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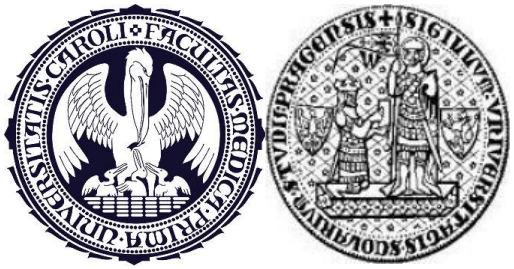

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Eye Movement Metrics in the Differentiation of Parkinsonian Syndromes

A thesis submitted for the degree of Ph.D.

Thesis Director and Supervisor Prof. Evžen Růžička, MD, DSc.

Prague, Czech Republic

2017

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I'll like to dedicate this thesis to my family and to Professor Flamand-Roze:

My beautiful kids Solène and Antoine, who are my best work, my inspiration and main reason to live. My gorgeous, brilliant and strong husband Henri for his love, thanks for making my life so wonderful. My mother Angelika for the moral education, philosophy and principles she leaves to me, which help me understand my patients.

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ABSTRACT

In this thesis we investigated conjugate and dis-conjugate eye movements (EM) in Parkinson's disease (PD) and other parkinsonian syndromes aiming to characterize and differentiate some aspects of their oculomotricity using infrared video-oculography.

First of all we published a practical review for medical students and clinicians describing clinical examination of eye movements, and interpretation of principal findings. Then we examined principal saccadic eye movements and smooth pursuit in the horizontal and vertical directions with video-oculography in a large group of healthy subjects, aiming to help new oculomotor laboratories in the constitution of their own norms. We conclude that age influence EM metrics but not gender or education level. The latency of saccades and the error rate of antisaccaes increases, while the velocity and gain diminishes with age. Saccades should be investigated in the horizontal and vertical plane because they are influenced by the direction of the target, resulting in a right/left and up/down asymmetry.

In a third project we focused on a frequent complain of PD patients, namely blurred near vision and visual discomfort during reading. We objectively assess for the first time vergence eye movements (VEM) in PD patients using VOG. Patients show increased latency of VEM and the divergence is slow and hypometric. In an unpublished part of this study, we additionally provided evidence in favour of disrupted VEM in Ephedrone parkinsonism (EP), Multisystem atrophy (MSA) and Progressive supranuclear palsy (PSP). EP patients had long latencies, slow velocity and reduced gain of VEM. MSA and PSP patients had also longer latencies, but the velocity, similar to PD patients, was only decreased for divergence. MSA patients exhibited reduced gain of VEM, while this was not observed in PSP.

Rapid eye movement sleep behaviour disorder (RBD) is by far the strongest clinical marker of prodromal PD. We investigate EM a group of patients diagnosed as idiopathic RBD (iRBD), aiming to detect prodromal PD. We found two groups of patients: i) iRBD composed of patients, free from any parkinsonian sign with EM similar to controls; and ii) RBD with possible PD, composed of patients with disrupted EM. We concluded that EM abnormalities could be considered as an additional early diagnostic marker of PD.

Intraoperative microelectrode recording of single neuronal activity at the basal ganglia in PD patients, was used to identify neurons participating in scanning eye movements based on specific electrophysiological pattern. We found that twenty percent of the neurons of the subthalamic nucleus, substantia nigra pars reticulata and globus pallidus showed eye movement related activity. Neurons related to scanning eye movements differ from neurons related to saccades, suggesting a functional specialization and segregation of both systems of eye movement control.

A recently described secondary toxic parkinsonian syndrome due to Ephedrone abuse draws the attention of the movement disorders community, because of its particular rapid onset and severe evolution. Horizontal and vertical eye movements were recorded in EP patients. We found slow and hypometric horizontal saccades, an increased occurrence of square wave jerks, long latencies of vertical antisaccades and high error rate in the antisaccade task. Patients make more errors than controls when pro- and antisaccades are mixed. Based on oculomotor performance, a direct differentiation between EP and PD was possible, EP patients presenting extensive oculomotor disturbances probably due to the manganese induced damage to the basal ganglia.

Finally we were interested in the improvement of some symptoms of PSP patients to Zopiclone, which is a gamma-aminobutyric acid (GABA) analogue. GABA levels were measured with spectroscopy and correlated to an eye movement paradigm, the remote distractor effect (RDE). Thus we did not find any significant difference in GABA level at the frontal cortex, or an increased RDE in our patients compared to controls, probably due to a low number of patients.

This thesis provides additional evidence about the importance and clinical utility of EM examination in the differentiation of PD and other parkisnonian syndromes, gaining insights into the physiology of the basal ganglia.

LIST OF ABREVIATIONS

AS:	Antisaccades
BRF:	Bulbar reticular formation
DW:	downward
EBN:	Exitatory Burst neurons
EM:	Eye movements
EP:	Ephedrone Parkinsonism
FEF:	Frontal eye field
GABA:	Gamma-aminobutyric acid
IBN:	Inhibitory burs neurons
H:	horizontal
IT:	Infratemporal Cortex
l:	left.
LW:	leftward
Mn:	manganese
MSA:	Multisystem atrophy
MST:	medial superior temporal area
MT:	middle temporal area
OPN:	omnipause neurons
PD:	Parkinson's disease
PFC:	prefrontal cortex
PPRF:	Paramedian pontine reticular formation
PS:	prosaccades
PSP:	Progressive supranuclear palsy
PPC:	Posterior Parietal Cortex
RBD:	REM sleep behaviour disorders
RDE:	remote distractor effect
SRT:	Saccade reaction time
OT:	Overlap task
RW:	rightward
r:	right
SEF:	Supplementary eye field
SC:	superior colliculus
SP:	Smooth pursuit
UW:	upward
V:	vertical
VM:	Vergence eye movements
VOG:	videoocculography
VOR:	vestibular ocular reflex
V1:	primary visual cortex

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PART I

GENERAL INTRODUCTION

Eye movement examination is an essential tool, which places few cognitive demands on subjects and provides a lot of information about brain anatomy and physiology. It also gives information about cognition, memory, adaptation and learning representing a large span of brain functions that may be probed, thus explaining why they have been and remain extensively used in both research and clinical practice.

It has been particularly used in movement disorders clinics providing key contributions for the diagnosis of some neurodegenerative (e.g. parkinsonian syndromes), hereditary (e.g. spino-cerebellar ataxias) or metabolic (e.g. Nieman-Pick disease) disorders and in neurophysiological departments to explore sensory, motor, and cognitive neural systems.

A large choice of video based infrared eye trackers is now available allowing to easily and non-invasively record saccades and smooth pursuit. Hence, an increasing number of neurophysiological departments tend to include eye movements (EM) setups in their equipment for the investigation of the central nervous system.

The movement disorders department of the Neurological Clinic of the first faculty of medicine, in Prague, created an eye movement laboratory for clinical diagnosis and research in 2010.

We began by publishing a paper describing how eye movements should be assessed in clinical practice. As a first step for a rational diagnostic approach, the accurate and detailed clinical examination of a patient is crucial. Physicians should be comfortable with neurological examination of eye movements and physical diagnosis. This first paper was oriented to students, residents, ambulant and hospital neurologists and was published in Cesk and Slov Neurol in 2011. We proposed a detailed description of the clinical examination at

the bedside, an algorithm for interpretation of the findings and the anatomy and physiology of eye movements.

A second step was to create our own laboratory norms. We examined 145 control healthy subjects and published our experience giving some clues for researchers who aim to create their own laboratory for research in EM. This paper was published in an important neurophysiological journal in 2013 and remains one of the Top Ten most downloaded papers on this field.

As a national expert centre on movement disorders, we were interested to enlarge the clinical utility of eye movement study in the differentiation between Parkinson disease (PD) and atypical or secondary parkinsonian syndromes.

Although the motor symptoms of PD have long been documented, the non-motor aspects do not seem to be as well recognized in clinical practice. Conjugate EM in Parkinson disease were widely studied in the past, however despite the frequent viewing complain of PD patients, no studies about convergent eye movements were performed. No objective characterization of EM was available at that time. We conducted with Jaromir Hanuska, former student of medicine, an important VOG study of vergence eye movements (VM) study in 18 PD patients (published in 2015). We additionally described VEM in EP, MSA and PSP and compared these results with our PD patients and a matched control group.

In collaboration with the sleep department we examined patients with idiopathic REM sleep behaviour disorders (RBD) aiming to explore a possible early diagnostic marker for PD. This paper was never published, but it serves as pilot study for an on-going study on the field.

During the exploration phase of DBS surgery, alert PD patients were shown a series of photographs and some of them underwent a visually guided saccade task. We analyze the relation between scanning EM and the activity of neurons in the STN, the substantia nigra pars reticulata (SNr) and the internal pallidum (GPi) in PD patients. Extracellular neuronal activity and electrooculography were recorded during the task. Action potentials of individual neurons were identified and the relation between the activity of individual neurons and the EM was assessed.

The Ephedrone project was an international collaboration between Czech Republic, Republic of Georgia and France. Our VOG device was exported, four of our researchers collaborated with seven other neurologist and residents, and examined 27 patients with EP in Tbilisi. Our team published two papers in 2014 that enlarge the knowledge on EP and the pathophysiology of the basal ganglia.

We have investigated a group of PSP patients with VOG and with spectroscopy to measure the correlation between low GABA levels and the remote distractor effect. The paper was submitted on 2016 and is under review at the Revue Neurologique.

We believe that VOG as a not invasive tool is an interesting method for diagnostic, follow up, and research of several movement disorders and other pathologies.

BRIEF HISTORY OF EYE MOVEMENT RESEARCH

In ancient Mesopotamia and Egypt eye specialist performed operations on cataracts, supporting interest of eye misalignment.[1]

Claudius Galen describes in the second century (130-200) basic aspects of the six extra ocular muscles and their functions based on dissections on rhesus monkey.[1] Aristotle more than 2000 years ago (384-322) distinguished conjugate and disconjugate (convergence) eye movements. He described many features of perception, strabismus and diplopia.

Five centuries later Galen's medical works were translated into Arabic by Hunain Ibn Is-Hâk (807-877). Alhazen (965-1039) made an extended description of coordinated eye movements and Christoph Schreiner (1571-1650) draws the differences between internal and external musculature.

The seventeen century was dedicated to the description of accommodation and alignment. The Scottish physician William Porterfield (1696-1771) distinguished the term accommodation and displayed some appreciation of the dynamic aspects of eye movements.[2] This English mathematician described a simple procedure for verifying the joint movements of the eyes with the fingers of the examiner. He also postulated that distinct vision is restricted to the central region of the retina (called fovea today) and that both eyes move in union to retain correspondence and alignment.[3]

William Charles Wells (1757-1817)[4] and Bell's (1823) experiments later distinguished between voluntary and involuntary eye movements.[5] Ewald Hering (1834-1918) a physiologist living in Prague, enunciated the principle of equal innervation, postulating that eyes moved as a single union not dependent of learning or association, and he was interested on special sense and eye movements.

Jan Evangelista Purkinje (1787-1869) described the images reflected from the surfaces of the eye, called later purkinje images, which were used to track eye position with great accuracy.[6] He also examined the nystagmus induced by a galvanic or electrical stimulation of the ears and the nystagmus produced by the rotating device created by Erasmus Darwing (1801) to examine the optokinetic reflex.[7] Several years later, Robert Barany (1896 - 1936),

son of the Prague scientist Maria Hock, received the Nobel Prize in 1914 for his vestibular researches and descriptions of nystagmus.

