subject of 15 min and 25 s. Visual stimuli were generated using Presentation software (Version 14.9; Neurobehavioral Systems Inc., CA) on a personal computer. Images were back-projected onto an MRI-compatible high-contrast screen pad positioned at the rear of the magnet bore. Subjects viewed the images via a reflection mirror located above their eyes. Movements of the left eye were recorded using an MRI-compatible infrared oculographic pupil tracker (SMI SensoMotoric Instruments) positioned on the head coil, at a sampling rate of 60 Hz. First run consisted of a prosaccade task containing 3 blocks of horizontal prosaccades (6 trials each) and 3 blocks of vertical prosaccades (6 trials each), randomly interleaved and separated by 15 s of blank screen (baseline condition). Each block lasted 27s. Trials were 4500 ms in length (including intertrial interval). The trials had identical stimulus characteristics to those displayed in the behavioral task outside MRI (see above), although target amplitude was shorter (8°) to accommodate screen size. Also here, target location was randomized and counterbalanced in each block (i.e., 3 right, 3 left in horizontal prosaccades; 3 up, 3 down in vertical prosaccades), The second run consisted on the same stimulus but subjects were instructed to perform antisaccades (**Fig. 1.b**). During the baseline condition participants were instructed to keep their eyes centered on the screen.

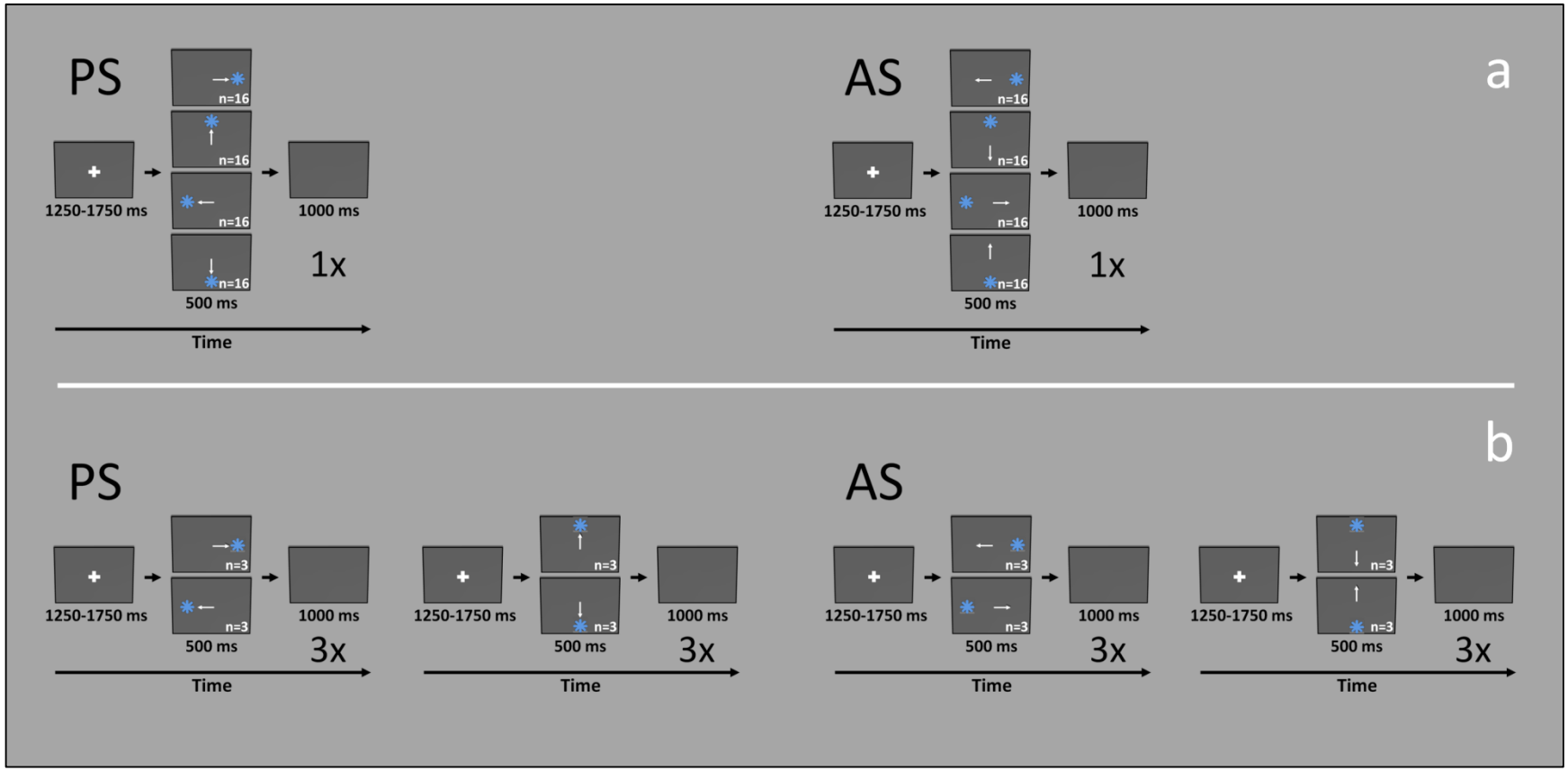

Figure 1. Schematic representation of stimuli and time course of the oculomotor tasks outside (**a**) and inside the scanner (**b**). Participants were instructed to keep foveating a central fixation cross until its extinction (total fixation time, 1250-1750 ms) (left, on each panel). Immediately after, participants were required to make a saccade towards (prosaccade, PS) or to the opposite mirror location (antisaccade, AS) of an eccentric target, randomly appearing for 500 ms on one of four locations (10° up, 10° down, 10° left, or 10° right) (center, on each panel). The screen was then cleared for 1000 ms (right, on each panel). An intertrial interval of 1500 ms was interposed between trials (not shown). Note that the paradigms outside and inside fMRI exhibited slight differences. Thus, while outside fMRI, saccade trials were interleaved between four random directions during 1 block comprising 64 trials (**a**), inside fMRI, blocks comprising 6 horizontal saccade trials were interleaved with blocks comprising 6 vertical saccade trials (**b**). (arrows in saccade trials represent instructed eye movement and not actual stimuli)

2.3. Data processing and analysis.

2.3.1. Behavioural data.

Eye movement data were analyzed off-line using the SMI BeGaze 3.4TM software (SensoMotoric Instruments Inc, Teltow, Germany) and in-house-written scripts in MATLAB 8.0 (The MathWorks Inc., Natick, MA, USA). For analysis, the following parameters were extracted from the left eye recordings in prosaccade and antisaccade tasks: latency (time [ms] between target onset and saccade onset], direction error (primary saccades directed away from the target in prosaccade task and towards the target in antisaccade task) rate, peak velocity (maximum velocity [°/s]), and amplitude (saccade amplitude [°] to target eccentricity [10°]). Saccades were automatically detected using a velocity (>40°/s) and duration (22 ms) criterion, with the possibility for manual correction. Saccades were rejected from the analysis if: 1) latency <120 ms (anticipatory saccade) or >800 ms (delayed saccade); 2) preceded by a blink within 100 ms before target onset; 3) saccade starting point fell outside a >1.5° circle centered on fixation cross; 4) saccade landing point fell outside a 7° circle centered on the mirror location of the target in antisaccade trials. The percentages of rejected trials from the analysis were significantly higher for PSP patients (12.1%) than for controls (3.5%) (p=0.001). Mean latency, peak velocity, amplitude and direction errors of both prosaccades and antisaccades were then calculated for each participant. Each parameter was extracted separately for horizontal and vertical direction. We were not able to carry out an offline analysis of the eye-movement data recorded inside the scanner due to technical limitations. However, participant´s task compliance "online" was verified in all subjects by monitoring eye movement performance with a video camera, during blocks and baseline condition.

2.3.2. fMRI data.

Imaging data pre-processing and analysis was carried out using BrainVoyager Qx 2.60 Software Package (Maastricht, the Netherlands) and included motion correction using a trilinear interpolation, slice-scan time correction using a cubic-spline interpolation, smoothing with 4-mm full-width at a half maximum isotropic Gaussian kernel, and temporal high-pass filtering using general linear model with a fourier basis set with a window of 2 cycles plus temporal smoothing (0,001 data points). After inhomogeneity correction and transformation into the coordinate system of Talairach space, functional images were coregistered to the anatomical scan.

2.3.3. Statistical analysis.

For behavioral analysis, within-groups comparison of saccade latency, peak velocity, amplitude and direction error rate both for horizontal and vertical directions and for prosaccade and antisaccade task was performed by using a Kruskal–Wallis test. For

within-groups comparison of the same variables between horizontal and vertical directions, the Wilcoxon test was used. Bonferroni correction, according to the number of comparisons was further utilized. All statistical tests were two tailed with criterion for statistical significance set at $p<0.05$, unless otherwise stated.

For first level fMRI analysis at (single subject) we applied a general linear model (GLM) considering BOLD signal as the dependent variable, individual blocks as predictors (regressors) and a constant term related to overall mean signal. The 15 s of blank screen (baseline condition) was not modelled explicitly and thus served as the implicit baseline. We performed a whole-brain fixed-effects group analysis , to increase sensitivity due to our small sample size, assuming the drawback of not being able to generalize our results to the population. Group contrast maps were obtain for horizontal and vertical prosaccades and horizontal and vertical antisaccades, as well as vertical>horizontal saccades contrast in each run, followed by multiple comparison correction with a cluster-extent base thresholding ($p <0.01$). An examination of between- and within-group activation was conducted by using T-tests on the comparisons of interest.

3. Results.

3.1. Clinical and demographic data.

PSP patients showed a significantly lower median MMSE score than controls (26 vs 29.5, $p=0.019$). However, MMSE score showed no correlation with any of the oculomotor parameters, irrespective of plane, task or group (data not shown). Age, gender and GDS score on the other hand, were not significantly different between groups (see **Table 1**).

3.2. Behavioral data.

Table 2 displays the results of the saccade behavioral data. In the prosaccade task, peak velocity and amplitude were significantly lower in PSP patients than controls, both in the horizontal and vertical plane (208 °/s vs. 346°/s, $p=0.001$; 5.6° vs. 10.4°, $p<0.001$; 124°/s vs. 289°/s, $p=0.001$; 2.9° vs. 8.7°, $p=0.001$, respectively), while latency of prosaccades only in the vertical plane, tended to be more prolonged in the PSP group ($p=0.026$). In the antisaccade task, the amplitude of vertical antisaccades was significantly lower in the PSP group, when compared to controls (4.3° vs. 11.3°, $p=0.002$), and peak velocity of horizontal antisaccades tended to be significantly lower in the PSP group ($p=0.027$). Within each group, vertical prosaccades were significantly or near-significantly more delayed, slower and shorter than horizontal prosaccades (PSP: 419ms vs. 288ms, $p=0.012$; 124°/s vs. 208°/s, $p=0.012$; 2.9° vs. 5.6°, $p=0.017$, respectively) (controls: 311ms vs. 266ms, $p=0.009$; 346°/s vs 289°/s, $p=0.005$; 8.7° vs.

10.4°, p=0.005, respectively). Within groups, vertical antisaccade parameters did not significantly differ from that of horizontal antisaccades.

Table 2. Behavioral Data

	PSP (n=8)	CTL (n = 10)	p value* between groups	p value** within groups PSP; CTL
Horizontal Prosaccades				
Latency, ms	288 (60)	266 (41)	0.534	0.012; 0.009
Peak Velocity, °/s	208 (64)	346 (60)	**0.001**	0.012; **0.005**
Amplitude, °	5.6 (2.0)	10.4 (1.6)	<**0.001**	0.017; **0.005**
Direction Error, %	1.9 (2.7)	1.6 (3.6)	0.574	0.465; 0.343
Vertical Prosaccades				
Latency, ms	419 (160)	311 (81)	0.026	
Peak Velocity, °/s	124 (72)	289 (50)	**0.001**	
Amplitude, °	2.9 (1.7)	8.7 (1.6)	**0.001**	
Direction Error, %	3.1 (6.4)	2.4 (2.7)	0.613	
Horizontal Antisaccades				
Latency, ms	395 (153)	381 (91)	0.624	0.109; 0.036
Peak Velocity, °/s	212 (112)	356 (91)	0.027	0.109; 0.092
Amplitude, °	8.0 (4.5)	12.8 (5.3)	0.178	0.109; 0.050
Direction Error, %	50.9 (37.9)	51.9 (31)	0.922	0.600; 0.285
Vertical Antisaccades				
Latency, ms	457 (49)	366 (179)	0.606	
Peak Velocity, °/s	251 (150)	314 (44)	0.071	
Amplitude, °	4.3 (2.5)	11.3 (2.8)	**0.002**	
Direction Error, %	62.0 (22.1)	37.8 (42)	0.200	

Values are means (standard deviations).

*Statistical difference between groups (PSP vs. CTL); Significant values, $p < 0.003$ (after Bonferroni correction) are marked in bold.

**Statistical difference within groups (horizontal vs. vertical PS, 1st to 4th row; horizontal vs. vertical AS, 8th to 12th row; PSP, left column; CTL, right column); Significant values, $p < 0.006$ (after Bonferroni correction) are marked in bold; note that within-groups comparison was only performed between horizontal and vertical directions, and not between tasks, i.e., prosaccade vs. antisaccade.

PSP, progressive supranuclear palsy; CTL, controls.

3.3. fMRI data.

Six PSP patients and ten controls performed similar behavioural paradigms inside the scanner. We first compared perisaccadic BOLD activation between groups (both for horizontal prosaccades, vertical prosaccades, horizontal antisaccades, and vertical antisaccades), followed by the within groups comparison between vertical versus horizontal saccades BOLD activation during performance of prosaccades and antisaccades. We first established in the control group the presence of key areas showing greater activation during saccadic performance (prosaccades + antisaccades) when compared to the baseline condition. As expected, functional activations were predominantly found in the FEF, SEF, PEF/precuneus, dlPFC, basal ganglia (caudate; putamen; thalamus), occipital cortex (middle and inferior occipital gyrus; cuneus; right lingual gyrus), temporal lobe (fusiform gyrus), and cerebellum (dorsal vermis; cerebellar anterior lobe; cerebellar uvula, left inferior semilunar lobule). This extensive network is known to be involved in oculomotor and visual processing. Importantly, task-deactivation areas overlapping the DMN were also revealed, including the anterior and posterior cingulate gyrus, ventromedial and dorsomedial prefrontal cortex (vmPFC; dmPFC), orbitofrontal cortex, inferior parietal lobe, insula, middle temporal gyrus, and left parahippocampal gyrus (**Supplemental Fig. 1**) [2, 3].

3.3.1. Prosaccades.

When comparing horizontal prosaccades BOLD activity between groups, controls exhibited significant greater activations in the left FEF and SEF, right caudate and both inferior occipital gyrus, while showing higher deactivations in areas overlapping the DMN (i.e., left orbitofrontal cortex, right insula and inferior parietal lobe). Of note, cerebellar tonsils showed less BOLD activity in controls. Vertical prosaccade findings paralleled those of horizontal prosaccades, but additional activation clusters in the left FEF, right fusiform gyrus and right cerebellar posterior lobe and uvula were found in controls. Stronger deactivations in controls were now evidenced in the left cuneus and in the vicinity of the insula. Talairach coordinates of the peak activations for all key regions in between-groups analysis for the horizontal and vertical prosaccade tasks are presented in **Table 3**. Activation maps comparing horizontal prosaccade and vertical prosaccade contrasts between the two groups are displayed in **Fig. 2**.

Table 3. Talairach coordinates (x,y,z) and number of voxels (v) of peak activations in GLM contrast maps for horizontal prosaccades (PS) and vertical PS contrasts between controls (CTL) and PSP group*

			Horizontal PS					Vertical PS				
				Local maxima peak coordinates (TLS)								
Anatomical Region or Functional Label	Side	BA	Dir	x	y	z	v	Dir	x	y	z	v
CTL > PSP												
FEF	L	6						(+)	-31	-8	58	757
		4	(+)	-40	-11	54	165	(+)	-43	-11	48	380
SEF	L	32/6	(+)	-7	10	42	380	(+)	-4	10	48	1684
Caudate	R		(+)	20	-5	21	211	(+)	14	4	9	676
iOG	R	18	(+)	32	-86	-6	1544	(+)	32	-86	-6	1711
	L	18	(+)	-40	-83	-13	986	(+)	-34	-89	-6	1584
FuG	R	37						(+)	47	-47	-18	352
lOFC	R	47	(-)	26	34	-9	286					
	L	47	(-)	-28	31	0	170					
Insula	R	13	(-)	44	-8	18	465					
iPL	R	40	(-)	56	-20	24	319					
Cuneus	L	18						(-)	-13	-86	24	517
Cerebellar tonsil	R		(-)	11	-56	-33	189					
	L		(-)	-13	-56	-39	262					
Cerebellar pL	R							(+)	41	-65	-15	323
Cerebellar uvula	R							(+)	5	-80	-37	425

PSP, progressive supranuclear palsy; FEF, frontal eye field; SEF, supplementary eye field; iOG, inferior occipital gyrus; FuG, fusiform gyrus; lOFC, lateral orbitofrontal cortex; iPL, inferior parietal lobule; pL, (cerebellar) posterior lobe; TLS, Talairach standard; BA, Brodmann area

"Peak" refers to location of voxel with most significant activation

"Side" refers to the location of the activation: R, right hemisphere; L, left hemisphere.