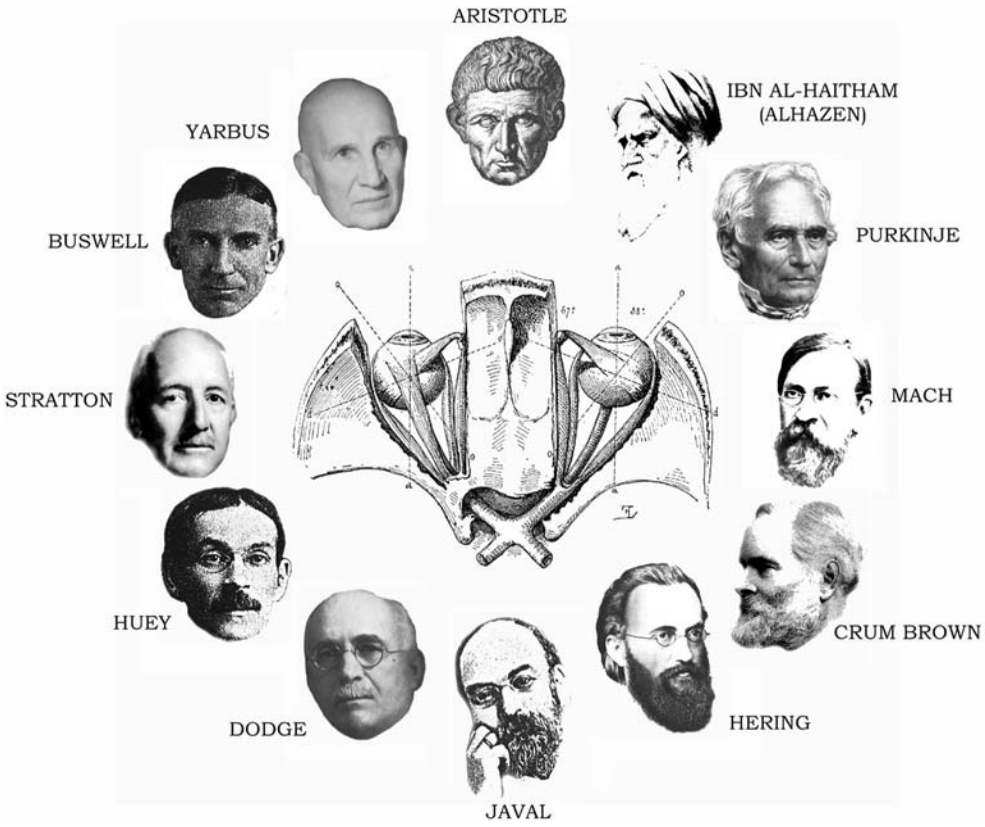

Figure 1. Some pioneers of eye movement research. They are arranged in a clockwise chronological sequence from Aristotle to Yarbus. The central diagram of the eyes and their musculature is from Landolt; it was published in 1879, the year in which Hering described the discontinuous movements of the eyes during reading (© Nicholas Wade).

The term saccade was introduced by Emil Javal (1839-1909) in Paris and later described at-large by Crum Brown (1878). The word originates from the old French word "sachier", meaning to shake. A saccade in horse-riding means to reduce the brisk movements of the reins during riding. Javal wrote a total of eight articles on the physiology of reading. Brown (1838-1922) also recorded nystagmus during body rotation and introduced the term "jerk" to describe saccades.

Objective eye trackers were developed in the late 19th and early 20th centuries allowing crucial new insights into the nature of eye movements. Edmund Huey (1870-1913) recorded eye movements during reading, and noticed that the eye moved by little jerks and not in a

continuous line. Dodge (1901) developed photographic devices that required no attachment on the eye for EM recording, giving the first step for the development of the VOG devices. In 1903 he established a taxonomy of eye movements, indicating that saccades disrupt vision, aiming to project the image to the central part of the retina called fovea.[8]

Later the research on eye movements became more complex by analysing eye movements during reading or viewing a picture, relating perception and cognition. In fact Stratton (1906) explored the relationship between eye movements and perception when viewing simple patterns and line illusions. Guy Buswell (1891-1994) was the first to explore how people view complex pictures rather than geometrical figures, he distinguished between children and adults, between Western and Oriental participants. Alfred Yarbus (1914-1986) investigated how an instruction given to an observer can radically change the places upon which he fixates his eyes. He called this behavior “stimulus driven guidance of attention”.

The scleral search coil technique, developed by David Robinson in 1963, hailed the advent of more accurate, higher resolution, and really more terrifying eye movement measurements.

Oculomotor research and behaviour constitute a fundamental feature of our exploration of the world. Eye movements give insights about perception, cognition, association, and reflects pathophysiology of several brain areas.

EYE MOVEMENTS STUDIED IN THIS WORK

We investigate two types of conjugate eye movements: saccades and smooth pursuit, and one disconjugate eye movement: vergence.

Saccades are the faster movements we are able to perform, which can reach 800 degrees/second in about ten milliseconds.[9] Two basic saccadic eye movement paradigms are currently used in clinical practice. Visually guided prosaccades (PS), elicited by instructing a subject to look at a suddenly presented peripheral target, and antisaccades (AS) with the instruction to look in the direction opposite to the suddenly presented peripheral target.[10-19] These tasks may be performed with either horizontal or vertical targets or a combination of both spatial locations.

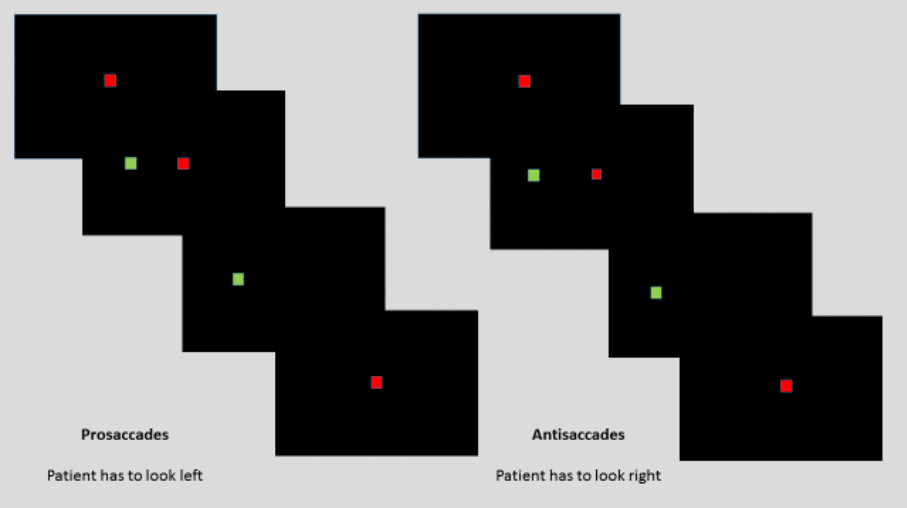

Figure 2. Example of a saccade and antisaccade task

In a saccade task, usual investigated metrics are saccade reaction time (SRT) or saccade latency, saccade velocity and saccade accuracy (Figure 2 and 3).

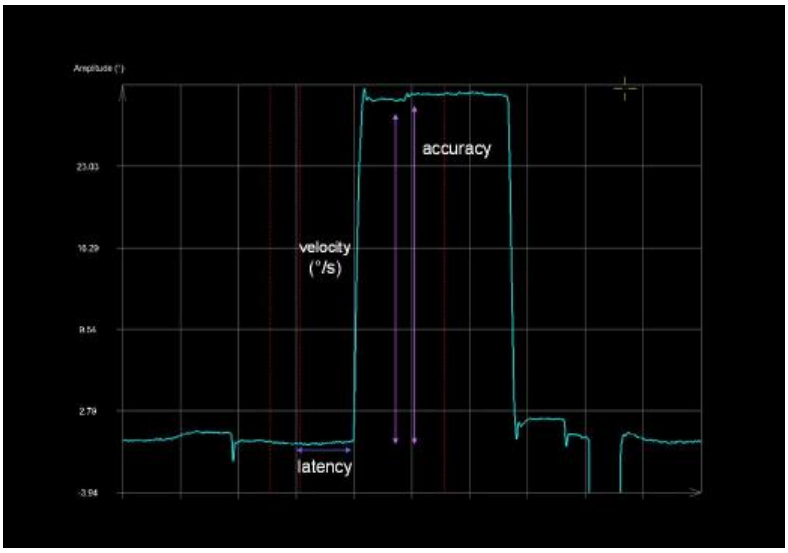

Figure 3. Metrics of saccades

Smooth pursuit eye movements (SP) allow the image of a moving target to remain on the fovea. Such movements are under voluntary control in the sense that the observer can choose whether or not to track a moving stimulus (Figure 4).

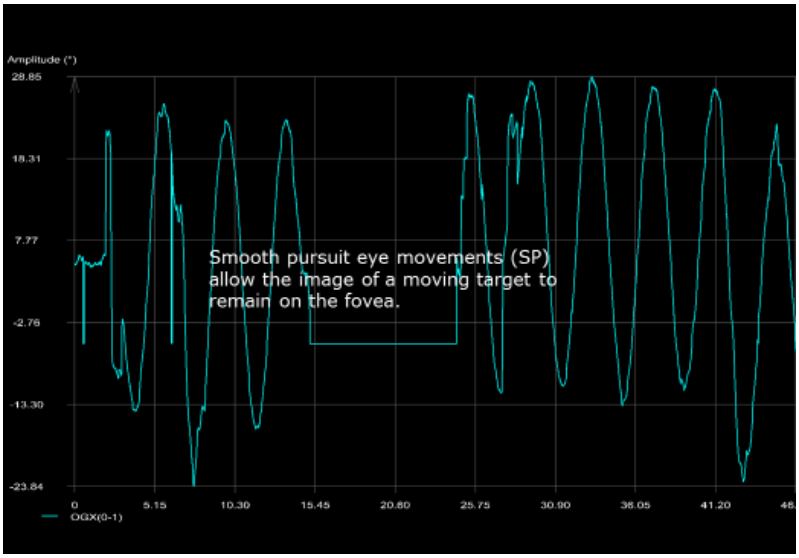

Figure 4. Smooth pursuit

Vergence eye movements (VM) are slow, disjunctive movements of the eyes necessary to read and to track objects moving in depth maintaining a fused and single percept.[20, 21]

NEUROPHYSIOLOGY OF SACCADES

Two parameters are important in the study of saccades: velocity and duration, both related to amplitude. Velocity and duration are driven by different cognitive processes, while amplitude is independent of the context of saccade realisation.[22] The size of a saccade is determined by the visual object that will be brought onto the fovea.

Three different forces drive saccades: pulse, slide and step. Pulse is a phasic activity that will create the strength to move the eye against the resistance of the tissues and the viscosity of the eye socket. This phasic activity is qualified as velocity. The pulse is critical because saccade size is determined by saccade duration that, in turn, is determined entirely by the neural pulse duration. This element is called spatial-temporal translator.[23] The input to the spatial-temporal translator comes from regions of the visual system such as the superior colliculus (SC) in which exists a retinotopic map, thus, activity in the colliculus may be said to be spatially coded.[24, 25] The step is a tonic activity which aims to maintain the eye at a determined position in spite of the viscoelasticity forces. Finally the slide is a command to come in between the pulse and step that aims to neutralize the viscoelastic forces.

These three forces are generated at a complex of premotor neurons called saccade generators localized at the paramedian pontine reticular formation (PPRF), the bulbar reticular formation (BRF).[26, 27] These neurons discharge about 10 ms before the saccadic movement and stop to discharge at the end of the saccade. Phasic neurons of the PPRF are excitatory called Excitatory Burst neurons (EBN) and Inhibitory Burst neurons (IBN). Both produce a burst of actions potentials that slightly precedes the activity of motoneurons, and are in contact with the ipsilateral motoneurons, activating ipsilateral saccades. This formation is often referred as the horizontal gaze centre and contains neurons which project to the lateral rectus motoneurons[28] and lesions in this region produce horizontal gaze deficit.[29] Phasic neurons of the BRF are inhibitory and contact contralateral motoneurons. Phasic neurons of the PPRF control ipsilateral horizontal saccades, while phasic neurons of the BRF control ipsi lateral, contralateral horizontal saccades but also vertical saccades.[26] Phasic excitatory neurons for the control of vertical saccades are localized at the rostral interstitial nucleus of the medial longitudinal fasciculus (riMLF). This region receives inputs from both cortical and

midbrain regions, as well from the omnipause neurons (OPN). The tonic signal for vertical saccades is thought to be provided by the interstitial nucleus of Cajal.[27, 28]

Premotor neurons are under inhibitory control of a neuronal network localized at the PPRF, called omnipause neurons, which discharge at the fixation and stop to discharge during the saccadic movement.[26]

The step response is believed to be controlled by the nucleus prepositus hypoglossus and the vestibular medial nucleus.[26-28] The PPRF receives input from the SC, which is intimately involved in the interplay between visual fixation and saccadic eye movements. The intermediate layers of the SC have neurons that discharge with visual fixation, called fixation neurons.

Cortical control of saccades

Except for the VOR and quick phases of all nystagmus, which are generated in the brain stem, the other eye movements are triggered and controlled by the Cerebral hemispheres.

Visual information originates at the retina and is relayed through the optic nerve, lateral geniculate nucleus to the primary visual cortex which has a direct excitatory connection to the SC that also receives direct retinal input.[30] The SC has also connections with extra striate areas that include Posterior Parietal Cortex (PPC). Three cortical regions control eye movements, the posterior part of the frontal lobe, the posterior part of the parietal and the temporal lobes.[31, 32]

The posterior part of the frontal lobe, has three areas that are involved in eye movement control: i) The frontal eye field (FEF), located in the precentral sulcus, and the adjacent parts of the precentral gyrus and middle frontal gyrus. ii) The supplementary eye field (SEF), which is a part of the supplementary motor area, located in the posterior part of the superior frontal gyrus. iii) The prefrontal cortex (PFC), located in the superior part of area 46 of Brodmann, i.e., just anterior to the FEF in the middle frontal gyrus (Figure 5).[33, 34]

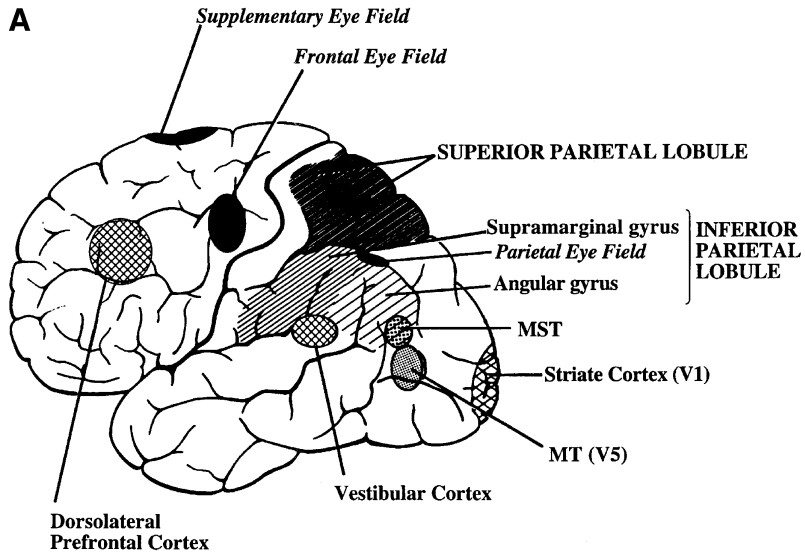

Figure 5. Cortical areas involved in saccade control. Leigh and Kennard (Brain 2004)

The FEF controls latency of reflexive visually guided saccades in the overlap task but not in the gap task.[35] The difference between the two tasks is the extinguishing of the central fixation point 200 ms (gap) before the appearance of the lateral target in the gap task, and the persistence of the central point in the overlap task. The FEF is needed to disengage actively fixation (from the central point), but is not crucial for triggering reflexive visually guided saccades.[33] On the other hand, the FEF is involved in the triggering of intentional saccades, it controls the intentional exploration of the visual environment, and is involved in smooth pursuit control and slow phase of ipsilateral optokinetik nystagmus.[33]

The SEF appears to be involved in the preparation of motor programs combining either a saccade with another body movement or several successive saccades, it controls the initial learning phase of a sequence, the launching of the motor program (just before saccade triggering).[36] However, the memorization phase (placed between the learning phase and the launching of the motor programme) is under the control of the hippocampal region (working memory). The SEF is connected to the FEF, the superior colliculus and premotor reticular formations.

The PFC is involved in spatial memorization, prediction and inhibition of saccades,[37] exerted through direct projections to the FEF and superior colliculus. The parietal eye field (PEF), located in the intraparietal sulcus [38] controls the latency of reflexive visually guided

saccades (in the gap and overlap tasks), [37] suggesting that the PEF is mainly involved in reflexive exploration of the visual environment, whereas, the FEF mainly controls the intentional exploration of this environment.[39] The posterior parietal cortex is also involved in visuomotor integration (Figure 6).[38]

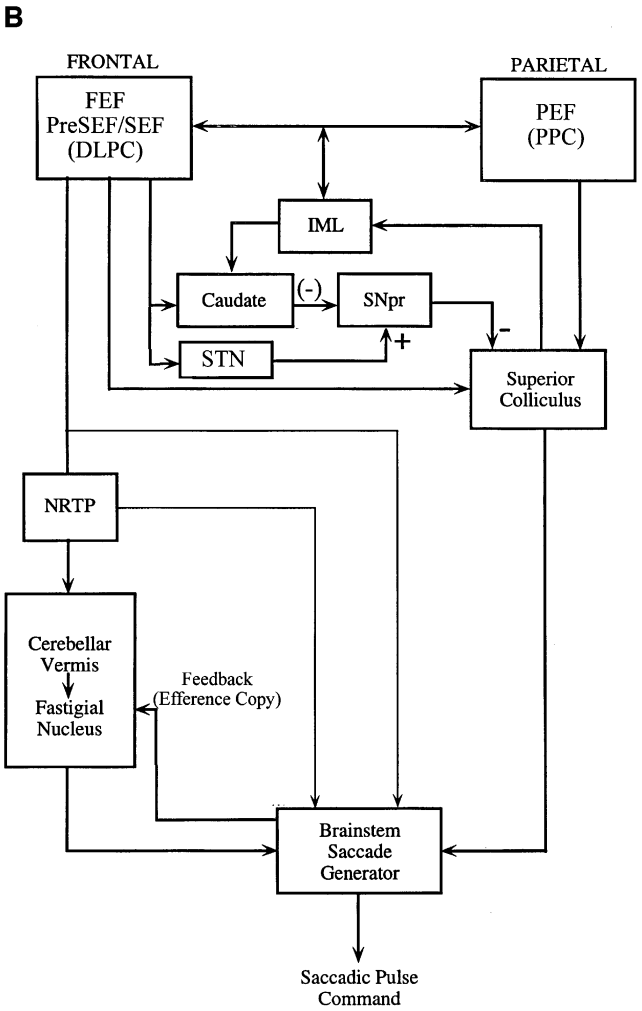

Figure 6. Interconnection of saccade related brain areas. Leigh and Kennard (Brain 2004)

The middle temporal area (area MT) and the medial superior temporal area (area MST) located near the parieto-temporo-occipital junction are involved in the control of smooth pursuit.[40]

OBJECTIVES OF THIS THESIS

We aimed to investigate conjugate and dis-conjugate eye movements (EM) in Parkinson's disease (PD), REM sleep behaviour disorder (RBD), Ephedrone Parkinsonism (EP), Progressive supranuclear Palsy (PSP) and Multisystem atrophy (MSA).

As a result of the collaboration between our department, the Neurology Department S. Khechinashvili University Clinic, Tbilisi Georgia, and the Pitié Salpetrière Hospital Paris, France, we conducted six important studies and created a VOG laboratory in our Faculty.

We hypothesized that

- Blurred vision in PD patients is due to dysfunction of the visual pathways responsible for VM and could be objectively demonstrated.
- If idiopathic REM sleep behaviour disorder (RBD) is a pre-clinical non-motor sign of PD, patients with RBD should have similar EM as PD patients, in order to identify occulomotricity as possible marker for pre or sub-clinical pathology of meso-pontine structures.
- Basal ganglia structures are involved in scanning eye movements, and this could be demonstrated by intraoperative microelectrode recordings.
- Eye movements in EP should be different from PD due to a secondary toxic large brain dysfunction.
- Patients with progressive supranuclear palsy (PSP) are more distractible, and should have a bigger remote distractor effect (RDE) due to lower Gamma-aminobutyric acid (GABA) levels.

PART II

2.1 From the beginning: How to examine eye movements as clinician

Eye Movement Examination in Neurological Practice. *Bonnet C, Hanuška J, Dombrowski A and Růžička E.. Cesk Slov Neurol N 2011; 74/107(5): 518-526.*[41]

A neurologist must be able to perform in a few minutes a systematic examination and to interpret the results driving the diagnostic approach. The test of integrity of the extra ocular eye muscles and their innervation is followed by the exploration of the different functional classes of eye movements.

The examination of eye movements should place few cognitive demands on subjects. It provides a lot of information about function of the central and peripheral nervous system, eye muscles and orbit. We published an algorithm of eye movement exam with a video example, which was published online and the first faculty of medicine. Students could systematically and accurately examine in few minutes the ocular motility. An anatomic, physiologic approach of eye movements is followed by description of clinical examination of eye movements and the most common abnormalities found during the examination.

Eye Movement Examination in Neurological Practice

Vyšetření očních pohybů v neurologické praxi

Abstract
Examination of eye movements is an essential part of any comprehensive neurological examination, providing important information about the function of the central and peripheral nervous systems, the eye muscles and the orbit. A careful, systematic and precise examination may be carried out within only a few minutes. The neurologist should be able to interpret signs, to adopt a topological or syndrome-based approach and to direct diagnostic procedures. The aim of this review is to provide a basic anatomo-physiological account of eye movements, followed by an account of the examination of eye movements and a summary of the principal abnormalities that may be disclosed.

Souhrn
Vyšetření očních pohybů je důležitou součástí neurologického vyšetření, které poskytuje významné poznatky o funkci centrálního i periferního nervového systému, okohybných svalech a orbitě. Pečlivé, systematické a přesné vyšetření musí být provedeno během několika minut. Neurolog by měl být schopen interpretovat jeho nález, provést syndromologickou a topickou diagnostickou rozvahu a naplánovat další cílená vyšetření. Cílem tohoto přehledu je připomenout anatomicko-fyziologické podklady očních pohybů, ukázat postup jejich vyšetření a prezentovat hlavní patologické nálezy.

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Klíčová slova
vyšetření očních pohybů – nervus oculomotorius – nervus trochlearis – n. abducens – vestibulo-optokinetický systém

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Introduction

Eye movements can be measured with extreme precision and provide a wide range of important information. The results of such eye examinations are usually rich in terms of derivable parameters, are well-documented in normal adults and in patients suffering from brain lesion, and may be used as one means of testing the functional integrity of the cortico-cortical and cortico-subcortical circuits. Examination of eye movements is an essential part of the neurological examination and places only few cognitive demands on subjects. The neurologist must be able to interpret the results that drive the diagnostic approach. The aim of this review is to provide practical information about the clinical examination of eye movements and the interpretation of potential findings.