"Dir" refers to the direction of contrast: greater (+) or less (-) saccade activation for CTL than PSP

"/" between two anatomical regions and/or functional labels indicates clusters involving two contiguous areas

* Fixed effects analysis, $p<0.01$, corrected for multiple comparisons at the cluster level

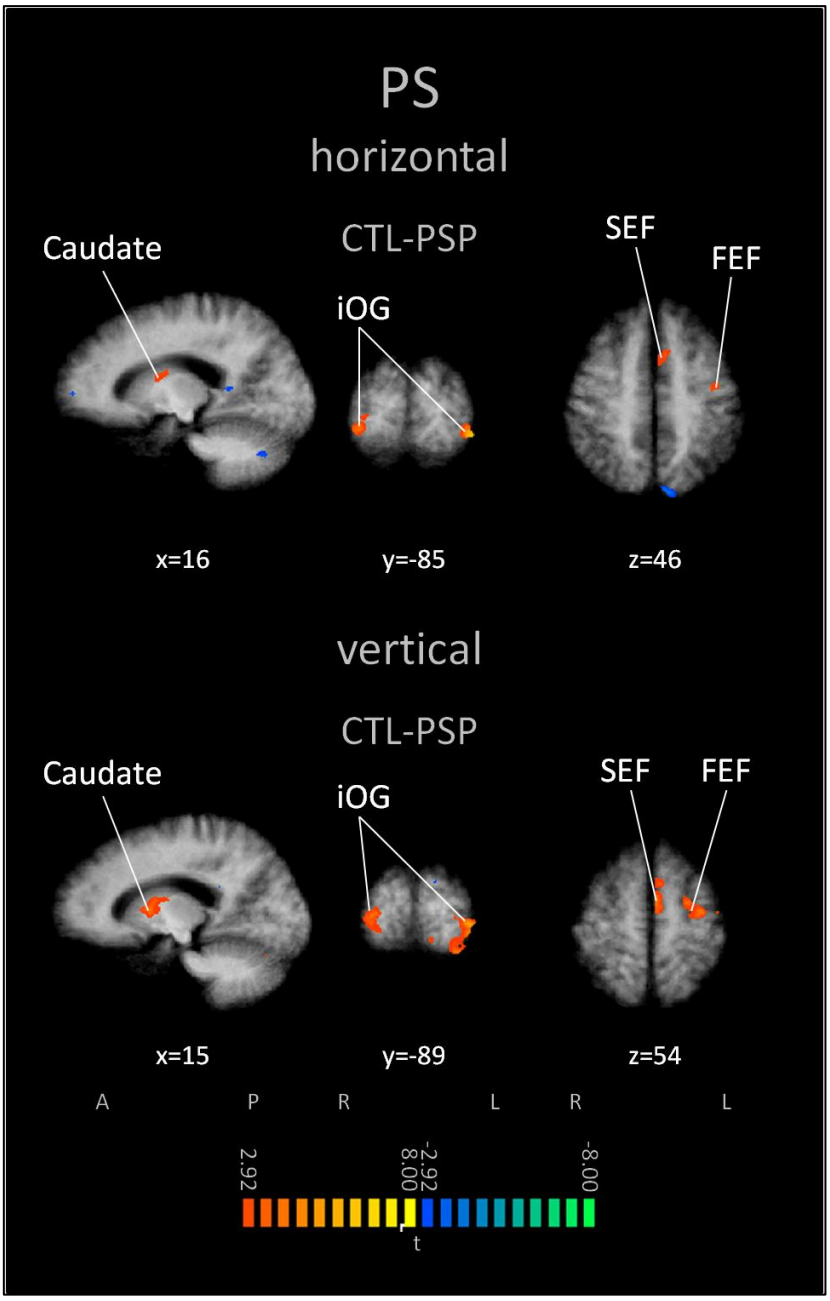

Figure 2. Group t-statistical activation maps comparing horizontal prosaccades (PS) and vertical PS between controls (CTL) and PSP group (single-voxel p-value < 0.01, cluster level-corrected). Yellow/red regions represent greater saccade activation for CTL than PSP group. Blue/green regions represent less saccade activation for CTL than PSP group. A and P denote anterior and posterior. L and R denote left and right. Coordinate values of planes in Talairach space are indicated. iOG, inferior occipital gyrus; SEF, supplementary eye field; FEF, frontal eye field; PSP, progressive supranuclear palsy

3.3.2. Antisaccades.

When comparing horizontal antisaccades BOLD activity between groups, controls demonstrated greater right middle occipital gyrus activity, lesser right cerebellar tonsil activity, and greater DMN (i.e., right posterior cingulate gyrus and dmPFC) deactivation. Vertical antisaccades comparison between groups demonstrated additional activations of the left FEF and SEF, right thalamus, right inferior occipital gyrus and cerebellum in controls, and stronger DMN (i.e., left vmPFC, left parahyppocampal gyrus and right posterior cingulate gyrus) deactivations in the same group. Talairach coordinates of the peak activations for all key regions in between-groups analysis for the horizontal and vertical antisaccade tasks are presented in **Table 4**. Activation maps comparing horizontal antisaccade and vertical antisaccade contrasts between the two groups are displayed in **Fig. 3**.

Table 4. Talairach coordinates (x,y,z) and number of voxels (v) of peak activations in GLM contrast maps for horizontal antisaccades (AS) and vertical AS contrasts contrast between controls (CTL) and PSP group*

			Horizontal AS					Vertical AS				
			Local maxima peak coordinates (TLS)									
Anatomical Region or Functional Label	Side	BA	Dir	x	y	z	v	Dir	x	y	z	v
CTL > PSP												
FEF	L	6						(+)	-16	-11	57	568
SEF	L	24						(+)	-4	1	45	292
Thalamus	R							(+)	23	-14	15	2383
mOG	R	18	(+)	29	-83	-6	167					
iOG	R	18						(+)	29	-86	-6	266
vmPFC	L	10						(-)	-6	61	18	234
dmPFC	R	8	(-)	20	22	45	199					
	L	8	(-)	-22	26	51	181					
pCG	R	30/31	(-)	2	-44	21	357	(-)	5	-59	24	1270
PHG	L	35						(-)	-25	-8	-21	239
Cerebellar pL	R							(+)	20	-65	-21	1847
	L							(+)	-22	-68	-24	261
Cerebellar nodulus	R							(+)	2	-47	-30	296
Cerebellar tonsil	R		(-)	26	-62	-36	185					

PSP, progressive supranuclear palsy; FEF, frontal eye field; SEF, supplementary eye field; mOG, middle occipital gyrus; iOG, inferior occipital gyrus; vmPFC, ventromedial prefrontal cortex; dmPFC, dorsomedial prefrontal cortex; pCG, posterior cingulate gyrus; PHG, parahyppocampal gyrus; pL, (cerebellar) posterior lobe; TLS, Talairach standard; BA, Brodmann area

"Peak" refers to location of voxel with most significant activation

"Side" refers to the location of the activation: R, right hemisphere; L, left hemisphere

"Dir" refers to the direction of contrast: greater (+) or less (-) saccade activation for CTL than PSP

"/" between two anatomical regions and/or functional labels indicates clusters involving two contiguous areas; "/" between two Brodmann areas separates BA indicated by Talairach coordinates in left and right columns, in case of different areas

* Fixed effects analysis, $p<0.01$, corrected for multiple comparisons at the cluster level

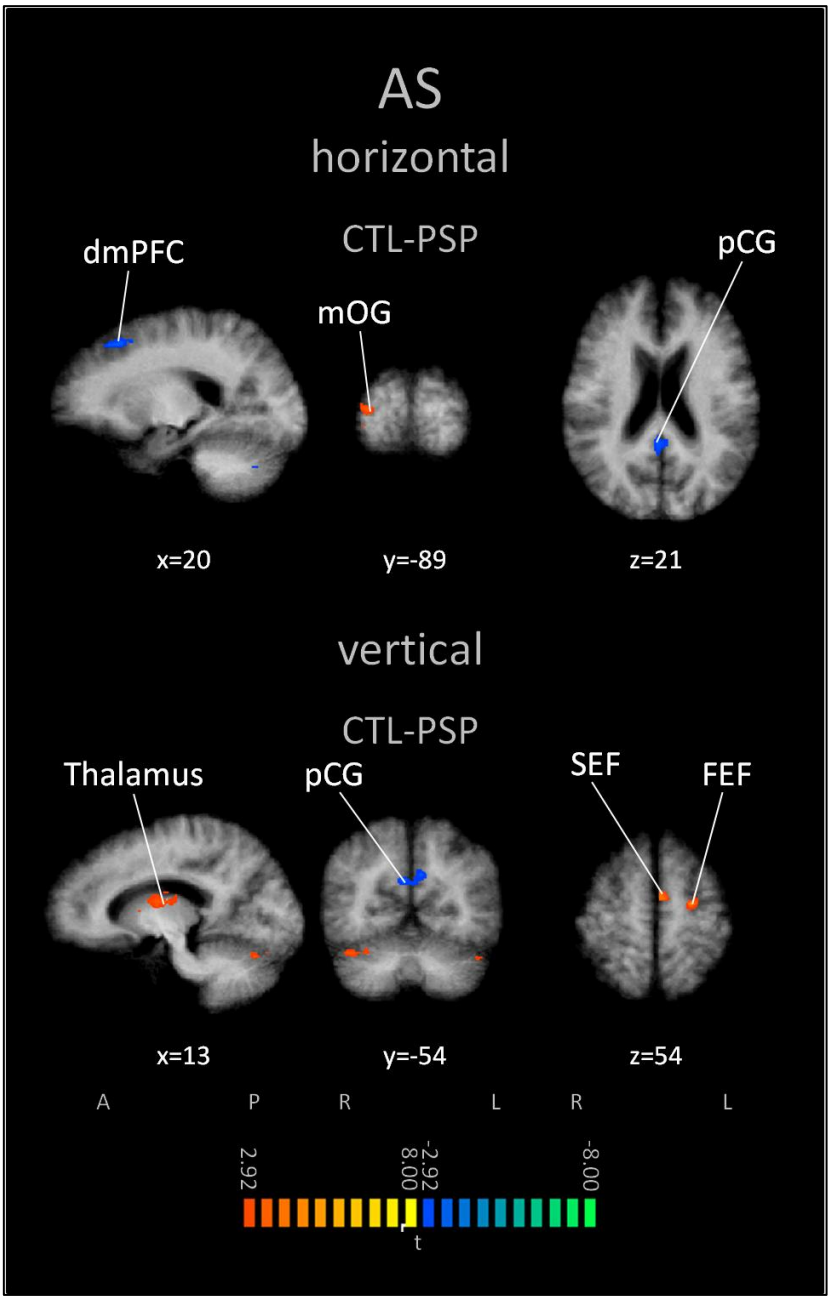

Figure 3. Group t-statistical activation maps comparing horizontal antisaccades (AS) and vertical AS contrasts between controls (CTL) and PSP group (single-voxel p-value < 0.01, cluster level-corrected). Yellow/red regions represent greater saccade activation for CTL than PSP group. Blue/green regions represent less saccade activation for CTL than PSP group. A and P denote anterior and posterior. L and R denote left and right. Coordinate values of planes in Talairach space are indicated. dmPFC, dorsomedial prefrontal cortex; mOG, middle occipital gyrus; pCG, posterior cingulate gyrus; SEF, supplementary eye field; FEF, frontal eye field; PSP, progressive supranuclear palsy

3.3.3. Vertical > horizontal saccades.

There were no significant differences in BOLD activation of vertical prosaccades versus horizontal prosaccades in controls. On the contrary, PSP group exhibited stronger right middle temporal gyrus, superior temporal gyrus and inferior parietal lobe deactivations during vertical prosaccades. During antisaccades run, the differences between vertical and horizontal plane involved several areas in each group. In controls, there was lesser left cuneus and right lingual gyrus BOLD activity and greater left vmPFC deactivation during vertical antisaccades. In the PSP group, there was lesser left FEF, left putamen, and right thalamus BOLD activity and greater right vmPFC deactivation during vertical antisaccades. Talairach coordinates of the peak activations for all key regions in within-groups analysis for the horizontal > vertical prosaccades and horizontal > vertical antisaccades contrasts are presented in **Table 5**. Activation maps comparing vertical prosaccades to horizontal prosaccades and vertical antisaccades to horizontal antisaccades for all groups are displayed in **Fig. 4** and **Fig. 5**, respectively.

Table 5. Talairach coordinates (x,y,z) and number of voxels (v) of peak activations in GLM contrast maps for vertical prosaccades (PS)-horizontal PS and vertical antisaccades (AS)-horizontal AS contrasts within controls (CTL) and PSP group*

			Vertical PS > Horizontal PS					Vertical AS > Horizontal AS				
			Local maxima peak coordinates (TLS)									
Anatomical Region or Functional label	Side	BA	Dir	x	y	z	v	Dir	x	y	z	v
CTL												
vmPFC	L	9						(-)	-13	52	27	2120
Cuneus	L	18						(-)	-10	-92	21	359
		23						(-)	-16	-71	9	308
LG	R	18						(-)	14	-74	6	486
PSP												
mTG	R	21	(-)	62	-35	-9	442					
		21	(-)	44	1	-30	494					
iPL	R	40	(-)	62	-29	34	189					
sTG	R	22	(-)	50	-59	15	217					
FEF	L	6						(-)	-25	-11	45	314
Putamen	L							(-)	-28	13	0	885
								(-)	-22	1	21	308
Thalamus	R							(-)	20	-5	12	1517
vmPFC	R	10						(-)	23	46	6	381
	L	10						(-)	-31	40	21	262

PSP, progressive supranuclear palsy; FEF, frontal eye field; LG, lingual gyrus; vmPFC, ventromedial prefrontal cortex; mTG, middle temporal gyrus; iPL, inferior parietal lobe; sTG, superior temporal gyrus; TLS, Talairach standard; BA, Brodmann area

"Peak" refers to location of voxel with most significant activation

"Side" refers to the location of the activation: R, right hemisphere; L, left hemisphere.

"Dir" refers to the direction of contrast: greater (+) or less (-) activation for vertical than horizontal saccades

"/" between two anatomical and/or functional labels indicates clusters involving two contiguous areas

* Fixed effects analysis, $p<0.01$, corrected for multiple comparisons at the cluster level

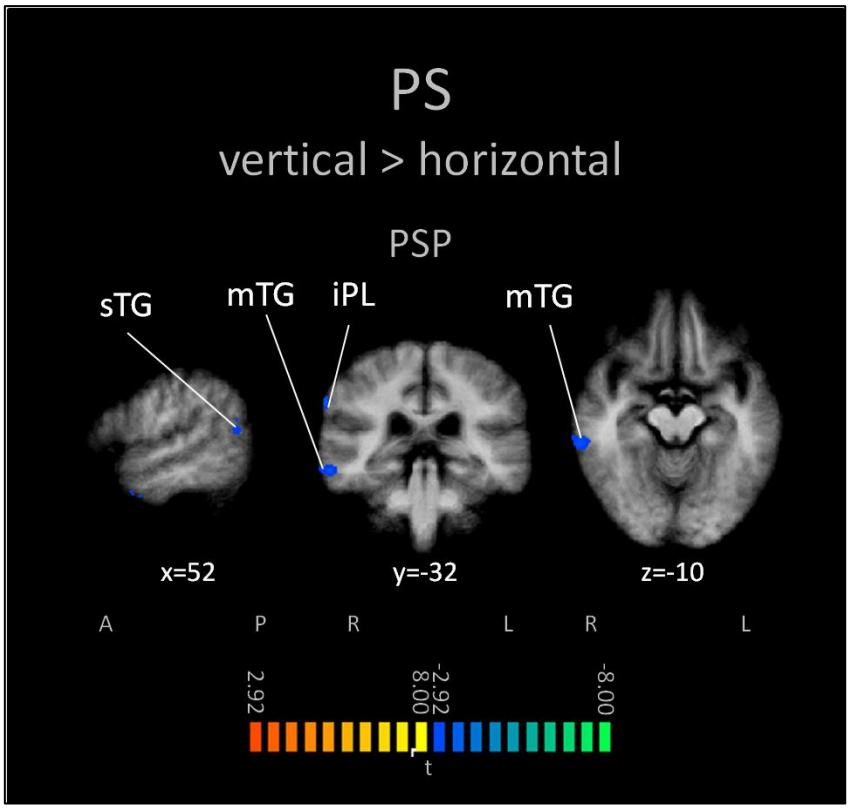

Figure 4. Group t-statistical activation maps comparing vertical prosaccades (PS)>horizontal PS within PSP group (single-voxel p-value < 0.01, cluster level-corrected). Yellow/red regions represent greater saccade activation for vertical PS than horizontal PS. Blue/green regions represent less saccade activation for vertical PS than horizontal PS. A and P denote anterior and posterior. L and R denote left and right. Coordinate values of planes in Talairach space are indicated. mTG, middle temporal gyrus; sTG, superior temporal gyrus; iPL, inferior parietal lobe; PSP, progressive supranuclear palsy

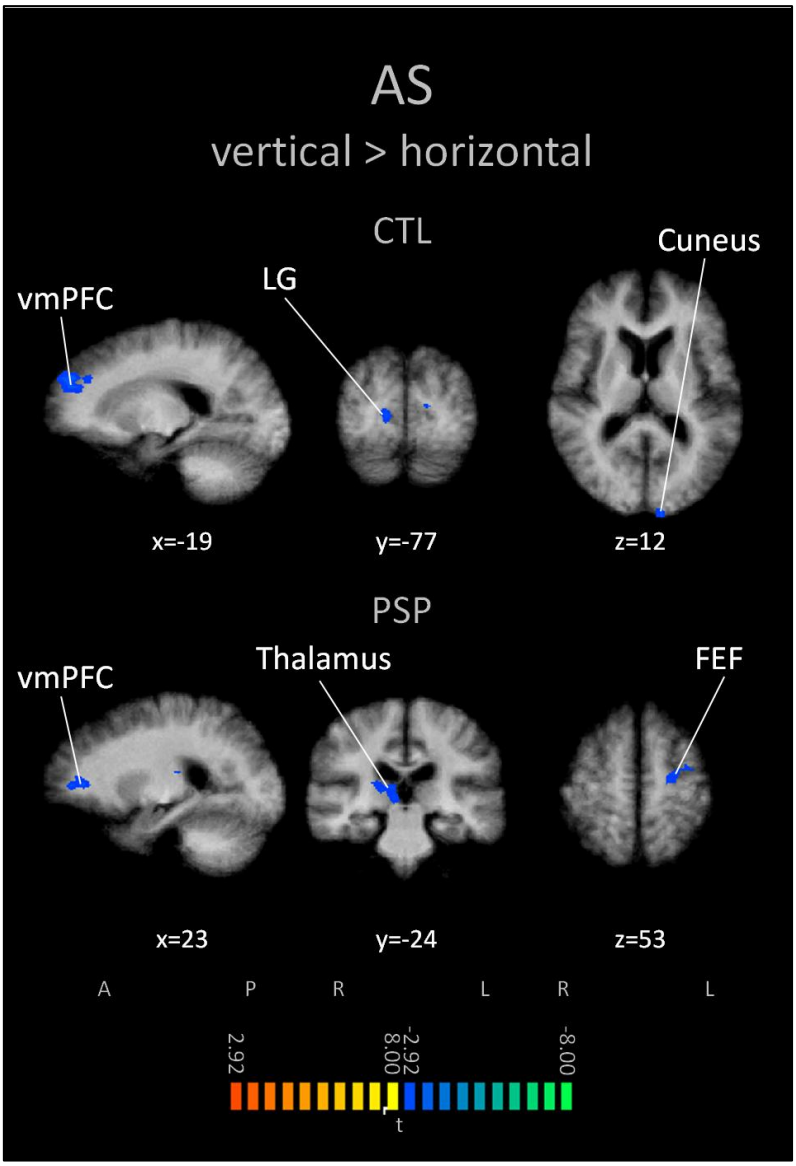

Figure 5. Group t-statistical activation maps comparing vertical antisaccades (AS)>horizontal AS within controls (CTL) and PSP group (single-voxel p-value < 0.01, cluster level-corrected). Yellow/red regions represent greater saccade activation for vertical AS than horizontal AS. Blue/green regions represent less saccade activation for vertical AS than horizontal AS. A and P denote anterior and posterior. L and R denote left and right. Coordinate values of planes in Talairach space are indicated. vmPFC, ventromedial prefrontal cortex; LG, lingual gyrus; FEF, frontal eye field; PSP, progressive supranuclear palsy

4. Discussion.

In the present study, we have shown that the PSP group, when compared to controls, demonstrated a significant BOLD-fMRI signal decrease in the frontal oculomotor cortical network (i.e., left FEF, left SEF) and basal ganglia (i.e., right caudate, right thalamus) during the execution of prosaccades along the vertical and horizontal plane and antisaccades along the vertical plane only. These new fMRI findings favour the hypothesis that a frontostriatal deficit also plays a role in saccadic disturbance in PSP, adding to the brainstem impairment. Interestingly in both groups, the execution of vertical saccades (both prosaccades and antisaccades) seemed to be more demanding than that of horizontal saccades, as shown by a greater DMN deactivation during vertical saccades performance. Only in PSP group however, we observed that vertical antisaccades performance additionally yielded less frontal (i.e., left FEF) and basal ganglia (i.e., left putamen, right thalamus) BOLD activity when compared to horizontal antisaccades, suggesting that in patients, the underlying cortical-subcortical neuronal populations generating vertical voluntary saccades evidence a greater impairment than those responsible for the execution of horizontal voluntary saccades. As expected, behaviourally, PSP patients demonstrated slower and shorter prosaccades than controls. These also tended to be more delayed in the former group, but only for the vertical plane. PSP patients also showed shorter vertical antisaccades. These findings will be further discussed below.