Anatomy

Eye movements are triggered and controlled by a number of cortical and subcortical areas, apart from the vestibulo-ocular reflex and quick phases of all nystagmus, which are generated in the brain stem.

Some of the cortical areas involved in the management of eye movement are the frontal eye field, the supplementary eye field, the pre-supplementary motor area, the parietal eye field, the dorsolateral prefrontal cortex and the posterior parietal cortex. These areas project directly to the superior colliculus and indirectly through basal ganglia and the substantia nigra pars reticulata (SNpr), to the pontine nuclei (nucleus reticularis tegmenti pontis) and the cerebellum [1].

The vertical and horizontal gaze centres are situated in the brainstem. Vertical and torsional conjugate eye movements are generated in the midbrain, in the rostral interstitial nucleus of the medial longitudinal fasciculus (riMLF) [2]. The paramedian pontine reticular formation (PPRF), known in the past as the para-abducens nuclear group, is the organizing centre for horizontal gaze [2]. Vertical and horizontal gaze centres project to neurons of the oculomotor, trochlear and abducens nuclei, where the corresponding cranial nerves arise (Fig. 1).

The oculomotor nerve begins in the midbrain at the level of the superior colliculus with a cluster of somatic and visceral nerve nuclei. Somatic motor nuclei provide fascicles to the extrinsic ocular muscles: the superior and inferior recti, the medial rectus, the inferior oblique and the levator palpebrae superioris. The visceral motor nucleus, also known as the Edinger-Westphal nucleus or the accessory oculomotor nucleus, provides parasympathetic fibres to intrinsic ocular muscles: the sphincter pupillae that regulates pupillary constriction in response to light, and the ciliary muscle that enables the lens to accommodate for near vision. The third intrinsic eye muscle, the dilatator pupillae, receives sympathetic innervation via the carotid plexus. Each subunit of the oculomotor nerve is paired, apart from the caudal subunit which supplies the two levator palpebrae superioris muscles. This interesting configuration explains why it is comparatively difficult to open only one eye while the other eye remains closed [3]. The oculomotor nucleus receives inputs from the contralateral abducens nucleus via the medial longitudinal fasciculus (MLF) for the innervation of the medial rectus muscle, allowing conjugate horizontal eye movements. The fascicles of cranial nerve (CN) III exit ventrally through the brainstem into the interpeduncular cistern [4,5], pass the basilar artery, superior cerebellar artery, and travel in close proximity to the posterior communicating artery. CN III then enters the cavernous sinus, where it is in close proximity to CN IV, CN V, CN VI, and the carotid artery. In the cavernous sinus, the nerve divides into superior and inferior elements and enters the orbital apex through the superior orbital fissure together with the ophthalmic artery, CN II, CN VI, and the nasociliary branch of CN V1 [4,5].

The nucleus of the trochlear nerve (CN IV) is located in the tegmentum of the midbrain, at the level of the inferior colliculus [6]. The trochlear nerves decussate in the roof of the aqueduct before exiting from the dorsal aspect of midbrain, and run between the posterior cerebral and superior cerebellar arteries before entering the cavernous sinus. CN IV enters the orbit through the superior orbital fissure and crosses medially over the levator palpebrae superioris and superior rectus muscles before reaching the superior oblique muscle.

The nucleus of the abducens nerve (CN VI) is located in the pons, ventral to the floor of the fourth ventricle and lateral to the MLF. The nerve contains two groups of neurons: the internuclear neurons and the motor neurons. The internuclear neurons cross the midline, ascend in the MLF to the oculomotor nerve nuclei and ensure innervation of the contralateral medial rectus muscle. The motor neurons are made up of the principal fascicle of the abducens nerve, which innervates the ipsilateral lateral rectus muscle [3]. CN VI travels between the pons and the clivus, then pierces the dura mater before continuing between the dura and the skull. At the top of the petrous temporal bone, the abducens nerve makes a sharp turn forwards to enter the cavernous sinus. In the cavernous sinus it runs alongside the internal carotid artery and enters the orbit through the superior orbital fissure.

The oculomotor, trochlear and abducens nerves control the six small extrinsic ocular muscles responsible for precise eye movement: four rectus muscles and two obliques. The medial rectus muscle adducts the eye (inwards) and the lateral rectus abducts it (outwards). The superior rectus

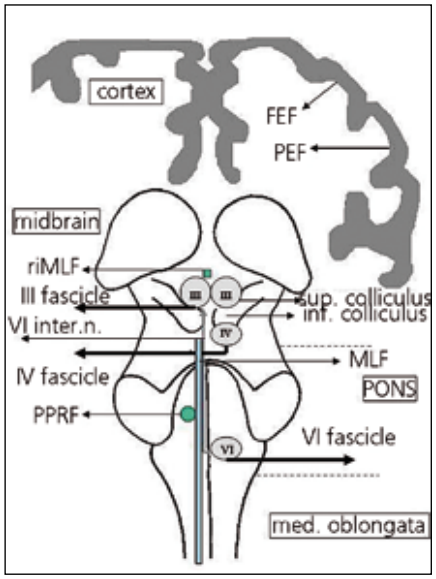

Fig. 1. Cortical and subcortical areas involved in eye movement control, coronal view.
FEF: frontal eye field; PEF: parietal eye field; MLF: medial longitudinal fasciculus; VI inter. n.: internuclear neurons of VI; PPRF: paramedian pontine reticular formation; riMLF: rostral interstitial nucleus of the medial longitudinal fasciculus; sup.: superior; inf.: inferior; med. oblongata: medulla oblongata; III: nucleus oculomotorius; IV: nucleus trochlearis; VI: nucleus abducens.

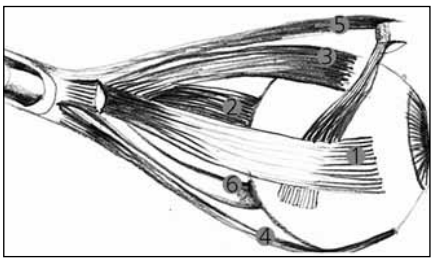

Fig. 2. Extrinsic ocular muscles, left eye viewed from centre.
1. M. medial rectus; 2. M. lateral rectus; 3. M. superior rectus; 4. M. inferior rectus; 5. M. oblique superior; 6. M. oblique inferior.

muscle elevates-adducts and slightly rotates the eye inwards, whereas the inferior rectus muscle depresses-adducts and slightly rotates the eye outwards. The oblique muscles have a predominantly rotatory pulling function. The superior oblique is the most important inward rotator, as well as depressing and slightly abducting the eye. The inferior oblique muscle rotates the eye outward, and also elevates and abducts (Fig. 2).

Neurophysiology

Clear imaging of an object requires steady fixation of its image on the central, foveal (macular) region of the retina, which contains the highest concentration of cone photoreceptors. Two main groups of eye movements facilitate this stabilization of an image on the retina and allow the line of sight to change when a new object of interest appears and needs to be directed to the fovea [2,7]. The eye movements that stabilize the image on the retina are the optokinetic reflex, the vestibulo-ocular reflex, and smooth pursuit:

- The optokinetic reflex (OKR) is induced when an entire visual scene drifts across the retina, eliciting eye rotation in the same direction at a velocity that minimizes the motion of the image on the retina. When the eyes rotate in the direction of a stimulus, their motion is periodically interrupted by rapid rotations in the opposite direction (quick phases or saccades), which reset the position of the eye for a new period of steady rotation [8].
- The vestibulo-ocular reflex (VOR) is a response analogous to head motion, with input coming from the vestibular system rather than the retina [7,8]. It responds to rotational and translational acceleration detected by two different structures, firstly the semicircular canals, which respond to angular (rotational) acceleration, with movement in the plane of the stimulated canal, and secondly the otolith organs, which respond to linear (translational) acceleration and sustained lateral tilt of the head. The VOR implies an intact trineuronal arc composed of the vestibular ganglion, the vestibular nuclei and the ocular motor nuclei [9].
- Smooth pursuit stabilizes an image when slow movement of an object is directed to the fovea [11]. Smooth pursuit needs to suppress the VOR because in moving the head, the VOR will normally move the eyes in the opposite direction to that of movement, disturbing vision [12].

The eye movements that change the line of sight are known as the saccades and vergence:

- Saccades are rapid eye movements that move the line of sight through successive points of fixation [2,13]. They are among the most well-understood eye movements, possessing dynamic properties that are easily measured, and have become a popular means of studying motor control, cognition and several neurological diseases [1]. Saccades are the quick phases of nystagmus. They may be reflexive, triggered externally by a visual target appearing suddenly, or intentional, triggered internally by a visual target already present for a period of time, perceived a moment before (memory-guided saccade), or expected at a specific location (predictive saccade). Antisaccades, made in the direction opposite to a suddenly-appearing visual target, are also voluntary [14].
- Vergence is the movement of the two eyes in different directions to enable binocular fixation of a single object. There are two main types of vergence movements, termed fusional and accommodative. The alignment of the eyes is maintained by fusional vergence and the reflex is driven by retinal image disparity. In the normal state, retinal image disparity produces diplopia. Motor fusion then triggers a vergence response to align the images of the object of regard on the two foveae. Accommodative vergence is stimulated by loss of image focus on the retina and occurs in association with accommodation of the lens and pupillary constriction [2,15–17].

Examination procedure

Patient history

An accurate patient history is essential to the examination of eye movements, including how long the symptoms have been present, whether pathological findings are evident in old photographs, and records of current medication.

Eye movement examination

(See also supplementary data: video on https://el.lf1.cuni.cz/ocularmovementsexam/)

The examiner should stand slightly to the side of the patient, since patients with cognitive impairment are accustomed to looking at the face or eyes of the examiner, and do not respond to verbal commands. The patient should be asked to sit or stand, hold the head erect and to look straight ahead. The examination starts with fixation, followed by pursuit, vergence, saccades and VOR.

1. Fixation

Examination of the eyes in primary position. The patient should view an object that requires visual discrimination on the other side of the room. Ocular alignment is determined by carefully observing the reflection of light on the cornea, which must be at the same height. This can be especially helpful in patients with ptosis or facial asymmetry. Careful inspection of the symmetry of both eyelids, pupils and head posture follows.

Each eye is then examined individually with the cover test, which may disclose heterotropia, a misalignment of the visual axes when both eyes are viewing a single target. A target that requires visual discrimination (e.g. an "E") is placed at a distance of 6 m and another at 35 cm. Firstly, with the eyes in the central position, the right eye is covered and corrective movements of the uncovered left eye are monitored. If no movement is detected, the cover is removed and the left eye covered. This test is repeated with the eyes brought to the nine cardinal positions of gaze and the whole process undertaken again with the near target [2].