4.1. Behavioural data.

In our sample, while the existence in PSP patients of markedly slow and small prosaccades especially in the vertical plane, primarily suggests midbrain (rostral interstitial nucleus of the medial longitudinal fasciculus [riMLF], superior colliculus [SC] and interstitial nucleus of Cajal [iNC]) and pontine (paramedian pontine reticular formation [PPRF], omnipause neurons) neuronal loss, the nature and contribution of cortical and/or subcortical damage for such deficit is largely unknown [12–14, 18, 25–28]. Prosaccades latency also tended to be more prolonged in PSP patients than controls, along the vertical plane. We believe that saccade latency in PSP is probably dependent upon the variable involvement of cortical and/or subcortical structures known to influence saccadic reaction time, including FEF, PEF and basal ganglia [15–18]. This may explain the heterogeneous results on saccade latency in PSP in the literature [14–18, 26, 29]. In any case, as suggested by our study, the latency of vertical prosaccades may be more sensitive than that of horizontal prosaccades to detect PSP oculomotor deficits. Notably within the PSP group, velocity and amplitude of vertical prosaccades, although lower than horizontal prosaccades, did not differ significantly. This is probably attributed to the concomitant deterioration of horizontal prosaccades in our patients, usually more pronounced later in the course of the disease [16, 24]. The only antisaccade parameter that significantly differed between PSP and the control group was gain along the vertical plane, being lower in PSP. Similarly, antisaccades latency and

directional errors rate, tended to be increased in PSP patients, only along the vertical plane. Increased number of directional errors is frequently reported in PSP and suggests dlPFC involvement [14, 15, 18, 26]. Taken together, these new findings stress the potential utility of adding vertical antisaccades to common saccade paradigms when evaluating patients with PSP and other neurodegenerative disorders that share asymmetric impairment of saccade planes.

4.2. Imaging data.

As we originally hypothesized, PSP patients showed frontal BOLD hypoactivity (left FEF and SEF) during prosaccades (horizontal and vertical) and antisaccades (vertical) when compared to controls. This finding further consolidates previous anatomical, pathological and functional evidence demonstrating frontal lobe dysfunction in PSP [20, 30, 31]. FEF and SEF activation during saccade execution is a consistent finding in fMRI studies and these areas seem to influence saccade latency and generate cognitively complex saccades, respectively [2]. Such hypoactivity in PSP is probably the result of a combination of reduced nigro-striatal and thalamic output to cortex and intrinsic cortical affection by tau pathology [20, 32]. Thus, while there is no doubt that the severe brainstem impairment in PSP largely contributes to the saccadic disturbance in these patients, our data supports the additional participation of the cortical frontal oculomotor areas. Interestingly in our study, basal ganglia BOLD differences between groups mirrored exactly FEF and SEF changes. Specifically, controls showed greater BOLD signal than PSP patients in the right caudate nucleus during prosaccades (horizontal and vertical) and in the right thalamus during antisaccades (vertical). Both these two structures play an important role in saccade generation. The caudate nucleus seems to modulate the latency of more voluntary saccades [33] while thalamus lesions have been associated with saccadic hypometria especially in the vertical direction [34]. Thus, our findings seem to extend recent evidence showing caudate and thalamic impairment in PSP during force production and mental imagery paradigms, respectively [35, 36]. Cerebellar findings in our study support the possibility of an anatomical and/or functional motor segregation for horizontal and vertical saccades in cerebellar specific areas. Notably, during horizontal saccades, controls evidenced less BOLD activity than the PSP group in the cerebellar tonsils, while during vertical saccades an opposite BOLD signal pattern occurred between groups, in the posterior lobe and nodulus/uvula complex. We think that the increased tonsilar (dorsal paraflocullus) activity in PSP patients may reflect a compensatory mechanism needed to overcome the progressive deterioration of horizontal saccades. Indeed, Purkinje cells in tonsil seem in to exhibit saccade directional selectivity [37]. In contrast with the dorsal paraflocculus, posterior lobe BOLD responses during vertical saccades in patients were reduced relative to controls. Cerebellar posterior lobe seems to play a role in gaze-shift orientation and sensory-motor adaptation of saccades, receiving important projections from the cortical eye fields and the superior colliculus [38, 39]. Therefore, we speculate that a critically diminished input from FEF and/of superior colliculus to cerebellum during vertical

saccades is the cause for such BOLD signal reduction in PSP patients. This last mechanism could also account for the nodulus/uvula complex findings in PSP patients, although the role of this complex in the execution of saccades remains elusive. Notwithstanding, the nodulus/uvula complex does exhibit a directional preponderance for the vertical plane in other types of eye movements (i.e., pursuit; translational vestibulo-ocular reflex) [40, 41]. Occipito-temporal BOLD activity (middle and inferior occipital gyrus, fusiform gyrus), was decreased in the PSP group relative to controls during saccades (vertical and horizontal), contrasting with previous functional studies demonstrating relative hyperactivity of the posterior parietal and occipital cortices in PSP, either reflecting compensatory or pathological activity [35]. It must be noted however that these areas not only participate in the generation of saccades, but also play a critical role in covert attention and in fact these two networks (oculomotor; attention) largely overlap and show striking similarities in BOLD signal modulation [42]. Since covert attention in PSP was shown to be disturbed and this finding is apparently independent from the oculomotor disorder, it is possible that the relative occipito-temporal BOLD hypoactivity seen in the PSP group could be due to a disturbed attentional network in these patients [43]. Task-negative areas thought to correspond to the DMN, showed greater deactivation in controls than PSP both for vertical and horizontal saccades. This result was rather expected, after recent evidence showing disrupted connectivity within DMN in PSP, specifically affecting premotor, temporal lobe, thalamus and striatum [44]. Therefore, PSP patients appear to be less able to deactivate the DMN when attentional and/or working memory demands are required.

Comparison of the BOLD signal between vertical and horizontal planes within each group provided important insights into the putative cortical/subcortical structural and/or functional directional bias occurring during the execution of saccades. During prosaccades, controls showed no significant BOLD difference between vertical and horizontal prosacades. However, it has been shown in young adults that even in the absence of eye movements, cortical BOLD differences between vertical and horizontal planes may be seen during covert attention paradigms, favouring the existence of the above-mentioned bias [45]. Aging in our sample may have prevented such BOLD differences by promoting a global reduction of the BOLD response in the oculomotor system [46]. PSP patients on the other hand showed greater DMN deactivation (i.e., right middle temporal gyrus and inferior parietal lobe) during vertical prosaccades when compared to their horizontal counterpart. This finding supports the idea that patients may use a greater amount of attentional resources during vertical prosaccades, since these are clearly more difficult to perform and more effortful than horizontal prosaccades. During antisaccades, controls showed greater DMN (i.e., vmPFC) deactivation during vertical antisaccades, which raises the possibility that the directional attentional bias between vertical and horizontal saccades in healthy individuals may only be observed when more demanding paradigms (e.g., antisaccades) are used. Cuneus and lingual gyrus decreased activity in this group during vertical antisaccades on the other hand could be related to the known underrepresentation of the vertical meridian in visual cortex [47]. In PSP, similarly to controls, there was greater vmPFC

deactivation during vertical antisacades, but notably in this group, three additional clusters within the oculomotor network showed less activity during vertical antisaccades: left FEF, left putamen, and right thalamus. Whether these clusters represent: (1) intrinsic malfunctioning of the cortical/subcortical oculomotor network, (2) feedback's loop decreased input from brainstem and/or cerebellar structures to FEF and basal ganglia, or (3) failure of covert attentional resources in the vertical plane during more complex oculomotor tasks, remain open questions. Nevertheless, this last finding raises at least the point that the pathopshysiology of vertical supranuclear palsy in PSP may significantly extend beyond the brainstem [43, 48] .

4.3. Study limitations.

Our study has some important limitations. The use of an fMRI block design, instead of an event-related design, does not allow to dissociate covert attention from eye movement evoked BOLD signals, reason why BOLD differences must be carefully interpreted, especially when clusters shared by both networks were involved and not separable at the event level. Also, we were not able to carry an off-line analysis of eye movement data inside fMRI, which prevented us from performing additional correlation analysis between BOLD signal and saccadic performance. Due to the same reason, we were not able to calculate the rate of antisaccade direction errors inside fMRI, which is known to influence the BOLD responses [49]. Some limitations are implicitly related to the exploratory nature of our study on a rare neurodegenerative disorder, namely the small number of patients in our sample (8 in the behavioural experiment and 6 in fMRI), demanding for a fixed effect group analysis, which does not allow us to generalize the findings in our specific group to the population level. Finally, since to our knowledge, this is the first fMRI study to address the cortical/subcortical control of vertical saccades in PSP patients, we decided to increase the sensitivity of our analysis by using a relatively liberal statistical threshold for fMRI analysis, even though at the cost of increasing the risk for type-I errors.

4.4. Concluding remarks.

We provided first time evidence for frontostriatal functional deficits in PSP patients during the execution of saccades, especially along the vertical plane. These findings stress the potential contribution of cortical and subcortical damage in saccadic disturbance in PSP. Additionally, we offered new evidence that not only in the brainstem, but also at a cortical and subcortical level, neuronal populations subserving vertical and horizontal saccadic eye movements seem to be distinctively impaired in PSP. The use of vertical antisaccades may prove to be a useful parameter in the detection of PSP and other neurodegenerative disorders showing predominant vertical saccadic impairment. This work opens new venues for PSP research by providing

further insight into the pathophysiologic mechanisms of saccadic impairment in these patients.

Conflicts of interest.

The authors declare that they have no conflicts of interest related to this manuscript.

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References.

1. Leigh RJ, Zee DS (2015) The neurology of eye movements, 5th ed. Oxford University Press, New York

2. McDowell JE, Dyckman KA, Austin BP, Clementz BA (2008) Neurophysiology and neuroanatomy of reflexive and volitional saccades: evidence from studies of humans. Brain Cogn 68:255–70. doi: 10.1016/j.bandc.2008.08.016

3. Domagalik A, Beldzik E, Fafrowicz M, et al (2012) Neural networks related to pro-saccades and anti-saccades revealed by independent component analysis. Neuroimage 62:1325–33. doi: 10.1016/j.neuroimage.2012.06.006

4. Jamadar SD, Fielding J, Egan GF (2013) Quantitative meta-analysis of fMRI and PET studies reveals consistent activation in fronto-striatal-parietal regions and cerebellum during antisaccades and prosaccades. Front Psychol 4:749. doi: 10.3389/fpsyg.2013.00749

5. Herweg NA, Weber B, Kasparbauer A, et al (2014) Functional magnetic resonance imaging of sensorimotor transformations in saccades and antisaccades. Neuroimage 102 Pt 2:848–60. doi: 10.1016/j.neuroimage.2014.08.033

6. Melamed E, Larsen B (1979) Cortical activation pattern during saccadic eye movements in humans: localization by focal cerebral blood flow increases. Ann Neurol 5:79–88. doi: 10.1002/ana.410050112

7. Bodis-Wollner I, Bucher SF, Seelos KC (1999) Cortical activation patterns during voluntary blinks and voluntary saccades. Neurology 53:1800–5.

8. Sparks DL (2002) The brainstem control of saccadic eye movements. Nat Rev Neurosci 3:952–964. doi: 10.1038/nrn986

9. Bender MB (1960) Comments on the physiology and pathology of eye movements in the vertical plane. J Nerv Ment Dis 130:156–66.

10. Litvan I, Agid Y, Calne D, et al (1996) Clinical research criteria for the diagnosis of progressive supranuclear palsy (Steele-Richardson-Olszewski syndrome): report of the NINDS-SPSP international workshop. Neurology 47:1–9.

11. Liscic RM, Srulijes K, Gröger A, et al (2013) Differentiation of progressive supranuclear palsy: clinical, imaging and laboratory tools. Acta Neurol Scand 127:362–70. doi: 10.1111/ane.12067

12. Bhidayasiri R, Riley DE, Somers JT, et al (2001) Pathophysiology of slow vertical saccades in progressive supranuclear palsy. Neurology 57:2070–7.

13. Chen AL, Riley DE, King SA, et al (2010) The disturbance of gaze in progressive supranuclear palsy: implications for pathogenesis. Front Neurol 1:147. doi: 10.3389/fneur.2010.00147

14. Rivaud-Péchoux S, Vidailhet M, Gallouedec G, et al (2000) Longitudinal ocular motor study in corticobasal degeneration and progressive supranuclear palsy. Neurology 54:1029–32.

15. Rivaud-Péchoux S, Vidailhet M, Brandel JP, Gaymard B (2007) Mixing pro- and antisaccades in patients with parkinsonian syndromes. Brain 130:256–64. doi: 10.1093/brain/awl315

16. Terao Y, Fukuda H, Shirota Y, et al (2013) Deterioration of horizontal saccades in progressive supranuclear palsy. Clin Neurophysiol 124:354–63. doi: 10.1016/j.clinph.2012.07.008

17. Pierrot-Deseilligny C, Rivaud S, Pillon B, et al (1989) Lateral Visually-Guided Saccades In Progressive Supranuclear Palsy. Brain 112:

18. Garbutt S, Matlin A, Hellmuth J, et al (2008) Oculomotor function in frontotemporal lobar degeneration, related disorders and Alzheimer's disease. Brain 131:1268–81. doi: 10.1093/brain/awn047

19. Amtage F, Maurer C, Hellwig S, et al (2014) Functional correlates of vertical gaze palsy and other ocular motor deficits in PSP: an FDG-PET study. Parkinsonism Relat Disord 20:898–906. doi: 10.1016/j.parkreldis.2014.05.013

20. Williams DR, Holton JL, Strand C, et al (2007) Pathological tau burden and distribution distinguishes progressive supranuclear palsy-parkinsonism from Richardson's syndrome. Brain 130:1566–76. doi: 10.1093/brain/awm104

21. Abed F (1991) Cultural Influences on Visual Scanning Patterns. J Cross Cult Psychol 22:525–534. doi: 10.1177/0022022191224006

22. Foulsham T, Kingstone A, Underwood G (2008) Turning the world around: patterns in saccade direction vary with picture orientation. Vision Res 48:1777–90. doi: 10.1016/j.visres.2008.05.018

23. Linzenbold W, Lindig T, Himmelbach M (2011) Functional neuroimaging of the oculomotor brainstem network in humans. Neuroimage 57:1116–23. doi: 10.1016/j.neuroimage.2011.05.052

24. Pinkhardt EH, Jürgens R, Becker W, et al (2008) Differential diagnostic value of eye movement recording in PSP-parkinsonism, Richardson's syndrome, and idiopathic Parkinson's disease. J Neurol 255:1916–25. doi: 10.1007/s00415-009-0027-y

25. Friedman DI, Jankovic J, McCrary JA (1992) Neuro-ophthalmic findings in progressive supranuclear palsy. J Clin Neuroophthalmol 12:104–9.

26. Vidailhet M, Rivaud S, Gouider-Khouja N, et al (1994) Eye movements in parkinsonian syndromes. Ann Neurol 35:420–6. doi: 10.1002/ana.410350408

27. Rottach KG, Riley DE, DiScenna AO, et al (1996) Dynamic properties of horizontal and vertical eye movements in parkinsonian syndromes. Ann Neurol 39:368–77. doi: 10.1002/ana.410390314

28. Marx S, Respondek G, Stamelou M, et al (2012) Validation of mobile eye-tracking as novel and efficient means for differentiating progressive supranuclear palsy from Parkinson's disease. Front Behav Neurosci 6:88. doi: 10.3389/fnbeh.2012.00088

29. Chu FC, Reingold DB, Cogan DG, Williams AC (1979) The Eye Movement Disorders of Progressive Supranuclear Palsy. Ophthalmology 86:422–428. doi: 10.1016/S0161-6420(79)35498-7

30. Foster NL, Gilman S, Berent S, et al (1988) Cerebral hypometabolism in progressive supranuclear palsy studied with positron emission tomography. Ann Neurol 24:399–406. doi: 10.1002/ana.410240308

31. Cordato NJ, Pantelis C, Halliday GM, et al (2002) Frontal atrophy correlates with behavioural changes in progressive supranuclear palsy. Brain 125:789–800.

32. Brooks DJ (1994) PET studies in progressive supranuclear palsy. J Neural Transm Suppl 42:119–34.

33. Nakamura K, Hikosaka O (2006) Role of Dopamine in the Primate Caudate Nucleus in Reward Modulation of Saccades. J Neurosci 26:5360–5369. doi: 10.1523/JNEUROSCI.4853-05.2006

34. Bellebaum C, Daum I, Koch B, et al (2005) The role of the human thalamus in processing corollary discharge. Brain 128:1139–54. doi: 10.1093/brain/awh474

35. Burciu RG, Ofori E, Shukla P, et al (2015) Distinct patterns of brain activity in progressive supranuclear palsy and Parkinson's disease. Mov Disord 30:1248–58. doi: 10.1002/mds.26294

36. Zwergal A, la Fougère C, Lorenzl S, et al (2011) Postural imbalance and falls in PSP correlate with functional pathology of the thalamus. Neurology 77:101–9. doi: 10.1212/WNL.0b013e318223c79d

37. Noda H, Mikami A (1986) Discharges of neurons in the dorsal paraflocculus of monkeys during eye movements and visual stimulation. J Neurophysiol 56:1129–46.

38. Pélisson D, Goffart L, Guillaume A (2003) Control of saccadic eye movements and combined eye/head gaze shifts by the medio-posterior cerebellum. Prog Brain Res 142:69–89. doi: 10.1016/S0079-6123(03)42007-4

39. Alahyane N, Fonteille V, Urquizar C, et al (2008) Separate neural substrates in the human cerebellum for sensory-motor adaptation of reactive and of scanning voluntary saccades. Cerebellum 7:595–601. doi: 10.1007/s12311-008-0065-5

40. Walker MF, Tian J, Shan X, et al (2008) Lesions of the cerebellar nodulus and uvula in monkeys: effect on otolith-ocular reflexes. Prog Brain Res 171:167–72. doi: 10.1016/S0079-6123(08)00622-5

41. Walker MF, Tian J, Shan X, et al (2008) Lesions of the cerebellar nodulus and uvula impair downward pursuit. J Neurophysiol 100:1813–23. doi: 10.1152/jn.01193.2007

42. Corbetta M, Akbudak E, Conturo TE, et al (1998) A common network of functional areas for attention and eye movements. Neuron 21:761–73.