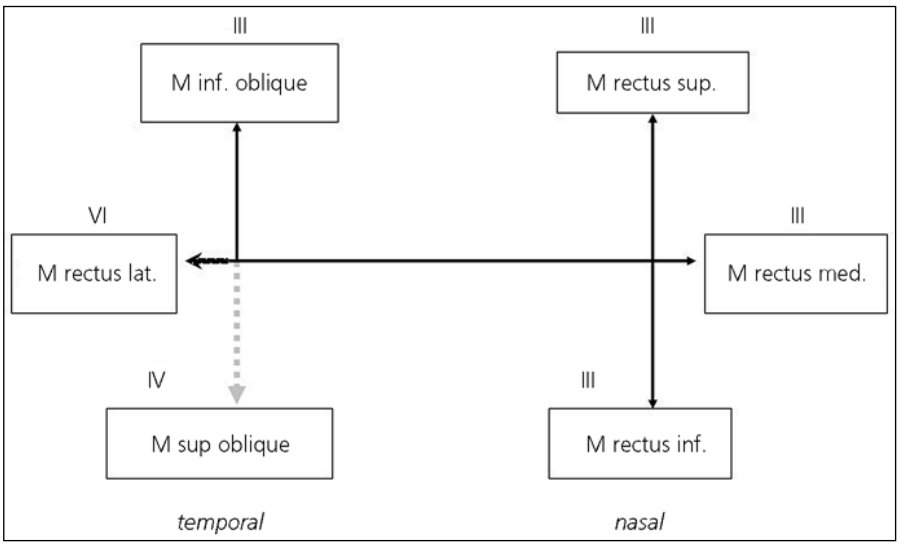

Fig. 3. Eye muscle examination.
It should be determined that each eye is able to trace a capital letter "H". In the horizontal plane, the M. medial rectus produces adduction (inward movement), and the M. lateral rectus produces abduction (outward movement). In examination of the M. superior and inferior recti, the patient is asked to look inward, then to look up and down. The M. superior rectus will elevate the eye and the M. rectus inferior will depress it. In examination of the M. superior and inferior obliquii, the patient is asked to look outwards, then to look up and down. The M. inferior oblique muscle will abduct, elevate and rotate the eye laterally and the M. superior oblique muscle will abduct, depress and internally rotate the eye.

2. Pursuit

The examiner's finger or a small target such as a pen, even a mirror, may be used for this part of the examination. The target is held approximately 60 cm in front of the patient's face and the patient asked to avoid moving the head. The target is moved horizontally then vertically, at a uniformly low speed (10–30/s), and the patient asked to follow it. Both eyes together are observed. The patient should be able to follow the target smoothly at an appropriate velocity. After this, each eye is observed separately to examine the eye muscles. Each eye is should be able to trace a capital "H" (Fig. 3).

Examination of the pursuit system includes assessment of VOR suppression, which is an essential component of the smooth pursuit task during motion. The patient is asked to follow a rotating object while the head is maintained in a fixed direction (e.g. the patient stretches the hands forward, holding them together, and maintains the gaze on them while seated in a chair that is rotating). The eyes should remain stable in the

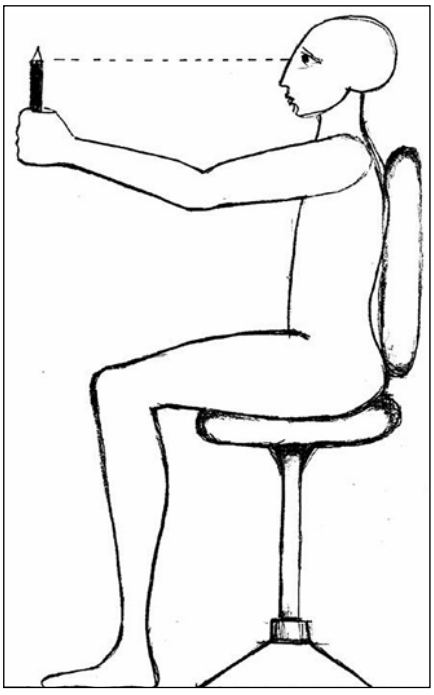

Fig. 4. VOR suppression.
The patient is asked to follow a rotating object while the head is maintained in a fixed direction (e.g. the patient stretches the hands forward, holding them together, and maintains the gaze on them while seated in a chair that is rotating).

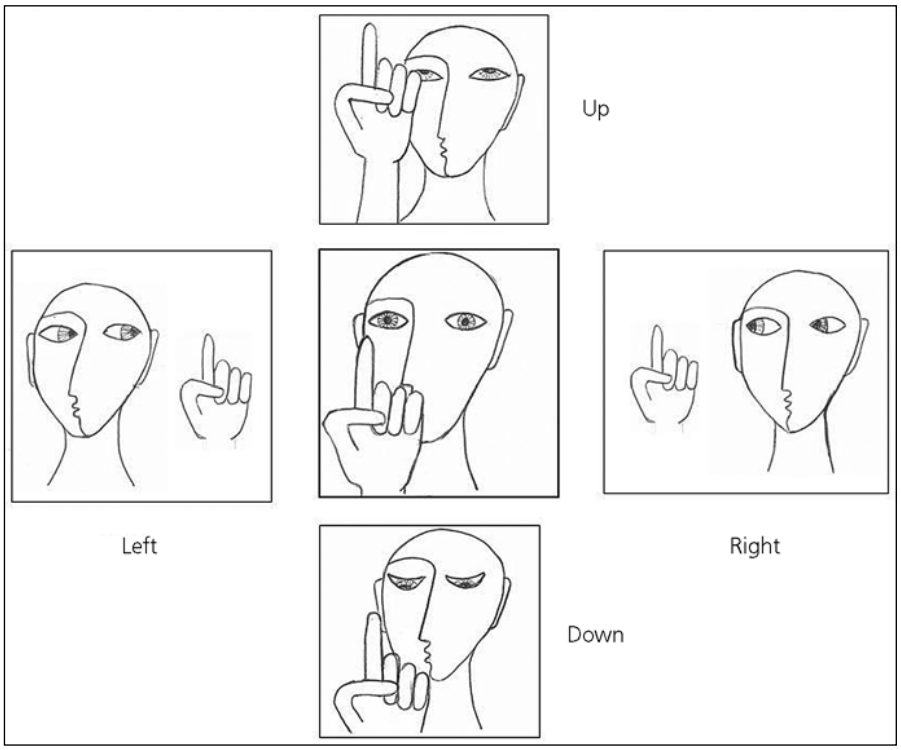

Fig. 5. Examination of saccades.
The target is placed in front of the patient and instructions given to look at it. The target is made to disappear from centre and re-appear to one side, when the patient is asked to change the line of sight and to view it. This procedure is repeated centre-right-centre-left-centre three or four times alternately. Vertical saccades are examined by alternating centre-up-centre-down-centre.

orbit, through visual fixation and suppression of VOR (Fig. 4).

3. Vergence

The patient is asked to maintain the gaze on an accommodative target that requires focus, something that is slowly brought to the bridge of the nose along the sagittal plane. The patient is also asked to shift the point of fixation alternately between a far target and a near target. Pupillary changes during convergence are noted.

4. Saccades

A target is placed approximately 60 cm in front of the patient's nose, and the patient asked to look at it. The target is moved away from centre and presented to one side, and the patient asked to change line of sight and view the target again. Avoiding large movements, this procedure is repeated centre-right--centre-left-centre, three or four times. The vertical saccades are then examined by alternating centre-up-centre-down--centre (Fig. 5). Saccades are usually fast, accurate and conjugate. Most patients are able to initiate the saccadic eye movement (latency) as quickly as they are asked to look at the target. Latency, velocity, accuracy and conjugated movement of both eyes are noted.

5. Vestibulo-ocular reflex and optokinetic reflex

Horizontal and vertical VOR are examined by means of the head-impulse test, after Halmagyi & Curthoys (Fig. 6) [18]. The patient's head is held still in two hands and the patient asked to fix the gaze on the examiner's nose; the head is then directed rapidly and horizontally to the left and to the right. To examine vertical VOR, the patient's head is directed vertically up and down. Rotation of the head in a healthy subject should lead to rapid compensatory eye movements in the opposite direction, leaving the patient still looking at the examiner's nose.

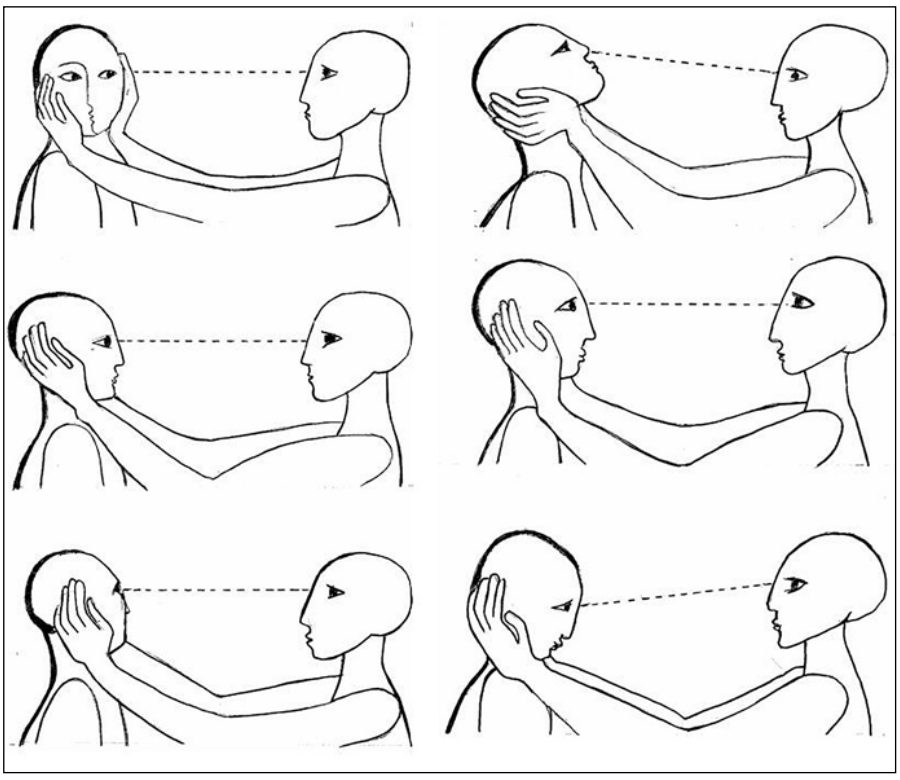

Fig. 6. VOR examination
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Dynamic visual acuity is the ability to resolve visual detail while the observer is moving. Motion reduces visual acuity relative to static conditions. This test provides a clinical functional measure of the vestibulo-ocular reflex (VOR) during horizontal or vertical sinusoidal head rotations at frequencies of at least 2 Hz and a rate greater than 120/s [10]. To start with, static acuity is assessed by means of the standard Snellen chart, asking the patient to read the smallest legible line on the chart. Then the patient's head is moved at about 2 Hz, at an amplitude of only 5–10 degrees in the horizontal and then the vertical, and the patient asked to read the smallest line on the chart that can be discerned. To avoid interference from the patient's memory, a different but equally difficult chart may be employed or the patient may be asked to read the line backwards during the dynamic part of the test. When the VOR system is impaired, visual acuity degrades during head movement [19].

A common method of testing the optokinetic reflex is to sit the patient inside a large, patterned optokinetic drum or to rotate the patient at a constant velocity for more than a minute with the eyes open in an illuminated room. Small-field motion induced by a drum or tape, with stripes that rotate horizontally or vertically, does not test the optokinetic system adequately, but primarily assesses the pursuit system [2].

Principal pathological findings

It is essential to record all information concerning signs and symptoms revealed by examination of eye movement. Definitions must be kept clear in order to avoid incorrect diagnosis. Definitions of the principal abnormalities and their origins appear above.

Patient history

Patients tend to seek medical advice when an eye movement disorder causes visual discomfort and/or leads to dizziness or instability.

Diplopia

Diplopia is the sensation of seeing an object at two different locations in space. The patient should be asked if it is hori-

zontal, vertical or torsional, and elicitation extended to the direction of gaze in which the diplopia is more marked, and if it is worse for near or distant viewing. Monocular diplopia is rare and may be caused by astigmatism, refractive errors, cataract, corneal irregularity, lens dislocation or eye trauma. Patients who complain of little or no visual disturbance despite an obvious ocular misalignment have usually had strabismus from an early age. Patients with acquired strabismus tend to close one eye to avoid diplopia [2].