43. Rafal RD, Posner MI, Friedman JH, et al (1988) Orienting of visual attention in progressive supranuclear palsy. Brain 111:

44. Whitwell JL, Avula R, Master A, et al (2011) Disrupted thalamocortical connectivity in PSP: a resting-state fMRI, DTI, and VBM study. Parkinsonism Relat Disord 17:599–605. doi: 10.1016/j.parkreldis.2011.05.013

45. Mao L, Zhou B, Zhou W, Han S (2007) Neural correlates of covert orienting of visual spatial attention along vertical and horizontal dimensions. Brain Res 1136:142–53. doi: 10.1016/j.brainres.2006.12.031

46. Raemaekers M, Vink M, van den Heuvel MP, et al (2006) Effects of aging on BOLD fMRI during prosaccades and antisaccades. J Cogn Neurosci 18:594–603. doi: 10.1162/jocn.2006.18.4.594

47. Janik J, Ropella K, DeYoe E (2003) Distortions of human retinotopy obtained with temporal phase mapped fMRI. In: Soc Neurosci Abstr. p 29:658

48. Lynch JC, Hoover JE, Strick PL (1994) Input to the primate frontal eye field from the substantia nigra, superior colliculus, and dentate nucleus demonstrated by transneuronal transport. Exp brain Res 100:181–6.

49. Ford KA, Goltz HC, Brown MRG, Everling S (2005) Neural processes associated with antisaccade task performance investigated with event-related FMRI. J Neurophysiol 94:429–40. doi: 10.1152/jn.00471.2004

Chapter VI

General discussion

1. Summary of findings

1.1. Experiment 1.

Our study yielded the following main findings (**Chapter 4, Section 3**):

(1) Saccade metrics did not differ significantly between Parkinson's disease (PD) and controls, except for vertical antisaccades latency, which tended to be longer in PD patients. Saccade findings in PD may indeed be subtle and may go unnoticed when comparing with healthy participants. This is particularly true at early stages of the disease, where motor and cognitive impairment is mild, as it was the case in our sample [1, 2]. Within each group however, vertical saccades showed significantly longer latencies than horizontal saccades, which is consistent with the previous literature and probably reflects the importance of the horizontal meridian in our quotidian [3, 4]. Of note, vertical antisaccades tended to be more delayed in PD patients, highlighting its potential use as a diagnostic marker.

(2) Strikingly contrasting with behavioural data, albeit not for all directions or tasks, PD group showed left frontal eye field (FEF) hypoactivity and right parietal eye field (PEF) hyperactivity relative to controls, and during antisaccade task only, controls showed greater default-mode network (DMN) deactivation than PD group. On the one hand, our data further extends Rieger et al. (2008) and Cameron et al. (2012) findings showing frontal hypoactiviy and putative compensatory hyperactivity in more posterior regions in PD, but importantly, our results also add new evidence for DMN dysfunction in PD during saccades [5, 6].

(3) Within each group, controls exhibited greater activation in the right FEF and cerebellum during vertical prosaccades while PD patients showed decreased cuneus activation during vertical prosaccades. Both groups showed greater DMN deactivation during vertical antisaccades, with the PD group demonstrating further hypoactivity in the left PEF. These novel findings suggest that vertical saccades require greater attentional demands than their horizontal counterpart. Importantly in PD, allocation of resources to task-necessary regions during vertical saccades seems to be either lost or changed.

1.2. Experiment 2.

In the present study, the overall results were as follows (**Chapter 5, Section 3**):

(1) Vertical prosaccades and antisaccades were significantly (or marginally significantly) slower, shorter and more delayed in progressive supranuclear palsy (PSP) patients, when compared to controls. Vertical prosaccades were significantly (or marginally significantly) slower, shorter and more delayed than horizontal prosaccades across groups, but only reaching significance within controls. Antisaccades showed no difference between planes across groups. While amplitude and velocity deficits in PSP

patients most probably reflect brainstem damage (i.e., rostral interstitial nucleus of the medial longitudinal fasciculus [riMLF] more than pontine paramedian reticular formation [PPRF]), greater latency (and eventually shorter amplitude) may indicate additional cortical and/or subcortical burden as suggested by the subsequent functional magnetic resonance imaging (fMRI) experiment (see below) [7–12].

(2) During prosaccades, frontal oculomotor areas (i.e., left FEF and supplementary eye field [SEF]) and basal ganglia (i.e., right caudate) showed less blood-oxygenation-level-dependent (BOLD) activity in PSP patients than controls. During antisaccades, controls showed greater DMN deactivation both in the horizontal and vertical plane, and higher activation of frontal oculomotor areas (i.e., left FEF and SEF) and basal ganglia (right thalamus) only in the vertical plane. These novel fMRI findings during saccade tasks constitute supporting evidence for the existence of a marked frontostriatal deficit in PSP, which seems to become even more evident during purposeful saccades along the vertical plane and probably influences behavioural performance [11].

(3) During prosaccades, there were no BOLD changes between saccade planes in controls while in PSP patients greater DMN deactivation (i.e., right middle temporal gyrus) was seen along the vertical plane. During antisaccades, greater DMN (i.e., ventromedial prefrontal cortex) deactivation was seen in both groups along the vertical plane, while additional BOLD hypoactivity in frontal oculomotor areas and basal ganglia (i.e., left FEF and putamen, right thalamus) was seen only the PSP group. From the above findings, it is striking that not only purposeful vertical saccades (i.e., antisaccades), but also vertical reflexive saccades (i.e., prosaccades) are associated with greater attentional demands in PSP patients, when compared with their horizontal counterpart in PSP. This clearly reinforces the idea that vertical saccades in these patients, even when reflexive, require substantial effort to be executed. Failure to properly activate the cortical and subcortical oculomotor network during the execution of purposeful saccades, specifically along the vertical plane, possibly aggravates saccadic deficits in PSP patients.

2. Thoughts on the field.

Our results provide further insights to the field of eye movement research in several ways. First, little is known about the cortical control of vertical saccades. Current knowledge derives mostly from electrophysiological data in animals and rarely in humans [13–18]. fMRI studies so far have mainly concentrated on the mapping of saccade direction and amplitude within several oculomotor cortical and subcortical areas (e.g., FEF, PEF, superior colliculus [SC]), in many ways corroborating previous work in monkeys using neuronal recordings [19–21]. Presumingly, for the execution of vertical saccades, activation of both hemispheres is required, while for horizontal saccades, this activation is mainly contralateral [13, 15, 22]. These assumptions are based on previous electrophysiological data showing that saccade direction mapping in FEF follows a consistent pattern at a local level. Thus, albeit not retinotopically coded

such as object location in the primary visual cortex, the coding of saccade direction in FEF seems to be organized in a systematic fashion [13]. Accordingly, cortical stimulation at different tangential depths in these areas originates saccades that progress from oblique upward to oblique downward, but always directed to the opposite side [13]. Additionally, the same direction seems to be coded in several different locations ("columns") along FEF [20]. In PEF, saccades are also coded contralaterally, albeit here similarities with the primary visual cortex retinotopy are greater, since the orientation of the visual field map follows an antero-posterior gradient representing upper and lower visual field targets, respectively [21, 23, 24]. Taken together, since (1) pure vertical saccades have been rarely obtained in monkeys trough unilateral electrophysiological stimulation but rather consistently with simultaneous bilateral stimulation, (2) fMRI mapping studies have shown mainly contralateral saccade activation, (3) and bilateral cortical lesions are usually required to cause clinically significant impairment of vertical saccades, vertical saccades are believed to require bilateral hemispheric activation [13, 15, 20, 21, 23, 25, 26].

2.1. Findings in healthy participants.

Is bilateral activation during vertical saccades, symmetric or lateralized? In our work (Experiment 1), a right predominance seemed to exist during vertical saccades in healthy individuals. Indeed, right FEF and cerebellar hemisphere clusters were more active during vertical than horizontal prossaccades (**Chapter 4, Section 3.4.3**). If one takes into account previous data demonstrating right FEF and cerebellar activity predominance during the execution of horizontal saccades, then their greater activity when comparing vertical to horizontal plane in our study suggests that also during vertical saccades, there might be a right cerebral and cerebellar predominance [27, 28]. Since there is a great overlap between saccadic and covert attention networks, this predominance may reflect an attentional bias [27–29]. Still, right FEF and cerebellar hyperactivity during vertical prosaccades is a notable finding. Since vertical and horizontal saccades are generated by overlapping cortical neuronal networks and fMRI temporal and spatial resolution might not be ideal to detect BOLD differences in this case, FEF and cerebellar findings become even more relevant [30]. BOLD hyperactivity in this case, particularly in FEF, may reflect greater recruitment of attentional resources during vertical saccades, which are less frequent than horizontal saccades, possibly due to the fact that most of the salient information in our quotidian is distributed along the horizontal dimension, and some aspects of our cultural adaptation also favour the horizontal plane [3, 29, 31, 32]. Alternatively but not mutually exclusive, FEF BOLD difference may be related to the greater latency of vertical prosaccades relative to horizontal prosaccades (Experiment 1) (**Chapter 4, Section 3.2**). The longer the latency, the more neuronal activation will be accumulated, causing a stronger BOLD response [33]. Since we did not perform simultaneous retinotopic mapping techniques, relating these findings to a distinctive topographical activation of vertical and horizontal saccades within FEF is far speculative. Moreover, it has been demonstrated that the

same target location is coded in several areas within FEF, which further poses difficulties for BOLD signal interpretation in this area [20]. Notably, during the antisaccades task, the execution of saccades along the vertical plane was associated with greater deactivation of the DMN (i.e., ventromedial prefrontal cortex; posterior cingulate gyrus) (**Chapter 4, Section 3.4.3**). These findings complement our BOLD findings during prosaccades and equally suggest that during vertical saccades, greater attentional and/or working memory resources are allocated to task-necessary regions [34–37]. Of note, amplitude and velocity of vertical prosaccades also tended to be lower relative to horizontal prosaccades (Experiment 1) (**Chapter 4, Section 3.2**). This was even more evident, reaching a significant level, in Experiment 2 (**Chapter 5, Section 3.2**). This was rather expected, since median age in control group in Experiment 2 was greater than that of control group in Experiment 1 (**Chapter 4, Section 2.1; Chapter 5, Section 2.1**), and previous oculomotor data suggests that there is a selective vulnerability of the vertical saccade system to aging, reflected both in lower amplitude and velocity [38, 39]. In experiment 2, in healthy participants, there were no BOLD differences between prosaccades planes, while greater DMN during vertical antisaccades was still demonstrated (**Chapter 5, Section 3.3.3**). It should be taken into consideration however that the number of control subjects in Experiment 2 was roughly half of the number included in Experiment 1, which may have precluded the detection of some of the above BOLD differences.

2.2. Findings in patients.

PD patients exhibited less left frontal (i.e., FEF) BOLD activity than controls during prosaccades (see **Table 1**) (Experiment 1). We reasoned that this finding most probably reflects a reduced nigro-thalamo-cortical output (**Chapter 4, Section 4.3**) [5, 6, 40, 41]. The right parieto-occipital cortex (i.e., PEF, cuneus) and the cerebellum (i.e., right anterior lobe and tonsil, and left inferior semilunar lobule) on the other hand showed *less* BOLD activity in controls than PD patients during the same task (PEF hyperactivity was also seen during antisaccades). This hyperactivation of cortico-cerebellar regions in PD patients has been previously demonstrated and is thought to reflect a functional compensation for the defective basal ganglia in motor control [5, 42]. Compensatory mechanisms in more posterior areas in PD patients, as supported by our work, may help to explain normal or marginally disturbed saccadic performance in PD in previous work and in our own data (**Chapter 4, Section 3.2**) [1, 2, 43–45]. During antisaccades, the most relevant finding was the inability of PD patients to deactivate the DMN (i.e., dorsomedial prefrontal cortex [dmPFC] and middle temporal gyrus) to the extent of that seen in controls (**Chapter 4, Section 3.4.2**). This was a novel finding in PD eye movement research and extends previous work showing DMN dysfunction in PD while performing other cognitive tasks [46]. It is currently thought that the presence of non-motor symptoms including pain, particularly during the OFF-state may interfere in DMN deactivation, and indeed our patients were evaluated in the OFF-state or at least, OFF-medication (**Chapter 4, Section 2.1** and **4.1**) [46]. Unlike healthy participants,

positive BOLD clusters were absent during vertical > horizontal prosaccades in PD patients (see above, **Section 2.1**). One may then speculate that PD patients are no longer able to recruit additional brain areas during vertical saccades, which apparently require greater attentional demands than horizontal saccades. This is an important finding in our research that may help to explain why vertical-horizontal asymmetry in PD is more marked than in healthy participants [47]. Still concerning vertical-horizontal saccade asymmetry, if frontal (i.e., FEF) hypoactivity is indeed being compensated by parietal (i.e., PEF) hyperactivity (see above), and if one takes into account previous neurophysiological data reporting the existence of a downward saccade bias activity in PEF [17], then the hypothetical existence of such bias also for saccade plane in PEF, could equally explain the enhancement of a vertical-horizontal saccadic asymmetry in PD. Cuneus hypoactivity during vertical prosacades on the other hand can merely indicate an underrepresentation of the vertical meridian in visual cortex, albeit weakened spatio-attentional processing, and/or reduced top-down control from FEF and/or PEF on stimulus direction-specific modulation by cuneus may have played a role (**Chapter 4, Section 3.4.3**) [41, 48–51]. Indeed, in experiment 2, including older healthy participants than those of Experiment 1, cuneus hypoactivity was also seen in this group during the vertical antisacades (not prosaccades), possibly highlighting the existence of weakened spatio-attentional processing in aging (**Chapter 5, Section 3.3.3**) [52]. In the vertical > horizontal antisaccade contrast, PD patients deactivated less DMN areas and showed left PEF hypoactiviy during vertical antisaccades, when comparing the same contrast in controls (**Chapter 4, Section 3.4.3**). These findings again support the view that PD patients probably fail to substantially change brain activity (i.e., oculomotor and default-mode networks) when performing vertical saccades, and this becomes even more evident for more purposeful saccades (i.e., antisaccades).

fMRI findings in PSP patients (Experiment 2) overlapped those of PD patients (Experiment 1), but into a greater extent (see **Table 1**). Thus, during prosaccades, not only FEF, but also SEF and caudate nucleus where hypoactive in PSP patients relative to controls. Importantly, the inability to deactivate the DMN was already present during prosaccades in PSP patients (Experiment 2) (**Chapter 5, Section 3.3.1**), and not only during antisaccades (Experiment 1), as in PD patients (**Chapter 4, Section 3.4.1**). The evidence we provide for DMN dysfunction already during prosaccades in PSP patients is remarkable and tells us in these patients how much voluntary, supposedly reflexive saccades turn into, due to a severe slowing of prosaccades. Findings in SEF and caudate speak in favour of greater cortical and subcortical damage in PSP than PD, as extensively shown in neuropathological and imaging studies [53–56]. Importantly, while in PD patients FEF hypoactivity during saccades may indicate reduced nigro-thalamo-cortical output to this structure and not intrinsic dysfunction *per se*, in PSP patients, FEF hypoactivity most probably relates to a combination of intrinsic involvement of FEF and basal ganglia [56]. As in PD, we speculate that increased cerebellar activity (i.e., dorsal paraflocullus) during prosaccades in PSP patients may also reflect a compensatory mechanism (**Chapter 5, Section 3.3.1**). In PSP patients however, such compensation clearly is insufficient to optimize behavioural performance

(**Chapter 5, Section 3.2**). When comparing antisaccades between the PSP group and controls, findings were roughly similar to prosaccade findings. Nevertheless, an interesting pattern was identified. Only in vertical (and not horizontal) antisaccades comparison, was FEF, SEF, and thalamus hypoactivity in patients demonstrated (**Chapter 5, Section 3.3.2**). Notably, this supports the idea that PSP patients fail to generate at least equal cortical and subcortical activity during vertical horizontal saccades, as compared to horizontal voluntary saccades. This was further corroborated during vertical > horizontal saccade contrasts, during which patients showed lesser left FEF, basal ganglia, and right thalamus BOLD activity and greater right DMN deactivation during vertical saccades, particularly during vertical antisaccades (**Chapter 5, Section 3.3.3**). Importantly, our data provided support for the participation of cortical frontal oculomotor areas in the saccadic disturbance of PSP patients, possibly influencing latency and amplitude data in our sample (**Chapter 5, Section 3.2**). So far, most of the PSP oculomotor studies have exclusively focused in the severe brainstem damage to explain saccadic deficits in these patients [8].

Table 1. Summary of fMRI findings in patients

	Between-Groups analysis (patients vs. controls)	Within-groups analysis (vertical vs. horizontal PS) (vertical vs. horizontal AS)
PD		
Vertical PS	↑ right PEF, ↑ cerebellum (left inferior lobe)	↓ cuneus
Horizontal PS	↓ left FEF ↑ right PEF, ↑ right cuneus ↑ cerebellum (right tonsil, left inferior lobe)	
Vertical AS	↓ DMN deactivation (left dmPFC, right vmPFC, left mTG)	↓ left PEF, ↓ lingual gyrus ↑ DMN deactivation (left pCG, right vmPFC)
Horizontal AS	↑ right PEF ↓ DMN deactivation (left dmPFC, left mTG) ↓ mOG	
PSP		
Vertical PS	↓ left FEF, ↓ left SEF ↓ right caudate, ↓ iOG, ↓ right FuG, ↓ left cuneus ↑ cerebellum (right uvula, right posterior lobe)	↑ DMN deactivation (right mTG)
Horizontal PS	↓ left FEF, ↓ left SEF ↓ right caudate, ↓ iOG, ↓ DMN deactivation (lOFC, right insula) ↑ cerebellum (tonsils)	
Vertical AS	↓ left FEF, ↓ left SEF, ↓ right thalamus, ↓ right iOG ↓ DMN deactivation	↓ left FEF, ↓ left putamen ↓ left thalamus ↑ DMN deactivation

	(left vmPFC, right pCG, left PHG) ↓ cerebellum (posterior lobe, right nodulus)	(vmPFC)
Horizontal AS	↓ right mOG ↓ DMN deactivation (dmPFC, right pCG) ↑ cerebellum (right tonsil)	

↓ decreased activity; ↑ increased activity; or lesser (↓) or greater (↑) DMN deactivation

fMRI, functional magnetic resonance; PD, Parkinson's disease; PSP, progressive supranuclear palsy; PS, prosaccades; AS, antisaccades; PEF, parietal eye field; FEF, frontal eye field; DMN, default-mode network; vmPFC, ventromedial prefrontal cortex; dmPFC, dorsomedial prefrontal cortex; mTG, middle temporal gyrus; pCG, posterior cingulate gyrus; mOG, middle occipital gyrus; SEF, supplementary eye field; iOG, inferior occipital gyrus; FuG, fusiform gyrus; lOFC, lateral orbitofrontal cortex; PHG, parahippocampal gyrus

2.3. Additional considerations.

We used a lenient threshold for the statistical fMRI analysis: $P < 0.01$, corrected for multiple comparisons using a spatial extent correction method, i.e., cluster-based multiple comparisons correction (1000 iterations), setting as voxel-level primary threshold $P < 0.01$ (**Chapter 4, Section 2.3.2**). Recent recommendations suggest decreasing P - value lower limit, as higher P – values may increase the rate of false positive results [57, 58]. Given that this was the first fMRI study comparing BOLD activity between vertical and horizontal saccades, and our study was powered by a sample size roughly approaching 20 participants in each group for Experiment 1, we decided to use the aforementioned cluster-extent based correction and threshold in order to increase sensitivity to detect BOLD activations [59]. Interestingly, using a different analysis (region-of-interest [ROI] analysis), less prone to the influence of multiple comparisons, Cameron et al. (2012), also found BOLD differences in some of the areas detected in our study (e.g., FEF hypoactivity), when comparing BOLD activity between PD patients and healthy controls [6]. In Experiment 2, albeit with a smaller sample size and thus a less powered study, we still used the same statistical threshold and correction (**Chapter 5, Section 2.3.3**). Still, it was interesting to see the validity and consistency of our fMRI results in PD patients (Experiment 1) being confirmed in another parkinsonian disorder (PSP) (Experiment 2). Namely, not only in PD, but also in PSP patients, we were able to demonstrate less DMN deactivation and/or FEF and/or PEF hypoactivity relative to controls, in both vertical and horizontal prosaccades and antisaccades. Additionally, we were able to show that both in PD and PSP patients (but not in controls), FEF and/or PEF were hypoactive during vertical purposeful saccades.