Oscillopsia

Oscillopsia is an illusion of movement of the perceived world, often associated with poor visual acuity, reported by many patients with neurological disorders. This commonly occurs while the patient is walking, during head movement, driving or even when the head is still. Oscillopsia very often results from an impaired vestibulo-ocular reflex due to bilateral, peripheral or central vestibular dysfunction; from abnormal eye movements such as nystagmus; or from paresis of the extraocular muscles [2].

Vertigo

Erroneous perception of self-motion or object-motion, as well as an unpleasant distortion of static gravitational orientation.

Eye movement examination

Fixation

Several complaints may be revealed by the clinical examination, among them eyelid abnormalities, ptosis, retractions, lid nystagmus, lid opening apraxia or synkinesis.

Strabismus

Horizontal misalignment of the visual axes causing the two images of an object to fall on non-corresponding areas of the two retinas, usually giving rise to diplopia.

Skew deviation

Vertical misalignment of the eyes caused by damage to the prenuclear vestibular input to the oculomotor nuclei. Skew deviation may arise out of a lesion in the utricle, the MLF, the midbrain, the cerebellum, the vestibular cortex or the thalamus. It may also be a transient finding associated with raised intracranial pressure associated with supratentorial tumours or pseudotumours.

Ocular tilt reaction

Head deviation, ear to shoulder, appears in some patients with skew deviation. This reaction is usually attributed to peripheral or central lesions disrupting otolithic inputs [2,20].

Nystagmus

Repetitive, rhythmic, involuntary oscillation of the eyes initiated by slow phases. Although the direction of nystagmus is defined by the direction of the fast corrective phase, it is the slow phase that reflects the underlying disorder [2].

Description of nystagmus needs to include the direction of beat, the degree, the effect of visual fixation and other provocation manoeuvres. The direction of eye motion should be described from the patient's perspective. For example, clockwise torsional rotations should correspond to rotation of the top poles of the patient's eyes to the patient's right. The planes in which the nystagmus occurs (horizontal, vertical, torsional, mixed) should be noted for each eye. Horizontal nystagmus is most often congenital or peripheral-vestibular in origin, commonly associated with a torsional component. Torsional nystagmus is usually associated with medulla oblongata lesions, such as syringobulbia and Wallenberg´s syndrome. Downbeat nystagmus commonly occurs with degeneration affecting the vestibulocerebellum, lesions near the craniocervical junction, vertebral ectasia and with drug intoxications, especially lithium. Upbeat nystagmus is less well localized than downbeat nystagmus, reported largely with paramedian lesions of the medulla oblongata, but also with pontine and midbrain abnormalities [2].

Nystagmus may be also of a pendular or jerk type: (1) pendular nystagmus: sinusoidal oscillations of approximately equal amplitude and velocity; (2) jerk nystagmus: slow initiating phase with a fast corrective phase [20].

The oscillations of each eye should be compared (synchrony or asynchrony). When the direction of the oscillations differ in each eye, the condition is known as disjunctive nystagmus.

The degree of nystagmus is defined as:

- 1st grade, nystagmus only in the direction of gaze;
- 2nd grade, nystagmus also present in primary position;
- 3rd grade, nystagmus also present in the opposite direction of gaze.

Further examination involves the effect of removing fixation with Frenzel goggles. This is a sensitive method for detecting spontaneous nystagmus, in fact nystagmus due to peripheral vestibular imbalance may only be apparent under these circumstances. Fixation can also be eliminated by examining one eye with an ophthalmoscope (while the other eye is covered) and simultaneously checking for movements of the optic papilla or retinal vessels. Since the retina is behind the axis of rotation of the eyeball, the direction of any observed vertical or horizontal movement is opposite to that of the nystagmus detected with this method, i.e. a downbeat nystagmus causes a rapid upward movement of the optic papilla or retinal vessels [21]. Other clinical tests, depending on the clinical question, are positional testing, hyperventilation, the Valsalva manoeuvre and head shaking. The neuro-otologist usually induces nystagmus by caloric, galvanic or vibratory stimuli [2].

Gaze-evoked nystagmus

A drift of the eye that is only present in certain directions of gaze, gaze-evoked nystagmus is often a side-effect of medication (anticonvulsants, benzodiazepines) or toxins (alcohol). A horizontal gaze-evoked nystagmus may indicate a structural lesion in the brain stem or cerebellum. A dissociated horizontal gaze-evoked nystagmus may be present in internuclear ophthalmoplegia [22], whereas vertical gaze-evoked nystagmus is observed in midbrain lesions involving the interstitial nucleus of Cajal.

Square-wave jerks

Square-wave jerks are small saccades, from 0.5° to 5°, that cause the eyes to oscillate around the primary position. They can increasingly occur in progressive supranuclear palsy and certain cerebellar syndromes.

Ocular flutter

Intermittent rapid bursts of oscillations without intersaccadic interval, occurring in only one direction, usually horizontal.

Opsoclonus

Bursts of oscillations without intersaccadic interval, occurring in combined hori-

zontal, vertical, and torsional directions; ocular flutter and opsoclonus occur in various settings, such as encephalitis, paraneoplasia (neuroblastoma in children or other tumours in adults), meningitis, intracranial tumours, hydrocephalus, thalamic haemorrhage, multiple sclerosis, systemic disease, and drug intoxication [22].

Pursuit

Smooth pursuit is a very brisk function that varies with subject age and the ability to direct visual attention, and is influenced by medication [23–28]. Even healthy persons exhibit a slightly saccadic smooth pursuit during vertical downward gaze. For these reasons, a saccadic smooth pursuit does not always allow either an exact topographical or etiological classification [21]. Pursuit movements that do not match the target velocity necessitate corrective saccades, making the pursuit saccadic. Impaired smooth pursuit is observed in intoxication (anticonvulsants, benzodiazepines, alcohol), cerebellar or extrapyramidal neurodegenerative disorders, hereditary cerebellar diseases, cerebral lesions, and even in extraocular muscle palsy [21,29–31]. Abnormalities of smooth pursuit may also be encountered in some individuals with congenital forms of nystagmus [2]. Failure of VOR suppression, investigated as a part of the examination of smooth pursuit, results in an incompletely cancelled VOR that appears as a jerk nystagmus beating in the direction of rotation.

CN III palsy

Palsy of CN III may be complete or partial. A complete CN III lesion causes ptosis, a fixed, dilated pupil with paralysis of accommodation, resting eye position ("down and out"), and the inability to elevate, depress or adduct the eye. The opposite eyelid may droop slightly, reflecting the bilateral innervation of the lids by CN III. Incomplete CN III palsy is more common and may result from lesion at various sites along the course of the nerve from the nucleus to the muscle [2,32].

CN IV palsy

Patients with CN IV palsy usually report vertical and torsional diplopia aggravated by looking downwards and inwards, especially when reading or climbing stairs [33]. The head may be tilted away from affected side to reduce blurred vision (the Bielschowsky sign). Accordingly, double vision increases markedly when the head is upright or tilted to the affected side (Bielschowsky test).

CN VI nerve palsy

Abducens nerve palsy is the most common of all ocular motor palsies. Patients usually present binocular, uncrossed, horizontal diplopia at its greatest when viewing distant objects and looking ipsilaterally. Abduction is restricted or slowed, and there is an esotropia (the eyes are "crossed" – while one eye looks straight ahead, the other eye is turned in toward the nose). Patients often turn the head to the affected side to minimize diplopia.

Internuclear ophthalmoplegia (INO)

This specific gaze abnormality, resulting from a lesion in the MLF, is characterized by impaired horizontal eye movement with weak adduction of one eye and abduction ataxic nystagmus of the contralateral eye. The adduction deficit identifies the INO as being either left or right, and is ipsilateral to the MLF lesion. Vertical saccades and convergence are normal.

One-and-a-half syndrome

This uncommon syndrome occurs if a lesion affects the paramedian pontine reticular formation (PPRF) and the medial longitudinal fasciculus on the same side. The eyes cannot move horizontally, except the eye contralateral to the lesion side, which can abduct. Convergence is unaffected.

Vergence

The most common vergence disorder is convergence insufficiency associated with diplopia, eye strain, fatigue, loss of concentration while reading, motion sickness, and headaches. Other vergence and accommodative anomalies are convergence excess, divergence insufficiency or excess, and vergence or accommodative insufficiency.

Convergence insufficiency may be present in some forms of childhood strabismus. Acquired disorders of vergence include the effect of some sedative drugs and alcohol, Parkinson's disease, progressive supranuclear palsy, midbrain lesions and parietal lesions [2]. One rare condition is spasm of convergence, in which the eyes intermittently converge or turn towards each other. This phenomenon causes diplopia, blurred vision, miosis and episodic adduction of one or both eyes. It may be a sign of an organic lesion or of a functional disorder. Organic forms include thalamic esotropia, brainstem and cerebellar disorders, Wernicke-Korsakoff syndrome, vertebrobasilar ischemia, Chiari malformations, posterior fossa tumours, multiple sclerosis, and metabolic disturbances [2].

Saccades

Disorders of saccadic velocity

- Saccades of small amplitude, appearing too fast (increased peak velocity-amplitude relationship), may be seen in myasthenia gravis, tumours of the globe, flutter and opsoclonus.
- Slow saccades are present in abnormalities of the extraocular muscles, oculomotor nerve palsy, internuclear ophthalmoplegia, in central neurological disorders and in pharmaceutical intoxication, especially that of anticonvulsants or benzodiazepines.
- Slowing of horizontal saccades is generally observed in pontine lesions after dysfunction of the ipsilateral PPRF.
- Slowing of vertical saccades indicates a midbrain lesion in which the rostral interstitial nucleus of the MLF is involved, such as in ischemic, inflammatory and neurodegenerative diseases, especially progressive supranuclear palsy.

Disorders of saccadic accuracy

- Hypermetric saccades are saccades of high amplitude; the patient will look over and past the target (overshoot), and will need a corrective back saccade to re-attempt to find the target. These indicate lesions of the cerebellum (especially the vermis) or the cerebellar pathways. Patients with Wallenberg syndrome make hypermetric saccades in the direction of the side of the lesion.
- Hypometric saccades are saccades of low amplitude; the subject will need to make more than one saccade to attempt to find the target. These occur in a variety of cerebellar, cerebral hemisphere and brain stem disorders.