Moreover, negative BOLD activity in basal ganglia during saccades was only seen in PSP patients (and not in PD), when compared to controls, which is in fair agreement with previous volumetric and functional imaging data showing greater dysfunction of basal ganglia in PSP (**Chapter 4, Section 3.4**; **Chapter 5, Section 3.3**) [53–55].

3. Future directions.

Our fMRI findings concerning vertical saccades pose several interesting questions. We hope these questions stimulate new research that can drive us closer towards a better understanding of the control of saccadic eye movements in health and disease.

We provided evidence for distinctive BOLD patterns during the execution of vertical versus horizontal saccades. Since the large majority of fMRI studies performed to date have used only horizontal saccades and most of the assumptions these provide were based on subjects' performance along one saccade plane only, should fMRI saccade paradigms in the future also include vertical (and/or oblique) saccades? We believe so, for several reasons. Although analysis concerning putative hemispheric contralaterality and hemispheric lateralization during saccades is easier and more intuitive if using only the horizontal plane [27, 60], adding the vertical plane (1) provides a wider perspective on brain dynamics and hemispheric interaction, (2) possibly puts in evidence the activity of brain networks (e.g., DMN) otherwise "silent" during the execution of horizontal saccades and thus clarifying their role in the execution of saccades, (3) may provide increased sensitivity for detecting BOLD differences in oculomotor and/or default-mode network areas, otherwise going unnoticed if only comparing horizontal saccades between groups, (4) raises several unanswered questions (e.g., how is vector inversion programmed in PEF for vertical antisaccades? Is shift in activity from one hemisphere to the other still required? [60]; Do oblique saccades directed to targets located close to the vertical meridian still evoke a predominant contralateral BOLD pattern? In other words, what is the saccade angle approaching verticality, beyond which bilateral symmetric hemispheric BOLD activity is achieved?). Choosing a blocked-design did not allow us to differentiate between covert attention-related activity and saccade-related activity [61]. Future studies, using paradigms including both saccade planes, but adapted to an event-related fMRI design, and possibly using memory guided saccades with long delays interposed between trial events, should clarify if greater BOLD activity during vertical saccades mainly occurs during the anticipation, preparation and/or execution of saccades. While our fMRI results shed light into the cortical mechanisms generating vertical and horizontal saccades, future fMRI studies should be combined and correlated with neurophysiological investigative approaches (e.g., transcranial magnetic stimulation; magneto-encephalography [16, 17]) in order to better detail the temporal events occurring within PEF and FEF during the execution of vertical and horizontal saccades. fMRI studies investigating brainstem activity are unquestionably needed and would be most useful to investigate and further clarify brainstem dysfunction in PSP patients. Moreover, being able to correlate BOLD

activity in FEF, SC and possibly riMLF and PPRF with saccade behavioural parameters in these patients would better elucidate the physiopathology of latency and amplitude disturbance in PSP and ascribed it to brainstem and/or cortical and subcortical damage [62]. From a pure clinical and diagnostic perspective, our work shows that vertical antisaccades may become a potential sensitive diagnostic and progression marker of PD in the future, since it was the only parameter that tended to be different between healthy participants and patients at a very early stage of the disease, lacking substantial motor and cognitive impairment (Experiment 1). We purposefully added a second dimension (vertical plane) for investigating purposeful saccades in PD, in order to potentially increase the difficulty of the task [4]. Moreover, we intermingled vertical with horizontal trials in randomized fashion outside the scanner aiming the same goal. The rate of directional errors in the antisaccade task possibly reflects these modulations (up to 50%), compared with the average number reported in the literature for horizontal antisaccades (~30%) [63]. Future research should investigate in more detail the role of adding the vertical dimension not only to reflexive saccades, but also to more voluntary saccades. This has only been rarely done so far [64–66]. We averaged together the results of upward and downward saccades because we were mainly interested in contrasting vertical with horizontal saccades. However, it will be most relevant in the future to investigate if BOLD differences between upward and downward saccadic movements also exist, not only in healthy participants [4, 65], but also in disease states. PSP patients for instance often show selective impairment of upward or downward saccades at early stages of the disease [10]. In our research, albeit no formal comparison was performed, there was a suggestion that also aging may change the pattern of BOLD asymmetries between vertical and horizontal saccades in healthy participants (Experiment 1 versus Experiment 2). This should be probed in future studies.

Basal ganglia and dopamine play an active role in reward processing [67]. Previous literature has shown that saccade parameters (e.g., velocity, latency) are modulated by reward expectations [68, 69]. PD patients however, particularly OFF medication (levodopa), often demonstrate a blunted response to reward. It has recently been shown that patients, when performing a saccade paradigm where reward expectation in each horizontal saccade trial is parametrically modulated, do not seem to show a significant increase in saccadic velocity as reward expectation increases [69]. Since saccades in PD show differences between the horizontal and vertical plane both at a behavioural and functional level, we recently designed an antisaccade task inside and outside the scanner to probe the effects of the interaction of saccade plane (vertical versus horizontal), medication status (ON versus OFF medication), and reward expectation (rewarded trials versus punished trials versus neutral trials) on saccade parameters and BOLD activity of PD patients and healthy participants. At the time of this writing, we are analyzing this data and hope to provide further insights on the processing of reward in PD.

4. Concluding remarks.

There are very few studies focusing on the cortical control of vertical saccades. In our research, we demonstrate distinctive cortical activation patterns for vertical versus horizontal saccades in critical areas within the default-mode and oculomotor networks, both in health and disease. These findings not only carry implications for future fMRI research of saccades and paradigm designs but importantly also open new venues for the diagnostic imaging of neurodegenerative diseases, particularly those predominantly affecting vertical saccades.

References.

1. Macaskill MR, Graham CF, Pitcher TL, et al (2012) The influence of motor and cognitive impairment upon visually-guided saccades in Parkinson's disease. Neuropsychologia 50:3338–47. doi: 10.1016/j.neuropsychologia.2012.09.025

2. Terao Y, Fukuda H, Yugeta A, et al (2011) Initiation and inhibitory control of saccades with the progression of Parkinson's disease - changes in three major drives converging on the superior colliculus. Neuropsychologia 49:1794–806. doi: 10.1016/j.neuropsychologia.2011.03.002

3. Abed F (1991) Cultural Influences on Visual Scanning Patterns. J Cross Cult Psychol 22:525–534. doi: 10.1177/0022022191224006

4. Dafoe JM, Armstrong IT, Munoz DP (2007) The influence of stimulus direction and eccentricity on pro- and anti-saccades in humans. Exp brain Res 179:563–70. doi: 10.1007/s00221-006-0817-8

5. Rieger JW, Kim A, Argyelan M, et al (2008) Cortical functional anatomy of voluntary saccades in Parkinson disease. Clin EEG Neurosci 39:169–74.

6. Cameron IGM, Pari G, Alahyane N, et al (2012) Impaired executive function signals in motor brain regions in Parkinson's disease. Neuroimage 60:1156–70. doi: 10.1016/j.neuroimage.2012.01.057

7. Garbutt S, Matlin A, Hellmuth J, et al (2008) Oculomotor function in frontotemporal lobar degeneration, related disorders and Alzheimer's disease. Brain 131:1268–81. doi: 10.1093/brain/awn047

8. Bhidayasiri R, Riley DE, Somers JT, et al (2001) Pathophysiology of slow vertical saccades in progressive supranuclear palsy. Neurology 57:2070–7.

9. Rivaud-Péchoux S, Vidailhet M, Brandel JP, Gaymard B (2007) Mixing pro- and antisaccades in patients with parkinsonian syndromes. Brain 130:256–64. doi: 10.1093/brain/awl315

10. Chen AL, Riley DE, King SA, et al (2010) The disturbance of gaze in progressive supranuclear palsy: implications for pathogenesis. Front Neurol 1:147. doi: 10.3389/fneur.2010.00147

11. Cordato NJ, Pantelis C, Halliday GM, et al (2002) Frontal atrophy correlates with behavioural changes in progressive supranuclear palsy. Brain 125:789–800.

12. Liscic RM, Srulijes K, Gröger A, et al (2013) Differentiation of progressive supranuclear palsy: clinical, imaging and laboratory tools. Acta Neurol Scand 127:362–70. doi: 10.1111/ane.12067

13. Bruce CJ, Goldberg ME, Bushnell MC, Stanton GB (1985) Primate frontal eye fields. II. Physiological and anatomical correlates of electrically evoked eye movements. J Neurophysiol 54:714–34.

14. Colby CL, Goldberg ME (1999) Space and attention in parietal cortex. Annu Rev Neurosci 22:319–49. doi: 10.1146/annurev.neuro.22.1.319

15. Bender MB (1980) Brain control of conjugate horizontal and vertical eye movements: a survey of the structural and functional correlates. Brain 103:23–69.

16. Tzelepi A, Yang Q, Kapoula Z (2005) The effect of transcranial magnetic stimulation on the

latencies of vertical saccades. Exp brain Res 164:67–77. doi: 10.1007/s00221-005-2250-9

17. Tzelepi A, Laskaris N, Amditis A, Kapoula Z (2010) Cortical activity preceding vertical saccades: a MEG study. Brain Res 1321:105–16. doi: 10.1016/j.brainres.2010.01.002

18. Blanke O, Seeck M (2003) Direction of saccadic and smooth eye movements induced by electrical stimulation of the human frontal eye field: effect of orbital position. Exp brain Res 150:174–83. doi: 10.1007/s00221-003-1395-7

19. Krebs RM, Woldorff MG, Tempelmann C, et al (2010) High-field FMRI reveals brain activation patterns underlying saccade execution in the human superior colliculus. PLoS One 5:e8691. doi: 10.1371/journal.pone.0008691

20. Kastner S, DeSimone K, Konen CS, et al (2007) Topographic maps in human frontal cortex revealed in memory-guided saccade and spatial working-memory tasks. J Neurophysiol 97:3494–507. doi: 10.1152/jn.00010.2007

21. Sereno MI, Pitzalis S, Martinez A (2001) Mapping of contralateral space in retinotopic coordinates by a parietal cortical area in humans. Science 294:1350–4. doi: 10.1126/science.1063695

22. Leigh RJ, Zee DS (2015) The neurology of eye movements, 5th ed. Oxford University Press, New York

23. Leoné FTM, Toni I, Medendorp WP (2014) Two-dimensional spatial tuning for saccades in human parieto-frontal cortex. Neuroimage 87:476–489. doi: 10.1016/j.neuroimage.2013.09.067

24. Blatt GJ, Andersen RA, Stoner GR (1990) Visual receptive field organization and cortico-cortical connections of the lateral intraparietal area (area LIP) in the macaque. J Comp Neurol 299:421–45. doi: 10.1002/cne.902990404

25. Mott FW, Schaefer EA (1890) On associated eye-movements produced by cortical faradization of the monkey's brain. Brain 13:165–173. doi: 10.1093/brain/13.2.165

26. Jadhav AP, Zenonos G, Pless M, et al (2013) A Variant of the Anterior Opercular Syndrome With Supranuclear Gaze Palsy. JAMA Neurol 70:800. doi: 10.1001/jamaneurol.2013.1947

27. Petit L, Zago L, Vigneau M, et al (2009) Functional Asymmetries Revealed in Visually Guided Saccades: An fMRI Study. J Neurophysiol 102:2994–3003. doi: 10.1152/jn.00280.2009

28. Dieterich M, Bucher SF, Seelos KC, Brandt T (2000) Cerebellar activation during optokinetic stimulation and saccades. Neurology 54:148–55.

29. Grosbras M-H, Laird AR, Paus T (2005) Cortical regions involved in eye movements, shifts of attention, and gaze perception. Hum Brain Mapp 25:140–54. doi: 10.1002/hbm.20145

30. Logothetis NK (2008) What we can do and what we cannot do with fMRI. Nature 453:869–878. doi: 10.1038/nature06976

31. Mao L, Zhou B, Zhou W, Han S (2007) Neural correlates of covert orienting of visual spatial attention along vertical and horizontal dimensions. Brain Res 1136:142–53. doi: 10.1016/j.brainres.2006.12.031

32. Foulsham T, Kingstone A, Underwood G (2008) Turning the world around: patterns in saccade direction vary with picture orientation. Vision Res 48:1777–90. doi: 10.1016/j.visres.2008.05.018

33. Ozyurt J, Greenlee MW (2011) Neural correlates of inter- and intra-individual saccadic reaction

time differences in the gap/overlap paradigm. J Neurophysiol 105:2438–47. doi: 10.1152/jn.00660.2009

34. Polli FE, Barton JJS, Cain MS, et al (2005) Rostral and dorsal anterior cingulate cortex make dissociable contributions during antisaccade error commission. Proc Natl Acad Sci U S A 102:15700–5. doi: 10.1073/pnas.0503657102

35. Raichle ME (2015) The Brain's Default Mode Network. http://dx.doi.org/10.1146/annurev-neuro-071013-014030

36. Mayer JS, Roebroeck A, Maurer K, Linden DEJ (2010) Specialization in the default mode: Task-induced brain deactivations dissociate between visual working memory and attention. Hum Brain Mapp 31:126–39. doi: 10.1002/hbm.20850

37. Persson J, Lustig C, Nelson JK, Reuter-Lorenz PA (2007) Age differences in deactivation: a link to cognitive control? J Cogn Neurosci 19:1021–32. doi: 10.1162/jocn.2007.19.6.1021

38. Huaman AG, Sharpe JA (1993) Vertical saccades in senescence. Invest Ophthalmol Vis Sci 34:2588–95.

39. Hotson JR, Steinke GW (2009) Vertical and Horizontal Saccades in Aging and Dementia: Failure to Inhibit Anticipatory Saccades. http://dx.doi.org/10.3109/01658108808996053

40. Alexander GE, DeLong MR, Strick PL (1986) Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu Rev Neurosci 9:357–81. doi: 10.1146/annurev.ne.09.030186.002041

41. Logothetis NK, Wandell BA (2004) Interpreting the BOLD signal. Annu Rev Physiol 66:735–69. doi: 10.1146/annurev.physiol.66.082602.092845

42. Tessitore A, Giordano A, De Micco R, et al (2014) Sensorimotor connectivity in Parkinson's disease: the role of functional neuroimaging. Front Neurol 5:180. doi: 10.3389/fneur.2014.00180

43. McDowell JE, Dyckman KA, Austin BP, Clementz BA (2008) Neurophysiology and neuroanatomy of reflexive and volitional saccades: evidence from studies of humans. Brain Cogn 68:255–70. doi: 10.1016/j.bandc.2008.08.016

44. Pflugshaupt T, Nyffeler T, von Wartburg R, et al (2009) Loss of exploratory vertical saccades after unilateral frontal eye field damage. BMJ Case Rep. doi: 10.1136/bcr.08.2008.0687

45. Wu T, Hallett M (2013) The cerebellum in Parkinson's disease. Brain 136:696–709. doi: 10.1093/brain/aws360

46. Delaveau P, Salgado-Pineda P, Fossati P, et al (2010) Dopaminergic modulation of the default mode network in Parkinson's disease. Eur Neuropsychopharmacol 20:784–92. doi: 10.1016/j.euroneuro.2010.07.001

47. Rottach KG, Riley DE, DiScenna AO, et al (1996) Dynamic properties of horizontal and vertical eye movements in parkinsonian syndromes. Ann Neurol 39:368–77. doi: 10.1002/ana.410390314

48. Janik J, Ropella K, DeYoe E (2003) Distortions of human retinotopy obtained with temporal phase mapped fMRI. In: Soc Neurosci Abstr. p 29:658

49. Nys GMS, Santens P, Vingerhoets G (2010) Horizontal and vertical attentional orienting in Parkinson's disease. Brain Cogn 74:179–85. doi: 10.1016/j.bandc.2010.07.004

50. McDowell JE, Kissler JM, Berg P, et al (2005) Electroencephalography/magnetoencephalography

study of cortical activities preceding prosaccades and antisaccades. Neuroreport 16:663–8.