Disorders of saccadic initiation

Saccadic latencies increase in patients with amblyopia and hemispheric lesions,

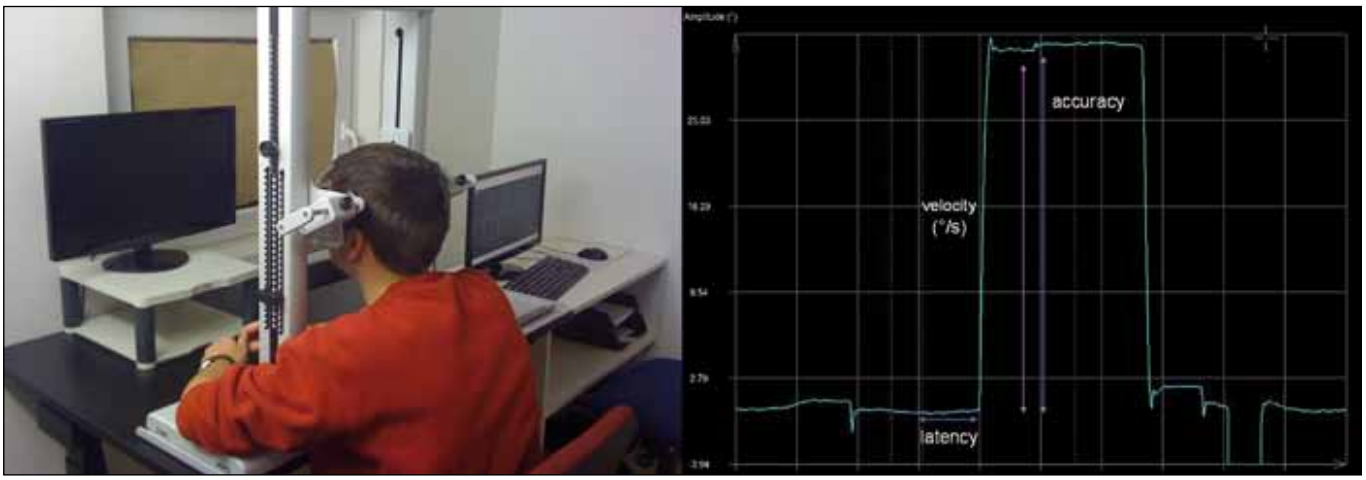

Fig. 7, 8. Eye movement recording with video-based infrared oculography (infra-red eye tracking).
The investigation takes place in an optically and acoustically shielded room. The patient sits, with the head stabilised by a chin rest, in a comfortable chair. The screen carried a horizontal and vertical array of red light-emitting diodes. Visually guided saccades are presented for horizontal and vertical directions. Movements of both eyes are recorded at a sampling rate of 300 Hz. The following parameters are extracted separately for left, right, up, and down: (1) gain or accuracy, (2) saccade peak velocity, (3) saccade latency.

especially those affecting the cortical eye fields. Bilateral frontoparietal lesions produce a severe defect of saccade initiation known as ocular motor apraxia. Other disorders causing increased latencies are Huntington's disease and corticobasal degeneration [2,21].

Vestibulo-ocular reflex (VOR)

Pathology involving the neuronal substrate of the VOR gives rise to changes in gain, direction of VOR and postural imbalance [2]. During the examination the patient will be not able to maintain target fixation. The patient will perform a corrective (catch-up) saccade to fix the target again, or will not move the eyes at all. Frequent causes of abnormalities of the VOR are:

- unilateral peripheral vestibular disorder, lesion of the labyrinth or of the vestibular nerve;
- bilateral peripheral vestibular disorders, due to bilateral eighth nerve section, aminoglycoside intoxication, or toxic, infectious, neoplastic, autoimmune, traumatic or inflammatory processes;
- central vestibular disorders due to infarct, haemorrhage, tumour, trauma or infection.

Eye movement recording

There are three main ways in which to record eye movements to a high degree of accuracy:

- electro-oculography: a large range of horizontal movements may be recorded by quantifying the corneo-retinal potential using skin surface electrodes. This method is applicable for children and poorly cooperative patients. Disadvantages are common lid artefacts, the requirement for repeat calibration, adaptation to level of ambient lighting and its inability to measure vertical eye movements;
- magnetic search coil technique: allows the measurement of eye movements in all directions using the scleral annulus, but is expensive and invasive;
- video-based infrared oculography (infra-red eye tracking) is the most frequently used method. A light source is used to produce reflections on the surface of the eye. Tracking the relative movements of these images gives an eye position signal. A video image is digitized and analyzed with computer software to calculate the position of the pupil and its centre. This method allows rapid and reliable recording of horizontal and vertical eye movements.

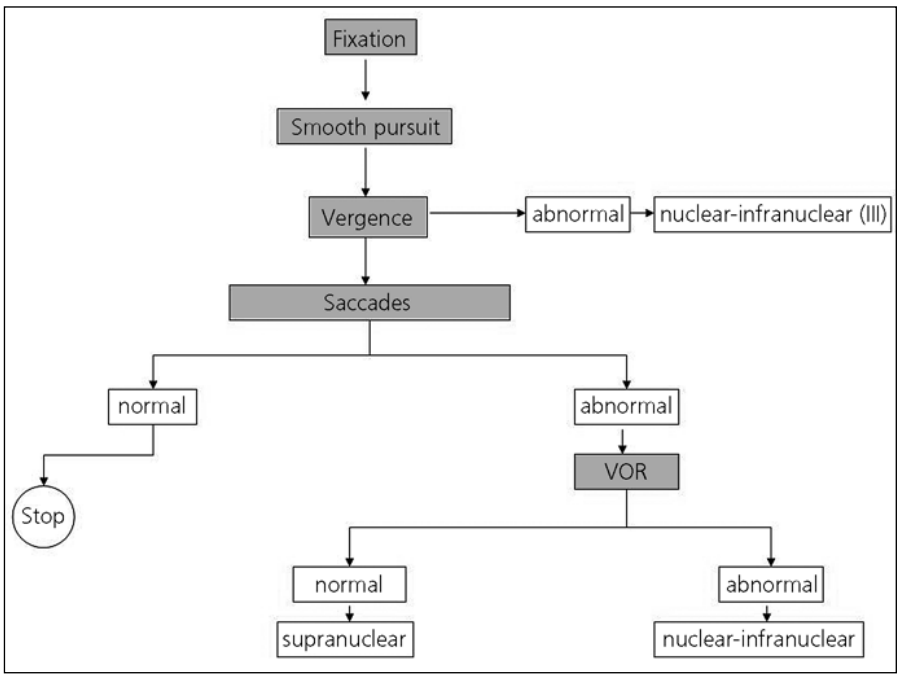

Fig. 9. Eye movement examination in clinical practice.

Different oculomotor paradigms are used in the laboratory to measure eye movements. We measure smooth pursuit and saccades (velocity, latency and accuracy). Saccades may be tested using visually stationary or moving targets, combined with head movements, both reflexive and memory-guided. The control of voluntary saccades may be tested with an antisaccade paradigm. In this task, the subject is required to suppress a saccade towards a stimulus that appears at the periphery of vision, and instead to generate a voluntary saccade of equal size towards the opposite side [2]. (Fig. 7, 8)

Conclusion

Examination of eye movement must be systematic, accurate, easy to perform, and place few demands on patients. The examination should begin by exploring fixation and smooth pursuit, and go on to investigate vergence. If vergence is abnormal the lesion will be probably be nuclear (CN III) or infranuclear. Finally, the saccades should be explored: if these are normal, the examination may cease. If they are abnormal, VOR is next. Should VOR be normal, the origin of the eye movement disorder will be probably be supranuclear, whereas if VOR is abnormal, the problem should be nuclear or infranuclear. (Fig. 9, adapted from Vignal et al [33])

Note: Supplementary data (video) associated with this article is available on the website of Charles University, Prague, First Faculty of Medicine and General University Hospital, Prague, Czech Republic: https://el.lf1.cuni.cz/ /ocularmovementsexam/.

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2.2 The normative study: How to examine eye movements as eye movement specialist.

Horizontal and Vertical Eye Movement Metrics: What´s Important?

Cecilia Bonnet, Jaromír Hanuška, Jan Rusz, Sophie Rivaud-Péchoux, Tomaš Sieger, Veronika Majerová, Tereza Serranová, Bertrand Gaymard and Evžen Růžička. Clin Neurophysiol. Volume 124, Issue 11, November 2013, Pages 2216–2229.[42]

The study of eye movement (EM) has been increasingly used as a model to test the sensory, motor, and cognitive neural systems. In neurological practice, EM recording complete the diagnostic of some neurodegenerative, metabolic, cerebellar hereditary diseases and is increasingly used in research to understand the function of the brain.[29] Numerous studies in a wide variety of disciplines have examined healthy subjects applying different paradigms, producing conflicting results and resulting in a lack of reliable normative data for the practitioner. The purpose of this study was to provide a detailed description of EM metrics of a large group of healthy subjects, using video based infrared videooculography, a photoelectric method that reflect an infrared light into the pupil, allowing rapid, reliable, and non-invasive recording of horizontal and vertical EM.

This paper has been downloaded and cited several times and helping clinicians in the creation of their own norms.

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Horizontal and vertical eye movement metrics: What is important?

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HIGHLIGHTS

- Latency of saccades lengthens with age, for targets presented left, up and down.
- The error rate of antisaccades may reach up to 80% by the seventh decade of life.
- Subjects of all age groups correct over 99% of the errors made on antisaccade task.
- Skewness of horizontal saccades is stable throughout the lifespan.
- The gain of horizontal and vertical smooth pursuit is not affected by senescence.

ABSTRACT

Objective: To assist other eye movement investigators in the design and analysis of their studies.
Methods: We examined basic saccadic eye movements and smooth pursuit in the horizontal and vertical directions with video-oculography in a group of 145 healthy subjects between 19 and 82 years of age.
Results: Gender and education level did not influence eye movement metrics. With age, the latency of leftward and vertical pro- and antisaccades increased ($p < 0.001$), velocity of upward prosaccades decreased ($p < 0.001$), gain of rightward and upward prosaccades diminished ($p < 0.001$), and the error rate of antisaccades increased ($p < 0.001$). Prosaccades and antisaccades were influenced by the direction of the target, resulting in a right/left and up/down asymmetry. The skewness of the saccade velocity profile was stable throughout the lifespan, and within the range of saccades analyzed in the present study, correlated with amplitude and duration only for antisaccades ($p < 0.001$).
Conclusions: Some eye movement metrics must be separated by the direction of movement, others according to subject age, while others may be pooled.
Significance: This study provides important information for new oculomotor laboratories concerning the constitution of subject groups and the analysis of eye movement metrics.

1. Introduction

The main objectives of voluntary eye movements (EM) are either to bring (saccades) or maintain (smooth pursuit) images of interest on the fovea; a small central retinal area of high visual acuity. Although saccades and smooth pursuit are controlled by different neural structures, common anatomical pathways may be distinguished, with several cortical areas being primarily concerned with the cognitive control of EM (e.g., visuospatial attention, decision making or inhibition), and brainstem structures being mainly concerned with the motor control of EM. The large span of brain functions that may be probed with the analysis of EM, from sensory to motor and cognitive functions, explains why they have been extensively studied in both neurophysiological research and clinical practice.

In the last decade, two factors have been especially responsible for the rapid expansion of oculomotor testing in clinical practice. First, the analysis of EM has been shown to provide key contributions to the diagnosis of some neurodegenerative (e.g., parkinsonian syndromes), hereditary (e.g., spinocerebellar ataxias) or metabolic (e.g., Niemann–Pick disease) disorders, and second, a large choice of video-based infrared eyetracking devices are now available, allowing easy and non-invasive recording of saccades

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and smooth pursuit. Hence, an increasing number of neurophysiological departments tend to include EM evaluation in their investigation of the central nervous system. Regardless of the neurophysiological testing that has been implemented, the first requisite step when developing a new technique is the acquisition of appropriate normative data. Although each lab should establish its own values, two main questions must nevertheless be answered in advance: which tests and parameters should be studied, and what criteria should be considered for the constitution of control groups?