51. Simpson G V, Weber DL, Dale CL, et al (2011) Dynamic activation of frontal, parietal, and sensory regions underlying anticipatory visual spatial attention. J Neurosci 31:13880–9. doi: 10.1523/JNEUROSCI.1519-10.2011

52. Gunter TC, Jackson JL, Mulder G (1996) Focussing on aging: an electrophysiological exploration of spatial and attentional processing during reading. Biol Psychol 43:103–145. doi: 10.1016/0301-0511(95)05180-5

53. Burciu RG, Ofori E, Shukla P, et al (2015) Distinct patterns of brain activity in progressive supranuclear palsy and Parkinson's disease. Mov Disord 30:1248–58. doi: 10.1002/mds.26294

54. Messina D, Cerasa A, Condino F, et al (2011) Patterns of brain atrophy in Parkinson's disease, progressive supranuclear palsy and multiple system atrophy. Parkinsonism Relat Disord 17:172–6. doi: 10.1016/j.parkreldis.2010.12.010

55. Oh M, Kim JS, Kim JY, et al (2012) Subregional patterns of preferential striatal dopamine transporter loss differ in Parkinson disease, progressive supranuclear palsy, and multiple-system atrophy. J Nucl Med 53:399–406. doi: 10.2967/jnumed.111.095224

56. Williams DR, Holton JL, Strand C, et al (2007) Pathological tau burden and distribution distinguishes progressive supranuclear palsy-parkinsonism from Richardson's syndrome. Brain 130:1566–76. doi: 10.1093/brain/awm104

57. Woo C-W, Krishnan A, Wager TD (2014) Cluster-extent based thresholding in fMRI analyses: Pitfalls and recommendations. Neuroimage. doi: 10.1016/j.neuroimage.2013.12.058

58. Silver M, Montana G, Nichols TE, Alzheimer's Disease Neuroimaging Initiative (2011) False positives in neuroimaging genetics using voxel-based morphometry data. Neuroimage 54:992–1000. doi: 10.1016/j.neuroimage.2010.08.049

59. Friston KJ, Worsley KJ, Frackowiak RS, et al (1994) Assessing the significance of focal activations using their spatial extent. Hum Brain Mapp 1:210–20. doi: 10.1002/hbm.460010306

60. Medendorp WP, Goltz HC, Vilis T (2005) Remapping the remembered target location for anti-saccades in human posterior parietal cortex. J Neurophysiol 94:734–40. doi: 10.1152/jn.01331.2004

61. Brown MRG, Vilis T, Everling S (2007) Frontoparietal activation with preparation for antisaccades. J Neurophysiol 98:1751–62. doi: 10.1152/jn.00460.2007

62. Neggers SFW, Raemaekers MAH, Lampmann EEL, et al (2005) Cortical and subcortical contributions to saccade latency in the human brain. Eur J Neurosci 21:2853–63. doi: 10.1111/j.1460-9568.2005.04129.x

63. Hutton SB, Ettinger U (2006) The antisaccade task as a research tool in psychopathology: A critical review. Psychophysiology 43:302–313. doi: 10.1111/j.1469-8986.2006.00403.x

64. Rupp J, Dzemidzic M, Blekher T, et al (2012) Comparison of vertical and horizontal saccade measures and their relation to gray matter changes in premanifest and manifest Huntington disease. J Neurol 259:267–76. doi: 10.1007/s00415-011-6172-0

65. Bonnet C, Hanuška J, Rusz J, et al (2013) Horizontal and vertical eye movement metrics: what is important? Clin Neurophysiol 124:2216–29. doi: 10.1016/j.clinph.2013.05.002

66. Bonnet C, Rusz J, Megrelishvili M, et al (2014) Eye movements in ephedrone-induced parkinsonism. PLoS One 9:e104784. doi: 10.1371/journal.pone.0104784

67. Hikosaka O, Nakamura K, Nakahara H (2006) Basal ganglia orient eyes to reward. J Neurophysiol 95:567–84. doi: 10.1152/jn.00458.2005

68. Harsay HA, Buitenweg JI V, Wijnen JG, et al (2010) Remedial effects of motivational incentive on declining cognitive control in healthy aging and Parkinson's disease. Front Aging Neurosci 2:144. doi: 10.3389/fnagi.2010.00144

69. Muhammed K, Manohar S, Ben Yehuda M, et al (2016) Reward sensitivity deficits modulated by dopamine are associated with apathy in Parkinson's disease. Brain 24:2175–2186. doi: 10.1093/brain/aww188

Appendix

Supplemental Table 1. Talairach coordinates (x,y,z) and number of voxels (v) of peak activations in GLM contrast maps for AS-PS contrast*

				AS>PS Local maxima peak coordinates (TLS)			
Anatomical Region or Functional Label	Side	BA	Dir	x	y	z	v
CTL							
pCG	L	31	(-)	-1	-55	22	4590
		31	(-)	-1	-30	35	891
vmPFC	R	32	(-)	6	43	4	3471
	L	32	(-)	-7	42	0	949
	10		(-)	-15	53	19	1959
dmPFC	L	8	(-)	-13	35	45	655
iPL	L	39	(-)	-43	-64	28	627
iTG/mTG	L	20	(-)	-50	-24	-11	626
Cerebellar aL	R		(+)	28	-47	-27	743
PD							
pCG	L	31	(-)	-10	-63	24	2221
CTL>PD							
dmPFC	L	8	(-)	-10	33	49	421
iTG/mTG	L	20	(-)	-49	-24	-11	606
lOFC	R	10	(-)	39	40	0	452

AS, antisaccade; PS, prosaccade; CTL, controls; PD, Parkinson's disease; pCG, posterior cingulate gyrus; vmPFC, ventromedial prefrontal cortex; dmPFC, dorsomedial prefrontal cortex; iPL, inferior parietal lobe; iTG, inferior temporal gyrus; mTG, middle temporal gyrus; lOFC, lateral orbitofrontal cortex; TLS, Talairach standard; BA, Brodmann area

"Peak" refers to location of voxel with most significant activation

"Side" refers to the location of the activation: R, right hemisphere; L, left hemisphere

"Dir" refers to the direction of contrast: greater (+) or less (-) antisaccade activation compared to prosaccade

"/" between two anatomical regions and/or functional labels indicates clusters involving two contiguous areas

* Random effects analysis, $p<0.01$, corrected for multiple comparisons at the cluster level

Here we comment on antisaccade (AS) > prosaccade (PS) contrast unexpected findings. Because this was not the primary interest of our study, the design chosen may not have been optimal to detect classical greater frontal eye field (FEF), supplementary eye field (SEF), parital eye field (PEF), dorsolateral prefrontal cortex (dlPFC), and anterior cingulate gyrus (aCG) BOLD activations in AS task (Jamadar et al., 2013). Actually, dlPFC actvation was already present in PS > baseline contrast, and no significant BOLD differences were found in AS > PS contrast in any of these areas. Additionally, in the latter contrast we found greater deactivation of areas that overlap with the defaut mode network (DMN) (e.g., dorsomedial prefrontal cortex [dmPFC], posteiro cingulate gyrus [pCG], middle emporal gyrus [mTG], inferior parietal lobe [iPL]), more so for controls (see **Figure 2** in the main manuscript and **Supplemental table 1**). Given that DMN areas usually show higher BOLD activity during rest than during cognitive tasks, one may assume that AS task in our paradigm was indeed more demanding than PS task, since it deactivated at a greater extent the DMN, as found by others (Buckner et al., 2008; Herweg et al., 2014). If so, why did we not find BOLD differences in oculomotor and executive areas in AS > PS contrast? One possible reason concerns methodology. Unlike standard saccade paradigms using 2 targets along the horizontal meridian, our saccadic paradigm consisted of 4 targets vertically and horizontally distributed. Thus, target number and/or saccade direction may have been associated with greater attentional load (most reflected in PS) and thus BOLD differences between PS and AS task may have been eliminated. In a recent work exploring the effects of attentional load on PS and AS paradigms, Chan et al. nicely demonstrated similar BOLD activities for oculomotor areas in PS and AS task if a rapid serial visual presentation (attentional load) was displayed during the saccade preparatory period (Chan et al., 2015). By analogy, having added two more targets (1 superior; 1 inferior) to a classical 2-target saccade paradigm in our case, might have increased attentional load. However, adding multiple targets with variable amplitude along the horizontal meridian to a classical PS – AS paradigm did not eliminate classical BOLD differences in AS > PS contrast in a recent study (Herweg et al., 2014). Curtis and Esposito on the other hand also used multiple locations (8) in an event related fMRI study, but here targets were distributed along horizontal, vertical and oblique meridians, sharing similarities with our paradigm. Interestingly, they found no differences in FEF or PEF in AS > PS contrast during the late preparatory period (Curtis and D'Esposito, 2003). Ford et al., only displaying 2 horizontal targets, showed greater activation in FEF, SEF and PEF during the same period in AS (Ford et al., 2005). Taken together, although target number and other factors may have accounted for these inconsistencies, the above findings and our own lead us to hypothesize that adding the vertical dimension to our paradigm may have increased attentional load and eliminated AS > PS BOLD differences. Moreover, randomizing vertical and horizontal saccade blocks order within PS and AS runs may have add uncertainty with respect to upcoming block direction, rendering PS task less "reflexive" (Cornelissen et al., 2002). One other aspect that may have played an additional role was the high percentage of AS direction errors outside the scanner. Although we were not able to calculate this parameter inside the scanner in order to draw firm conclusions, rapid event fMRI studies have clearly demonstrated that AS

direction errors are associated with lower BOLD activity in PEF, SEF, dlPFC, aCG during saccade preparatory period, when compared to correct AS (Cameron et al., 2010; Curtis and D'Esposito, 2003; Ford et al., 2005). Lastly, one cannot exclude an order effect, since with did not counterbalanced for AS and OS run order.

References.

Buckner, R.L., Andrews-Hanna, J.R., Schacter, D.L., 2008. The brain's default network: anatomy, function, and relevance to disease. Ann N Y Acad Sci. 1124**,** 1-38.

Cameron, I.G., Watanabe, M., Pari, G., Munoz, D.P., 2010. Executive impairment in Parkinson's disease: response automaticity and task switching. Neuropsychologia. 48**,** 1948-57.

Chan, J.L., Kucyi, A., DeSouza, J.F., 2015. Stable Task Representations under Attentional Load Revealed with Multivariate Pattern Analysis of Human Brain Activity. J Cogn Neurosci. 27**,** 1789-800.

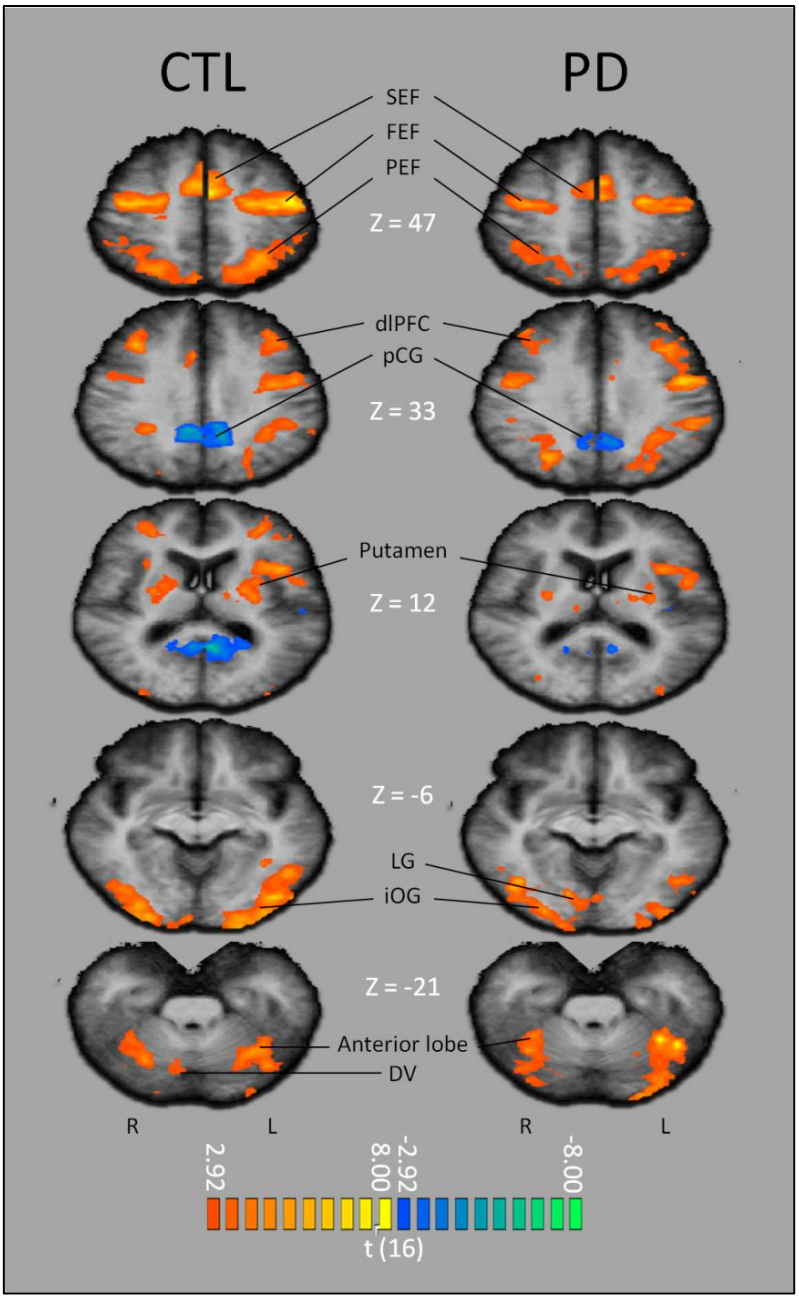
Cornelissen, F.W., Kimmig, H., Schira, M., Rutschmann, R.M., Maguire, R.P., Broerse, A., Den Boer, J.A., Greenlee, M.W., 2002. Event-related fMRI responses in the human frontal eye fields in a randomized pro- and antisaccade task. Exp Brain Res. 145**,** 270-4.

Curtis, C.E., D'Esposito, M., 2003. Success and failure suppressing reflexive behavior. J Cogn Neurosci. 15**,** 409-18.

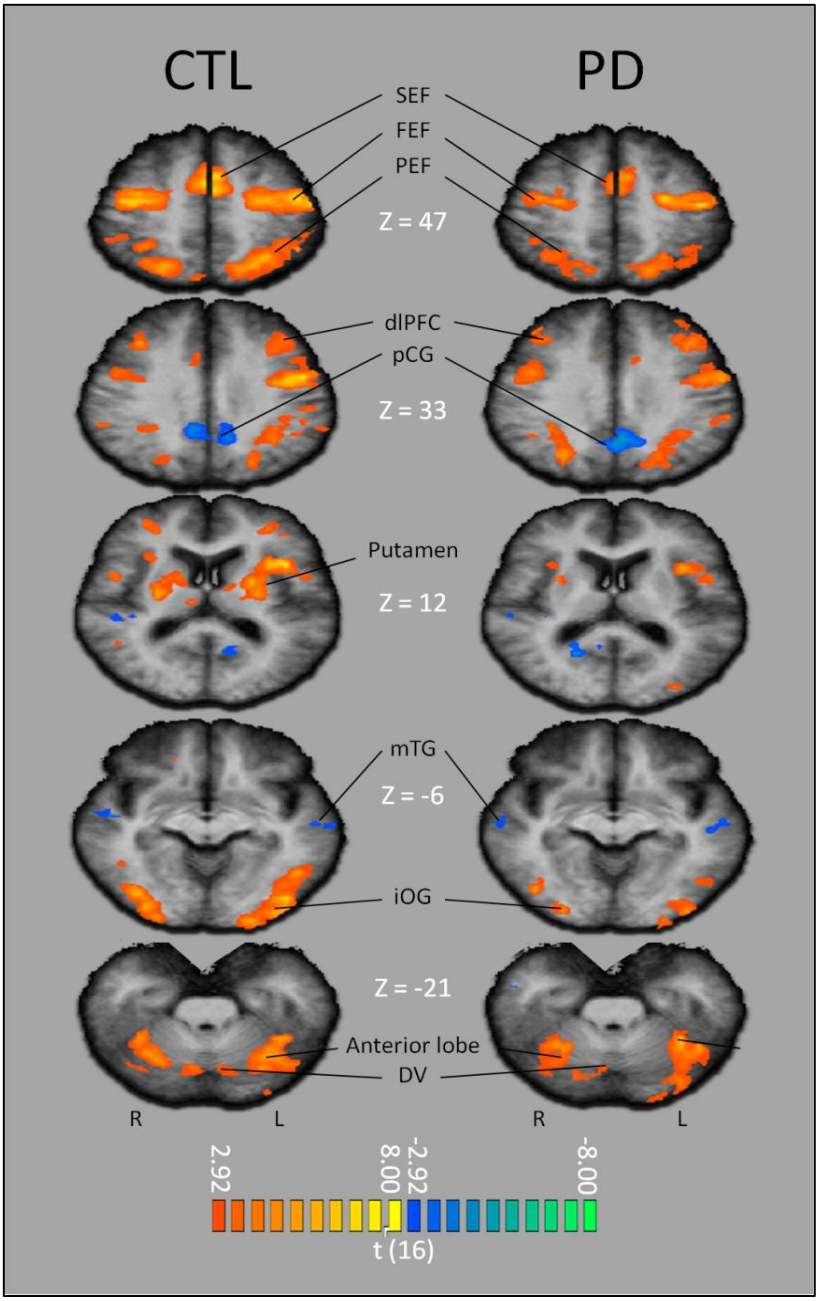
Ford, K.A., Goltz, H.C., Brown, M.R., Everling, S., 2005. Neural processes associated with antisaccade task performance investigated with event-related FMRI. J Neurophysiol. 94**,** 429-40.

Herweg, N.A., Weber, B., Kasparbauer, A., Meyhofer, I., Steffens, M., Smyrnis, N., Ettinger, U., 2014. Functional magnetic resonance imaging of sensorimotor transformations in saccades and antisaccades. Neuroimage. 102 Pt 2**,** 848-60.

Jamadar, S.D., Fielding, J., Egan, G.F., 2013. Quantitative meta-analysis of fMRI and PET studies reveals consistent activation in fronto-striatal-parietal regions and cerebellum during antisaccades and prosaccades. Front Psychol. 4**,** 749.



Supplemental figure 1. T-contrast maps of horizontal PS > baseline contrast in control group (CTL, n= 17, left panel) and PD group (PD, n=15, right panel) displayed on standard T1 image ($p < 0.01$, corrected for multiple comparisons as estimated by Brain Voyager's Cluster-level Statistical Threshold Estimator with 1000 iterations). Yellow/red and blue/green regions represent greater and less saccade activation than baseline, respectively. For sake of clarity, BOLD modulations in insula, mOG, right LG (only in CTL), cuneus, FuG, uvula, and inferior semilunar lobule are not shown/marked here. L, left hemisphere; R, right hemisphere; FEF, frontal eye field; SEF, supplementary eye field; PEF, parietal eye field; dlPFC, dorsolateral prefrontal cortex; pCG, posterior cingulate gyrus; iOG, inferior occipital gyrus; LG, lingual gyrus; DV, dorsal vermis; mOG, middle occipital gyrus; FuG, fusiform gyrus (see **Text** for details)

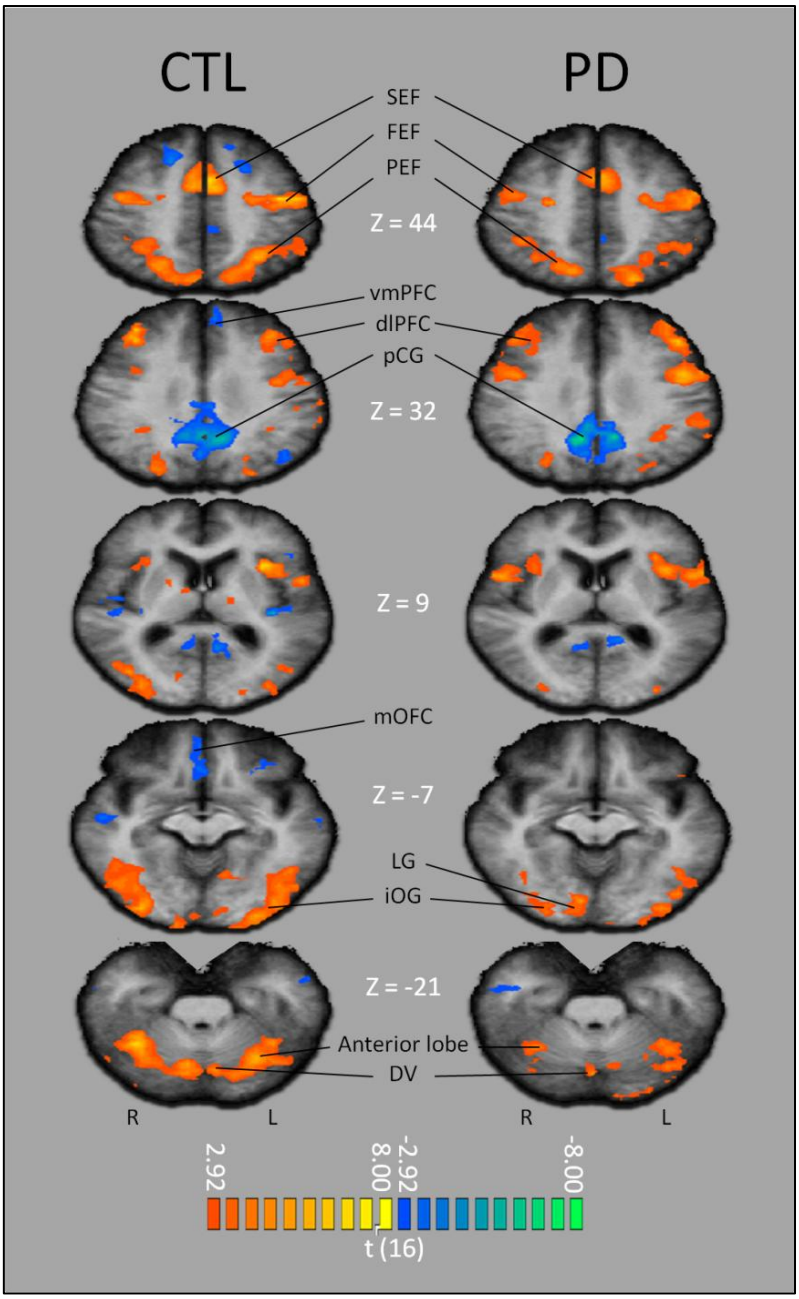


Supplemental figure 2. T-contrast maps of vertical PS > baseline contrast in control group (CTL, n= 17, left panel) and PD group (PD, n=15, right panel) displayed on standard T1 image ($p < 0.01$, corrected for multiple comparisons as estimated by Brain Voyager's Cluster-level Statistical Threshold Estimator with 1000 iterations). Yellow/red and blue/green regions represent greater and less saccade activation than baseline, respectively. For sake of clarity, BOLD modulations in insula, mOG, cuneus, FuG, LG, uvula, and inferior semilunar lobule are not shown/marked here. L, left hemisphere; R, right hemisphere; FEF, frontal eye field; SEF, supplementary eye field; PEF, parietal eye field; dlPFC, dorsolateral prefrontal cortex; pCG, posterior cingulate gyrus; mTG, middle temporal gyrus; mOG, middle occipital gyrus; iOG, inferior occipital gyrus; DV, dorsal vermis; FuG, fusiform gyrus; LG, lingual gyrus (see **Text** for details)

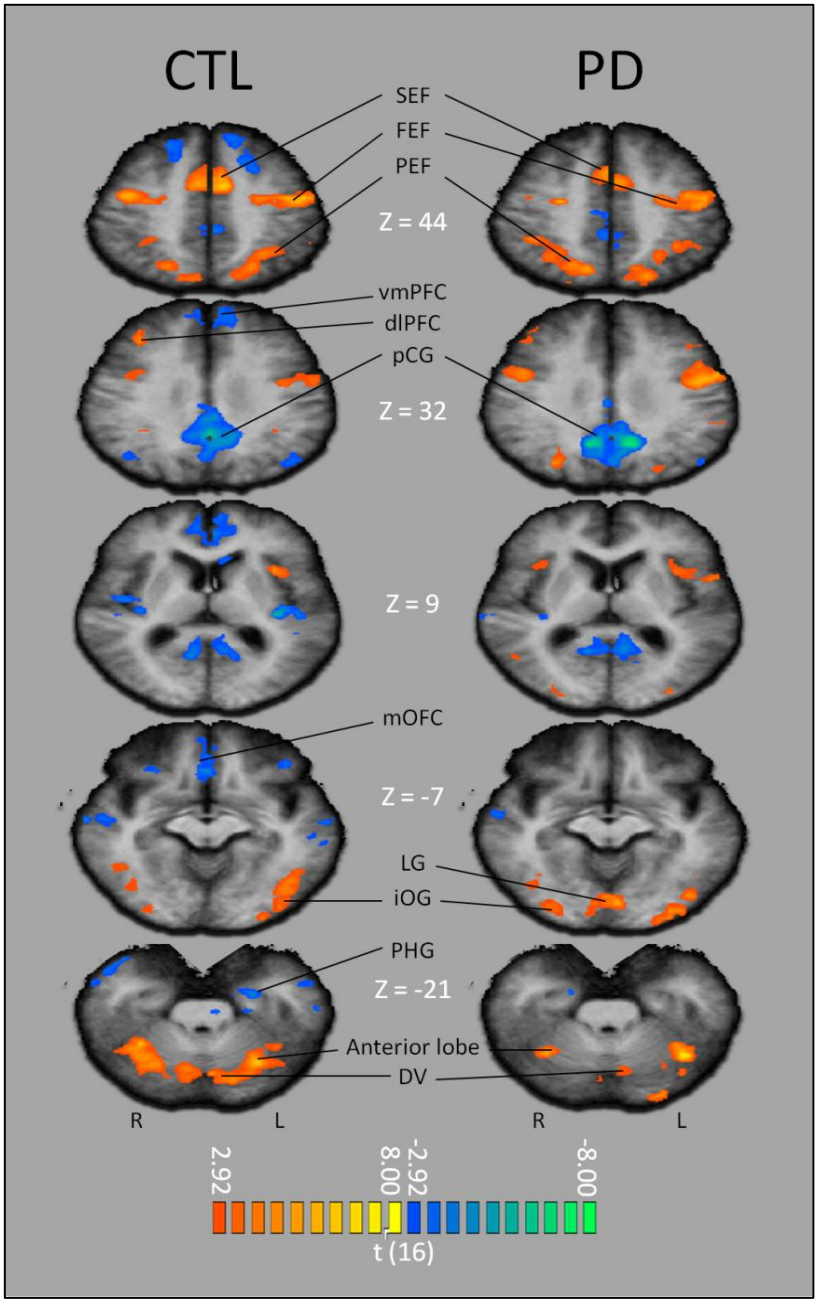
Prosaccades (PS) (within-groups analysis).

In within-groups analysis, horizontal PS > baseline and vertical PS > baseline contrasts showed bilateral activations of oculomotor areas (frontal eye field, FEF; supplementary eye field, SEF; and precuneus/parietal eye field, PEF) across groups, albeit apparently to a less extent in Parkinson's disease (PD) patients. However, there were distinctive patterns of activation for each group in other brain areas. **Supplemental Fig. 1** and **2** display the most relevant slices for these contrasts. Specifically, in horizontal PS > baseline contrast, controls (CTL) showed additional activations of the dorsolateral prefrontal cortex (dlPFC), putamen, occipital lobe (right lingual gyrus, LG; middle occipital gyrus, mOG; inferior occipital gyrus, iOG), temporal lobe (fusiform gyrus, FuG), and cerebellum (right uvula; anterior lobe; dorsal vermis, DV; right inferior semilunar lobule) and deactivations of the posterior cingulate gyrus (pCG) and insula. PD patients, while showing similar activations/deactivations, did not evidence mOG and DV positive BOLD activations, and showed additional positive BOLD activity in left LG. (see **Supplemental Fig. 1**).

In vertical PS > baseline contrast, CTL showed additional activations of dlPFC, putamen, occipital lobe (mOG; iOG), temporal lobe (FuG), and cerebellum (uvula, anterior lobe, DV, inferior semilunar lobule) and deactivations of pCG, anterior middle temporal gyrus (mTG) and insula. PD group showed the following differences when compared to CTL: (1) no significant putaminal activations were seen; (2) additional right LG activation was demonstrated; (3) insular deactivation and inferior semilunar lobule activation were unilateral (right) (see **Supplemental Fig. 2**).



Supplemental figure 3. T-contrast maps of horizontal AS > baseline contrast in control group (CTL, n= 17, left panel) and PD group (PD, n=15, right panel) displayed on standard T1 image ($p < 0.01$, corrected for multiple comparisons as estimated by Brain Voyager's Cluster-level Statistical Threshold Estimator with 1000 iterations). Yellow/red and blue/green regions represent greater and less saccade activation than baseline, respectively. For sake of clarity, BOLD modulations in caudate, aCG, anterior and posterior mTG, insula, mOG, FuG, uvula, and inferior semilunar lobule are not shown/marked here. L, left hemisphere; R, right hemisphere; FEF, frontal eye field; SEF, supplementary eye field; PEF, parietal eye field; vmPFC, ventromedial prefrontal cortex; dlPFC, dorsolateral prefrontal cortex; aCG, anterior cingulate gyrus; pCG, posterior cingulate gyrus; mOFC, medial orbitofrontal cortex; LG, lingual gyrus; iOG, inferior occipital gyrus; mOG, middle occipital gyrus; DV, dorsal vermis; mTG, middle temporal gyrus; FuG, fusiform gyrus (see **Text** for details)

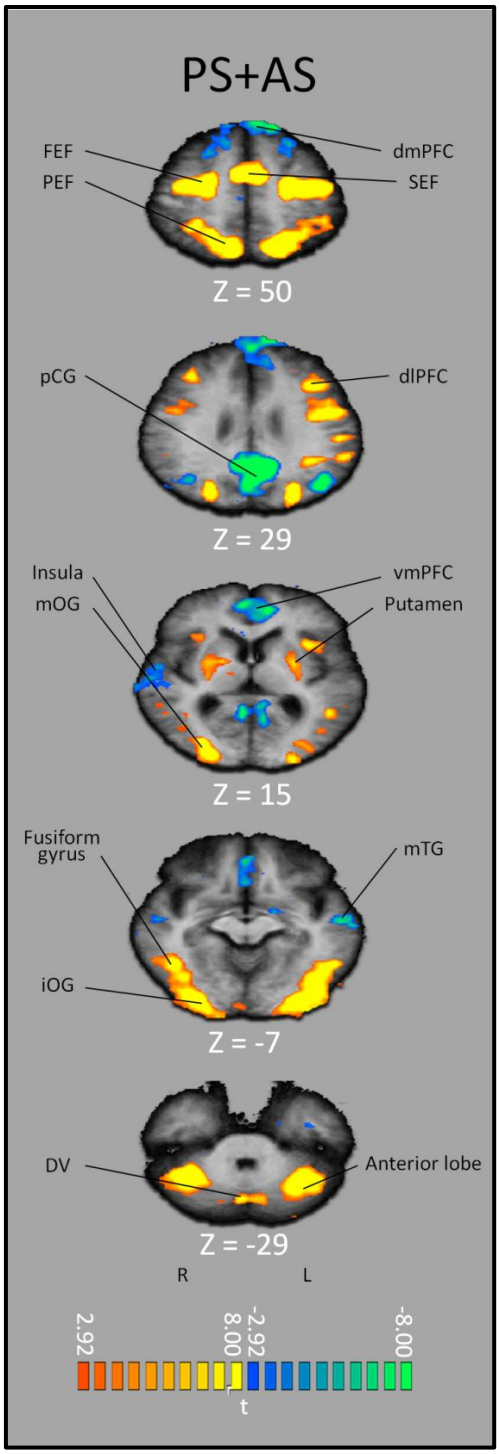


Supplemental figure 4. T-contrast maps of vertical AS > baseline contrast in control group (CTL, n= 17, left panel) and PD group (PD, n=15, right panel) displayed on standard T1 image ($p < 0.01$, corrected for multiple comparisons as estimated by Brain Voyager's Cluster-level Statistical Threshold Estimator with 1000 iterations). Yellow/red and blue/green regions represent greater and less saccade activation than baseline, respectively. For sake of clarity, BOLD modulations in anterior and posterior mTG, insula, mOG , FuG, uvula, and inferior semilunar lobule are not shown/marked here. L, left hemisphere; R, right hemisphere; FEF, frontal eye field; SEF, supplementary eye field; PEF, parietal eye field; vmPFC, ventromedial prefrontal cortex; dlPFC, dorsolateral prefrontal cortex; pCG, posterior cingulate gyrus; mOFC, medial orbitofrontal cortex; LG, lingual gyrus; mOG, middle occipital gyrus; iOG, inferior occipital gyrus; PHG, parahyppocampal gyrus; mTG, middle temporal gyrus; DV, dorsal vermis; FuG, fusiform gyrus (see **Text** for details)

Antisaccades (AS)(within-groups analysis).

In within-groups analysis, similarly to PS, horizontal AS > baseline and vertical AS > baseline contrasts showed bilateral activations of oculomotor areas (FEF, SEF and PCu/PEF) across groups. Also here, each group demonstrated different activation patterns in other brain areas. **Supplemental Fig. 3** and **4** display the most relevant slices for these contrasts. In horizontal AS, CTL showed additional positive BOLD modulations in frontal lobe (dlPFC), right caudate, occipital lobe (mOG, iOG), temporal lobe (FuG) and cerebellum (anterior lobe, DV, uvula, right inferior semilunar lobule), and further deactivations in pCG, left posterior mTG, ventromedial prefrontal cortex (vmPFC)/anterior cingulate gyrus (aCG), right medial orbitofrontal cortex (mOFC), insula, and anterior mTG. PD patients showed identical activations with the exception of right caudate, uvula and left inferior semilunar lobule, while most of the deactivations seen in CTL were remarkably absent in PD (i.e., vmPFC/aCG, left posterior mTG, insula, right mOFC, left anterior mTG). Right inferior semilunar lobule and right LG activations were additionally seen in PD, while not being present in CTL. (see **Supplemental Fig. 3**).

In vertical AS, CTL showed additional positive BOLD modulations in frontal lobe (right dlPFC), occipital lobe (left mOG, iOG), temporal lobe (FuG) and cerebellum (anterior lobe, DV, uvula, right inferior semilunar lobule), and several deactivations in pCG, posterior mTG, vmPFC/aCG, mOFC, insula, anterior mTG, and left parahyppocampal gyrus (PHG). Most of these deactivation foci were not present in PD group however (i,e., anterior mTG, vmPFC/aCG, right posterior mTG, mOFC, left PHG). Positive BOLD activations on the other hand, were similar between groups, although in PD, dlPFC activation was not present and LG activation was noted instead. Basal ganglia BOLD activity during vertical AS was not seen in either group (see **Supplemental Fig. 4**).



Supplemental Fig. 1. T-contrast maps of prosaccades (PS) + antisaccades (AS) > baseline contrast in control group (n= 10) displayed on standard T1 image ($p < 0.05$, corrected for multiple comparisons as estimated by Bonferroni correction). Yellow/red and blue/green regions represent greater and less saccade activation than baseline, respectively. For sake of clarity, BOLD modulations in caudate, thalamus, right inferior semilunar lobe, cerebellar uvula, right lingual gyrus, left parahypoccampal gyrus, posterior middle temporal gyrus, orbitofrontal cortex, inferior parietal lobe, cuneus, anterior cingulate gyrus are not shown/marked here. L, left hemisphere; R, right hemisphere; FEF, frontal eye field; SEF, supplementary eye field; PEF, parietal eye field; dmPFC, dorsomedial prefrontal cortex; dlPFC, dorsolateral prefrontal cortex; pCG, posterior cingulate gyrus; vmPFC, ventromedial prefrontal cortex; mOG, middle occipital gyrus; mTG, (anterior) middle temporal gyrus; iOG, inferior occipital gyrus; DV, dorsal vermis

Curriculum vitae

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2. University education.

2013	Neuro-ophthalmology Fellowship, Department of Neurology and Ophthalmology, Michigan State University, Michigan, USA
2012	Post-graduation studies - Statistical Package for the Social Sciences (SPSS) Basic and Advanced Courses, Faculty of Economics, Coimbra University, Coimbra, Portugal
2010 - date	Doctoral Programme in Health Sciences, Faculty of Medicine, Coimbra University, Coimbra, Portugal
2005 - 2006	Post-graduation Studies - Diploma in Clinical Neurology, Queen Square Hospital, University College London, London, England
1994 - 2001	Program in Medical Education, Faculty of Medicine, Coimbra University, Coimbra, Portugal

3. Certification and licensure.

2015 First International Masterclass Certification: Diagnosis and treatment of Peripheral, Central and Functional Vestibular Disorders Certificate, University Hospital Munich, Munich, Germany

2013 Neuro-ophthalmology Fellowship Certification, Department of Neurology and Ophthalmology, Michigan State University, Michigan, USA

2012 Statistical Package for the Social Sciences (SPSS) Basic and Advance Course Certification, Faculty of Economics, Coimbra University, Coimbra, Portugal

European University Professors of Ophthalmology (EUPO) Course on Neuro-ophthalmology Certification, Coimbra University Hospital Centre, Coimbra, Portugal

2010 Neurology Speciality Certification, Coimbra University Hospital Centre, Coimbra, Portugal

Teaching Course Neuro-ophthalmology Certification, 14th EFNS Congress, Geneva, Switzerland

2008 Teaching Course Neuro-ophthalmology Certification, 12th EFNS Congress, Madrid, Spain

2006 Diploma in Clinical Neurology, Queen Square Hospital for Neurology and Neurosurgery, University College London, London, England

2005 English Language Requirement Test Certification (ELRT), University College London, London, England

2004 General Internship Certification, Coimbra General Hospital, Coimbra, Portugal

2002 Medical Degree Certification, Faculty of Medicine, Coimbra University, Coimbra, Portugal

4. Professional career since graduation.

2015 - date Teaching Assistant, Faculty of Medicine, Coimbra University, Coimbra, Portugal

2013 - 2014 Neuro-ophthalmology Fellow, Department of Neurology and Ophthalmology, Michigan State University, Michigan, USA

2009 - date Neurology Assistant**,** Coimbra University Hospital Centre, Coimbra, Portugal

2004 – 2009 Neurology Resident, Coimbra University Hospital Centre, Coimbra, Portugal

2002 – 2004 General Resident**,** Coimbra General Hospital, Coimbra, Portugal

5. Peer-reviewed publications.

5.1. Journal Articles.

2016 ***Lemos J***, Pereira D, Almendra L, Rebelo D, Castelhano J, Cunha G, Patrício M, Januário C, Cunha L, Gonçalves AF, Castelo-Branco M. 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 Oct 1;1648(Pt A):469-84.

Lemos J, Pereira D; Castelo-Branco M. Visual cortex plasticity following peripheral damage to the visual system: fMRI evidence. Curr Neurol Neurosci Rep. 2016 Oct;16(10):89.

2015 Crandall E. Peeler, Lindsey B. De Lott, Lina Nagia, ***João Lemos***, Eric R. Eggenberger, Wayne T. Cornblath. Clinical Utility of Acetylcholine Receptor Antibody Testing in Ocular Myasthenia Gravis. Archives of Neurology. August 10, 2015.

Lemos J, Eggenberger E. Neuro-ophthalmological emergencies. Neurohospitalist. May 17, 2015 1941874415583117.

Nagia L, ***Lemos J***, Abusamra K, Cornblath W, Eggenberger E. Prognosis of Ocular Myasthenia Gravis: Retrospective two-center analysis. Ophthalmology. 2015 Apr 16.

2014 ***Lemos J***, Pereira D, Amorim M, Santiago B, Paiva A, Cunha L. Downbeat Nystagmus Elicited by Eyelid Closure. J Neuroophthalmol. 2014 Dec;34(4):350-3.

Lemos J, Eggenberger E. Supranuclear disorders of eye movements. Current opinion in Ophthalmology, 2014 Nov;25(6):471-9.

2013 ***Lemos J***, Eggenberger E. Clinical utility and assessment of cyclodeviation. Current Opinion in Ophthalmology. 2013 Nov;24(6):558-65.

Lemos J, Eggenberger E. Saccadic intrusions: review and update. Current Opinion in Neurology. 2013 Feb;26(1):59-66.

5.2. Conference papers.

2012 Pereira T, Barbeiro P, ***Lemos J***, Morgado M, Silva E. "Digital image acquisition for ophthalmoscope," Bioengineering (ENBENG), 2012 IEEE 2nd Portuguese Meeting in , vol., no., pp.1,6, 23-25 Feb. 2012

5.3. Book chapters.

2014 ***Lemos J***, Eggenberger E. "Central positional dizziness". in "Eye Movement Disorders". Nova Science Publishers, 2014

Lemos J. Supranuclear and internuclear ocular motor disorders. Evidence-based neuro-ophthalmology. Portuguese Society of Ophthalmology, 2014

6. Conferences and presentations. (selected work)

6.1. Invited presentations.

2016 ***João Lemos***. Supranuclear and internuclear eye movement disorders. European University Professors of Ophthalmology (EUPO) Course on Neuro ophthalmology, Coimbra, Portugal

João Lemos. Eye movement disorders. Reunião Anual da Sociedade Portuguesa de Otoneurologia, Luso, Portugal

João Lemos. Eye Movements for Clinicians. XVII Congresso Nacional de Ortoptistas, Sesimbra, Portugal

João Lemos. Video-oculography. Portugal. 2016. XVII Congresso Nacional de Ortoptistas, Sesimbra, Portugal

João Lemos. Atypical parkinsonian syndromes. Fórum de Neurologia, Leiria, Portugal

João Lemos. Movement Disorders and Neuro-ophthalmology. Fórum de Neurologia, Leiria, Portugal

João Lemos. Eye movement disorders in movement disorders. Sociedade Portuguesa de Doenças de Movimento, Torres Vedras, Portugal

2015 ***João Lemos***. Supranuclear and internuclear ocular motor disorders. Reunião Anual de GP de Neuroftalmologia, GP de Patologia, Oncologia e Genética Ocular e GP Inflamação Ocular, Curia, Portugal

João Lemos. Deadly causes of tinnitus. Reunião Anual da Associação Portuguesa de Otoneurologia, Albufeira, Portugal

João Lemos. Pharmacological treatment of vertigo, nystagmus and oscilopsia. Reunião Anual da Associação Portuguesa de Otoneurologia, Albufeira, Portugal

João Lemos. Central positional nystagmus. Reunião de Inverno da Associação Portuguesa de Otoneurologia, Angra do Heroísmo, Portugal

João Lemos. Pharmacological treatment of nystagmus and oscilopsia. IV Reunião Ibérica de Otoneurologia, Lisboa, Portugal

2014 ***João Lemos***. Pharmacological treatment of nystagmus. Reunião GP Oftalmologia Pediátrica e Estrabismo e Neuro-oftalmologia, Porto, Portugal

João Lemos. Central facial palsy. Reunião Anual da Associação Portuguesa de Otoneurologia, Albufeira, Portugal

João Lemos. Anatomy and physiology of the vestibular system. Reunião Anual da Associação Portuguesa de Otoneurologia, Albufeira, Portugal

2014 - date ***João Lemos***. Neuro-ophthalmological and neuro-otological examination. INeurologia - Introdução à Neurologia, Escola das Ciências da Saúde da Universidade do Minho, Braga

2012 ***João Lemos***. Oculomotor disturbances in neurodegenerative diseases. 55º Congresso da Sociedade Portuguesa de Oftalmologia, Lisboa, Portugal

2008 ***João Lemos***, Fernando Matias, Maria C. Macário, Lívia Sousa. Ocular movements in multiple sclerosis. Reunião do Grupo de Estudos de Esclerose Múltipla, Aveiro, Portugal

João Lemos. Vertigo - a practical approach. 23ª Jornadas de Medicina Geral e Familiar de Coimbra, Coimbra, Portugal

6.2. Conference presentations.

6.2.1. Platform presentations.

2016 ***João Lemos***, Joana Parra, César Nunes, Carla Nunes. It's a third! It's a sixth! No, is Superstrabismus. Encontro do Grupo Ibérico de Neuro-oftalmologia, Coimbra, Portugal

Martins I, Carvalho J, Geraldo A, ***Lemos J***. Disabling Positioning Upbeat Nystagmus associated with anti-GAD antibodies. Encontro Anual da Sociedade Portuguesa de Otoneurologia, Luso, Portugal

João Laffont, Margarida Amorim, ***João Lemos***, João Fonseca, Ana Sofia Melo, Ricardo Caiado, José Bastos, António Diogo Paiva. Is it inside or outside the cupula? Encontro Anual da Sociedade Portuguesa de Otoneurologia, Luso, Portugal

2015 ***João Lemos***, Adnan Subei, Mário Sousa, José Coelho, Luís Cunha, Christopher Glisson, Eric Eggenberger. Differentiating vertical misalignment using different head positions: a reappraisal. 67th American Academy of Neurology Annual Meeting, Washington, USA

João Lemos, Daniela Pereira, Luciano Almendra, Diliana Rebelo, João Castelhano, Gil Cunha, Cristina Januário, António Freire, Luís Cunha, Miguel Castelo-Branco. Cortical Control of Vertical versus Horizontal Saccades in Parkinsonian Syndromes: an fMRI study. 41th North American Neuro-ophthalmological Society (NANOS) Annual Meeting, San Diego, USA

Cristina Duque, Ana Novo, Joana Ribeiro, ***João Lemos***, Cristina Januário. Pinball nystagmus: a peculiar form of spontaneous nystagmus in neurodegenerative ataxia. 7º Curso de Neurofisiologia Clínica, Lisboa, Portugal

2014 Cristina Duque, Rui Bernandes, Luísa Ribeiro, António Correia, Pedro Fonseca, ***João Lemos***, Cristina Januário, António Freire Gonçaves. The utility of OCT in differentiating between Parkinson's disease genetic forms. Reunião da Secção Portuguesa de Doenças do Movimento, Albufeira, Portugal

Cristina Duque, Margarida Amorim, ***João Lemos***, Cristina Januário, Freire Gonçalves. Multiple system atrophy: a videonystagmographic study. Reunião Anual da Associação Portuguesa de Otoneurologia, Albufeira, Portugal

Cristina Duque, Clara Silva, Margarida Amorim, Sónia Batista, ***João Lemos***, António Paiva, Luís Cunha. The utility of the vestibulo-ocular reflex in the diagnosis of thiamine deficiency. Reunião Anual da Associação Portuguesa de Otoneurologia, Albufeira, Portugal

Miguel Silva, Cristina Duque, ***João Lemos***, Margarida Amorim, Luís Cunha, António Paiva. Pseudovestibular neuritis. Reunião Anual da Associação Portuguesa de Otoneurologia, Albufeira, Portugal

2012 ***João Lemos***, Margarida Amorim. Bilateral vestibular failure - case report. Reunião Anual da Associação Portuguesa de Otoneurologia, Porto, Portugal

Margarida Amorim, ***João Lemos***. Central positional vertigo in a patient with vertebral dissection. Reunião Anual da Associação Portuguesa de Otoneurologia, Porto, Portugal

2011 ***João Lemos***, Cristina Januário. Ocular movements in corticobasal degeneration. Reunião da Secção Portuguesa de Doenças do Movimento, Coimbra, Portugal

João Lemos, Fernando Matias, Maria C. Macário, Lívia Sousa. Ocular movements in multiple sclerosis - an update. 54º Congresso da Sociedade Portuguesa de Oftalmologia, Vilamoura, Portugal

2010 ***João Lemos***, Nuno Mendonça, Argemiro Geraldes, Cristina Januário. Corticobasal Degeneration: presentation of 4 atypical clinical cases, focusing on eye movement disturbance. Reunião do Grupo de Estudos de Envelhecimento Cerebral e Demências, Tomar, Portugal

2006 ***João Lemos***, Paula Bastos Lima, Cristina Januário. Progressive supranuclear palsy - case report. Fórum de Neurologia, Luso, Portugal

6.2.2. Poster presentations.

2016 Ana Margarida Novo, Cristina Duque, Joana Afonso Ribeiro, João Castelhano, ***João Lemos***, Cristina Januário. Disconjugate Horizontal Eye Movements in Spinocerebellar Ataxia Type 3 (SCA3). 2º European Academy of Neurology, Copenhagen, Denmark

Duque C, Coelho J, Marçal J, Ribeiro I, Melo A, Nunes C, Macário C, Batista S, Abreu L, ***Lemos J***. Exploring The Oculomotor Effects Of Sustained-Release Fampridine In Multiple Sclerosis Patients With Gait Impairment. Annual North American Neuro-ophthalmological Society (NANOS) Meeting, Arizona, USA.

Lemos J, Novo A, Duque C, Ribeiro J, Castelhano J, Januário C. A Detailed Analysis Of Oculomotor Function In 22 Patients With Spinocerebellar Ataxia Type 3. Annual North American Neuro-ophthalmological Society (NANOS) Meeting Arizona, USA

2015 ***João Lemos***, Daniela Pereira, Luciano Almendra, Diliana Rebelo, João Castelhano, Gil Cunha, Cristina Januário, António Freire, Luís Cunha, Miguel Castelo-Branco. Saccadic eye movements in Parkinson's disease: an eye-tracking and fMRI study. 67th American Academy of Neurology Annual Meeting, Washington, USA

Cristina Duque, Daniela Pereira, Margarida Amorim, Sónia Batista, ***João Lemos***. Superior cerebellar peduncle demyelination causing geotropic central positional nystagmus. 41th North American Neuro-ophthalmological Society (NANOS) Annual Meeting, San Diego, USA

Cristina Duque, Filipe Sobral, Ricardo Oliveira, ***João Lemos***. Post-cardiac catheterization skew deviation. Reunião Anual da Associação Portuguesa de Otoneurologia, Albufeira, Portugal

Cristina Duque, Ricardo Varela, Luciano Almendra, Daniela Pereira, Fernando Silva, Pedro Fonseca, Luís Cunha, ***João Lemos***. Neuro-ophthalmological manifestations of hematologic malignancies. Congresso da Sociedade Portuguesa de Neurologia, Lisboa, Portugal

2014 ***João Lemos***, Lina Nagia, Khawla Abusamra, Brooke T. Johnson, Wayne Cornblath, Jonathan Trobe, Christopher Glisson, Sunita Yedavally, David Kaufman, Eric Eggenberger. The course of ocular myasthenia

gravis: a retrospective study of 158 patients. 66th American Academy of Neurology Annual Meeting, Philadelphia, USA

Rui Araújo, Sónia Batista, Margarida Amorim, ***João Lemos***, António Paiva, Freire Gonçalves, Luís Cunha. Diagnostic utility of the head impulse test in patients with non encepalopathic presentation of thiamine deficiency. Reunião Anual da Sociedade Portuguesa de Neurologia, Lisboa, Portugal

2013 ***João Lemos***, Daniela Pereira, Taíssa Pereira, Paulo Barbeiro, Miguel Morgado, Eduardo Silva. Video-guided direct ophthalmoscopy improves medical student's skills and self-confidence. 65th American Academy of Neurology Annual Meeting, San Diego, USA

João Lemos, Filipe Blanco, Luís Isidoro, Dalila Coelho, Maria C Macário, João Figueira. Simultaneous paraneoplastic optic neuropathy and ocular flutter-myoclonus syndrome associated with lung adenosquamous carcinoma and circulating collapsin response-mediating protein (CRMP-5). 39th North American Neuro-ophthalmological Society (NANOS) Annual Meeting, Utah, USA

João Lemos, Daniela Pereira, Margarida Amorim, Beatriz Santiago. Downbeat nystagmus elicited only by eye closure caused by a paramedian pontine demyelinating plaque. 39th North American Neuro-ophthalmological Society (NANOS) Annual Meeting, Utah, USA

2012 Luís Isidoro, ***João Lemos***, Fernando Matias, Beatriz Santiago. Episodic ataxia type 2: a diagnostic challenge. 16th EFNS Congress, Stockholm, Sweden

Luís Isidoro, Filipe Blanco, ***João Lemos***, João Figueira, Carmo Macário. Opsoclonus-myoclonus, cerebelar ataxia, limbic encephalitis and bilateral optic neuropathy: a case report. 16th EFNS Congress, Stockholm, Sweden

Taíssa Pereira, ***João Lemos***, Paulo Barbeiro, Miguel Morgado, Eduardo Silva. Digital Image Acquisition for Ophthalmoscope. 2nd Portuguese BioEngineering Meeting, Coimbra

Daniela Pereira, ***João Lemos***. Eight and half syndrome - imaging review. Fórum de Neurologia, Lisboa, Portugal

Daniela Pereira, Gil Cunha, Luís Pedro, ***João Lemos***. Oculopalatal tremor in 2 patients. Fórum de Neurologia, Lisboa, Portugal

2010 ***João Lemos***, Cristina Januário, Luís Negrão. Ocular myasthenia gravis - pitfalls in clinical diagnosis. 14th EFNS Congress, Geneva, Switzerland

João Lemos, Fradique Moreira, Cristina Januário. Ocular movements in Corticobasal Degeneration. Movement Disorder Society, Toronto, Canada

6.3. Conference workshop monitoring.

2012 - 2014 Neuro-ophthalmology and Neurovestibular Exam Lab Skills Pavilion 64-66th American Academy of Neurology Annual Meeting

7. Peer review service.

7.1. Journal peer review.

2013 – date Journal of Neuro-Ophthalmology

2013 Frontiers in Neurology, Neuro-ophthalmology Section

7.2. Conference reviewing panel service.

2014 66th American Academy of Neurology Annual Meeting, Neuro-ophthalmology and Neuro-otology Section, Philadelphia, USA

Reunião Anual da Associação Portuguesa de Otoneurologia, Sesimbra, Portugal

7. Research projects.

2014 - date Diagnóstico diferencial de estrabismo vertical em diferentes posições cefálicas. Department of Neurology, Coimbra University Hospital Center. Coimbra, Portugal (main investigator)

2013 - 2014 Differentiating vertical misalignment using different head positions. Department of Neurology and Ophthalmology, Michigan State University. Michigan, U.S.A. (co-investigator)

The course of ocular myasthenia gravis. Department of Neurology and Ophthalmology, Michigan State University; Department of Ophthalmology and Visual Sciences, University of Michigan. Michigan, U.S.A. (co-investigator)

2012 - date Digital Image Acquisition for Ophthalmoscope. Department of Neurology, Coimbra University Hospital Center; Department of Ophthalmology, Coimbra University Hospital Center; Department of Physics, Coimbra University; Blueworks. Coimbra, Portugal (co-investigator)

2010 - date Functional imaging of saccades in parkinsonian disorders: a focus on verticality, dopamine and reward. Coimbra University, Faculty of Medicine; Portuguese Brain Imaging Network; Department of Neurology, Coimbra University Hospital Centre. Coimbra, Portugal (main investigator)

8. Research Supervision and Examination.

8.1. Co-supervision of MSc projects.

2016 Mário Carvalho. Parésias oculares motoras: Risco de AVC. Faculty of Medicine, Coimbra University, Coimbra, Portugal

2014 João Martins. Doença de Wilson: estudo retrospectivo. Faculty of Medicine, Coimbra University, Coimbra, Portugal

2012 Ricardo Varela. O Olfacto na Doença de Parkinson. Faculty of Medicine, Coimbra University, Coimbra, Portugal

Sílvia Alves. Fisiopatologia dos Gânglios da Base na Doença de Parkinson. Faculty of Medicine, Coimbra University, Coimbra, Portugal

Susana Lopes. A disfunção olfactiva na doença de Parkinson: um marcador evolutivo? Faculty of Medicine, Coimbra University, Coimbra, Portugal

2011 Taíssa Pereira. Digital image acquisition for ophthalmoscope. Department of Physics, Coimbra University, Coimbra, Portugal

8.2. Juri member of MSc projects.

2016 João Ramos. Dimensions of the olfactory bulb and sulcus and their relation with olfactory cortical regions in usher syndrome. Faculty of Medicine, Coimbra University, Coimbra, Portugal

Sara Pereira. Integrated Instrumentation of a Direct Ophthalmoscope. Physics Department, Coimbra University, Coimbra, Portugal

2015 Flávia Cunha. Apatia na doença de Parkinson. Faculty of Medicine, Coimbra University, Coimbra, Portugal

2014 Diogo Carneiro. Validation studies of the clock drawing test in mild cognitive impairment. Faculty of Medicine, Coimbra University, Coimbra, Portugal

2012 Ana Abreu. Processamento Emocional: Influência de Variáveis Demográficas. Faculty of Medicine, Coimbra University, Coimbra, Portugal

Diogo Branco. Synergistic roles of the proteasome and mytochondria in alpha-synuclein oligomerization: implications in Parkinson's disease. Faculty of Medicine, Coimbra University, Coimbra, Portugal

Hugo Ribeiro. Influência Hormonal na Enxaqueca. Faculty of Medicine, Coimbra University, Coimbra, Portugal

Sofia Almeida. Gait disorders in Parkinson's and Huntington's diseases. Faculty of Medicine, Coimbra University, Coimbra, Portugal

Telma Gameiro. Alterações imunológicas na Esclerose Múltipla e sua contribuição para o conhecimento da fisiopatologia da doença. Faculty of Medicine, Coimbra University, Coimbra, Portugal

8.3. Medical course activities.

2007 - date Biannual neurology lectures to the medical students at Coimbra University, covering hyperkinetic movement disorders and coma, and weekly clinical demonstrations in the clinic

8.3. Neurology residency activities.

2014 - date Coordinator of the Neurology Residents Annual Exam, Department of Neurology, Coimbra University Hospital Centre, Coimbra, Portugal

2006 - date Coordinator of the Weekly Neurology Residents Meeting, Department of Neurology, Coimbra University Hospital Centre, Coimbra, Portugal

Coordinator of the Neurology Department Webpage, www.neurohuc.com

9. Other work.

2015- date Coordinator of the Neurology of Vision and Balance Disorders Unit, Department of Neurology, Coimbra University Hospital Center, Coimbra, Portugal

10. Scolarships & awards.

2016 Second Best Presentation Award - Disabling Positioning Upbeat Nystagmus associated with anti-GAD antibodies. Martins I, Carvalho J, Geraldo A, ***Lemos J***. Encontro Anual da Sociedade Portuguesa de Otoneurologia, Luso, Portugal

Second Best Presentation Award - Is it inside or outside the cupula? João Laffont, Margarida Amorim, ***João Lemos***, João Fonseca, Ana Sofia Melo, Ricardo Caiado, José Bastos, António Diogo Paiva. Encontro Anual da Sociedade Portuguesa de Otoneurologia, Luso, Portugal

2015 Best Poster Award - Post-cardiac catheterization skew deviation. Cristina Duque, Filipe Sobral, Ricardo Oliveira, ***João Lemos***. Reunião Anual da Associação Portuguesa de Otoneurologia, Albufeira, Portugal

2013 American Academy of Neurology Institute's (AANI) Annual Meeting Fellow Scholarship

2011 Brain Functional Imaging National Association Scholarship

2008 Portuguese Movement Disorder Section Scholarship Award

2007 Portuguese Neurological Society Internship Scholarship Award

11. Affiliations.

2012 - date	American Academy of Neurology
	Portuguese Society of Otoneurology
2004 - date	Portuguese Neurological Society
2002 - date	Portuguese Medical Council

12. Interests.

Neuro-ophthalmology; Neuro-otology; Eye movement disorders; Neurodegeneration