Two basic saccadic eye movement paradigms are currently used in clinical practice. Visually guided prosaccades, elicited by instructing subjects to look at a peripheral target presented suddenly, and antisaccades (AS) elicited in the same manner as in the previous task, but with the subject instructed to look in the direction opposite of the peripheral target that is presented suddenly (Amador et al., 1998; Cherkasova et al., 2002; Curtis and D'Esposito, 2003; Edelman et al., 2006; Ettinger et al., 2005; Everling and Fischer, 1998; Gaymard et al., 1998; Guitton et al., 1985; Pierrot-Deseilligny, 1990; Schlag-Rey et al., 1997). Both tasks may be performed with either horizontal or vertical targets, or with a combination of both spatial locations. In a prosaccade task, the parameters typically analysed are saccade reaction time (SRT) or saccade latency, saccade velocity and saccade accuracy. Saccade latency mainly reflects the time required by cortical processes such as target selection and decision making. The posterior parietal eye field is more involved in the control of reflexive prosaccades (Braun et al., 1992; Gaymard et al., 1998; Pierrot-Deseilligny et al., 1991), whereas the frontal eye field (FEF) is more involved in the control of volitional saccades (Braun et al., 1992; Dias and Bruce, 1994; Gaymard et al., 1999; Rivaud et al., 1994). Two additional frontal areas are implicated in the control of volitional saccades. The supplementary eye field (SEF) is involved in higher-order oculomotor control, such as conditional oculomotor associations (Chen and Wise, 1995), the chronological control of sequential saccades (Gaymard et al., 1993) and the modulation of the oculomotor system according to error monitoring (Gaymard et al., 1990; Stuphorn et al., 2010) but lesions of this area would not affect basic saccade parameters. The dorsolateral prefrontal cortex (DLPFC) allows any unwanted reactive saccade to be suppressed (Condy et al., 2007; Ploner et al., 2005) and is also responsible for spatial working memory, which allows the triggering of memory-guided saccades (saccades towards a remembered location) and predictive saccades (saccades towards an expected location) (Funahashi et al., 1993; Gaymard et al., 1998; Guitton et al., 1985; Pierrot-Deseilligny et al., 2005, 2003a). It should be noted that the DLPFC is not a true oculomotor area, since it does not contribute to saccade triggering *per se*. Within subcortical structures, the superior colliculus (Leigh and Zee, 2006), the dorsal vermis (Sato and Noda, 1992a,b; Waespe and Wichmann, 1990), the fastigial nucleus (Robinson et al., 1993) and the brainstem saccade generator are more concerned with saccade velocity and accuracy. Although it is widely accepted that the analysis of both pro- and antisaccade paradigms is a good compromise that allows reflexive-like (prosaccades) and volitional (antisaccades) types of saccades to be analysed, several practical aspects remain to be determined concerning either the task design (e.g., should vertical AS be analysed?) or the relevant saccade parameters (e.g., is there any useful information provided by the analysis of vertical saccade latencies?).

Another interesting parameter of saccades that has received little attention and has only been studied in small groups of healthy subjects, is the skewness of the velocity profile (Collewijn et al., 1988a; Collins et al., 2008; Smit et al., 1987; Van Opstal and Van Gisbergen, 1987). Skewness is defined as the ratio of the time to reach maximal velocity (the acceleration phase) to the total duration of the saccade. From these results it was concluded that skewness is related to the amplitude (Baloh et al., 1975; Collewijn et al., 1988a; Hyde, 1959) and duration (Smit et al., 1987; Van Opstal and Van Gisbergen, 1987) of the saccade.

Smooth pursuit eye movements (SP) allow the image of a moving target to remain on the fovea. A widespread network of cerebral structures (visual cortex, middle temporal visual area and medial superior temporal area, the FEF, pontine nuclei, cerebellum, vestibular and ocular motor nuclei) contribute to the control of smooth pursuit (Buttner et al., 2008; Lisberger et al., 1987; Pierrot-Deseilligny and Gaymard, 1992). This volitional eye movement requires attention and motivation and may be influenced by the subject's age and affected by medication (Leigh and Zee, 2006). Controversial results of previous studies include stable (Bono et al., 1996; Moschner et al., 1994; Warabi et al., 1984) or decreased smooth pursuit gain with increasing age (Paige, 1994; Sharpe and Sylvester, 1978; Spooner et al., 1980; Zackon and Sharpe, 1987).

A large number of studies in a wide variety of disciplines have examined saccade parameters in healthy subjects but the results of these studies are inconsistent concerning the influence of age and direction of the target, either for horizontal and vertical saccade latencies (Abel et al., 1983; Bono et al., 1996; Fischer et al., 1997a; Huaman and Sharpe, 1993; Klein and Foerster, 2001; Moschner and Baloh, 1994; Munoz et al., 1998; Olincy et al., 1997; Peltsch et al., 2011; Pratt et al., 1997; Shafiq-Antonacci et al., 1999; Sharpe and Zackon, 1987; Spooner et al., 1980; Sweeney et al., 2001; Warabi et al., 1984; Yang and Kapoula, 2006, 2008), velocities (Abel et al., 1983; Bono et al., 1996; Fukushima et al., 2000; Moschner and Baloh, 1994; Munoz et al., 1998; Sharpe and Zackon, 1987; Spooner et al.,1980; Tedeschi et al., 1989; Warabi et al., 1984; Wilson et al., 1993) and accuracy (Abel et al., 1983; Bono et al., 1996; Irving et al., 2006; Moschner and Baloh, 1994; Munoz et al., 1998; Olincy et al., 1997; Sharpe and Zackon, 1987; Tedeschi et al., 1989; Warabi et al., 1984; Wilson et al., 1993). Similar discrepancies have been reported for antisaccades, concerning both AS latencies and error rates (Abel et al., 1983; Butler et al., 1999; Eenshuistra et al., 2004; Fischer and Weber, 1997; Klein et al., 2000; Munoz and Everling, 2004; Olincy et al., 1997; Pratt et al., 1997; Shafiq-Antonacci et al., 1999; Sweeney et al., 2001). Many factors may have contributed to this variability, among which the characteristics of the control groups (number of subjects, influence of age, handedness), the exact instruction given before each paradigm, or the task design (number of trials, periods of rest, interleaved conditions or blocks of similar trials, etc.). Studies simultaneously investigating horizontal and vertical EM are scarce and have included at most 66 subjects (Bono et al., 1996). The large majority have analysed only horizontal EM in less than 100 subjects (Abel and Douglas, 2007; Butler et al., 2006; Butler et al., 1999; Edelman et al., 2006; Fischer and Weber, 1997; Honda, 2002; Klein et al., 2000; Klein and Foerster, 2001; Peltsch et al., 2011; Taylor and Hutton, 2009; Warabi et al., 1984), rarely more (Evdokimidis et al., 2002; Fischer et al., 1997a,b; Munoz et al., 1998; Smyrnis et al., 2002), while others have analysed only vertical EM in at most 40 participants (Collewijn et al., 1988b; Goldring and Fischer, 1997; Huaman and Sharpe, 1993; Yang and Kapoula, 2006). To the best of our knowledge, no special attention has been paid to the influence of gender on EM metrics.

The main purpose of the present study was to analyse two basic saccade paradigms, namely prosaccades and antisaccades, as well as smooth pursuit in an especially large number of healthy subjects in order to determine the most relevant criteria regarding the constitution of control groups and eye movement analysis.

We hypothesized that (i) aging similarly influences the SRT of horizontal and vertical prosaccades, as they share cortical structures undergoing progressive degenerative changes (Head et al.,

2004; Salat et al., 2001); (ii) the velocity and gain of saccades dependent on topographically segregated subcortical structures (Leigh and Zee, 2006), are less and variably affected according to the direction of movement; (iii) smooth pursuit and saccade skewness driven by the adaptive capacity of the cerebellum should be less influenced by senescence. We expected to confirm that eye movements are dependent on the paradigm used and on the direction of target presentation.

2. Methods

2.1. Subjects

Subjects were recruited through local advertisements and examined at our video-oculography laboratory. All subjects were determined by a questionary to be free of any neurological or psychiatric illnesses and denied the intake of any medication acting on the central nervous system. All subjects provided signed, informed consent and received an adequate flat fee to compensate for their time and travel expenses. The study was approved by the local ethics committee and was in compliance with the Declaration of Helsinki.

2.2. Experimental paradigm

Subjects were seated in a calm, dark room with their chin supported by a chin strap and their forehead in contact with a frontal support. They faced a flat, 26 in. LCD screen (ProLite, Iiyama model PL 2600, size 550 mm × 344 mm) located 60 cm in front of them at eye level. Each recording session started with a calibration procedure during which the subject was instructed to accurately look at 16 consecutive targets presented over the entire screen. A complete recording session consisted of 16 blocks of trials and lasted 20 min.

2.2.1. Prosaccades

This task started with the onset of a green central fixation point (size: 15 × 15 pixels; luminance: 120 cd/m^2) that was presented for a pseudorandom duration of 2800, 3200, 3500, 3800, 4000 or 4100 ms. The rationale of varying the fixation time was to avoid anticipations of the subject. The fixation point was then turned off and 200 ms later, a red peripheral target (15 × 15 square, luminance 120 cd/m^2) appeared during 1000 ms at a 13° right or left location, or at a 13° up or down location. The rationale for the 200 ms gap period is to facilitate saccade triggering by an exogenous removal of the fixation activity prior to saccade onset. Subjects were instructed to look as fast and as accurately as possible to the peripheral target. A total number of 28 saccades were performed, horizontal targets being presented 6 times and vertical 8 times in each direction. Vertical saccades (which have been investigated less in the past, see above) were presented twice more than horizontal saccades in order to obtain more measurements for normative data.

Saccades were analysed for latency (or saccade reaction time – SRT), velocity and gain. Gain was defined as the ratio between initial saccade amplitude and target location. We calculated a SRT index for prosaccades to the right vs. prosaccades to the left. The same index was calculated for the vertical direction; the SRT of upward/downward prosaccades. These indicies were correlated with patient age.

2.2.2. Antisaccades

The task design was the same as in the prosaccade task, with the exception that the colour of the central fixation point was red. Furthermore, horizontal and vertical target locations were either presented in separate blocks of simple horizontal and vertical trials, or in mixed blocks of interleaved horizontal and vertical trials. Subjects were instructed to look as fast as possible in the direction opposite to the peripheral target. A total number of 48 saccades were performed. In the horizontal and vertical tasks, targets were presented 8 times in each direction. In the mixed task, horizontal targets were presented 8 times and vertical 8 times in each direction.

Latency, error rate and rate of corrected errors were extracted. We calculated a SRT index for antisaccades to the right versus antisaccades to the left. The same index was calculated for the vertical latency of upward/downward antisaccades. This index was correlated with patient age.

2.2.3. Skewness

Skewness of a saccade refers to the asymmetry of the velocity profile, and is simply estimated from the ratio of the time to reach maximal velocity (the acceleration phase) to the total duration of the saccade. Skewness was estimated for correct performed horizontal pro- and antisaccades with the before mentioned paradigms. Additionally, controls performed a prosaccade step task (central target disappearing simultaneously with target onset) in a variable angle of 5°, 15°, 10°, and 20°. The rationale to use variable angles to investigate skewness was to investigate the relation between the amplitude and duration of the saccade and the shape of the velocity profile. Subjects were instructed to look towards the peripheral stimulus as soon as it appeared. The experiment began with the fixation point (same characteristics as in gap task) presented for the periods of 2800, 3200, 3500, 4000 ms. The target was always a red square measuring 15 × 15 pixels, luminance 120 cd/m^2, presented for 1000 ms. Healthy volunteers performed this task twice, targets being presented 2 times for each angle, in each direction, for a total number of 32 saccades.

The skewness was estimated only for correct horizontal prosaccades performed in the gap and overlap task and for correct antisaccades performed in the simple antisaccade task.

2.2.4. Smooth pursuit

This task began with the presentation of a central red target (20 pixels diameter) for 1000 ms. It then started to move with a sinusoidal velocity profile, either horizontally or vertically, both directions being performed in separate blocks of trials. In the horizontal trial (horizontal smooth pursuit, HSP), two different target velocities were used, the maximum velocity (V_{max}) being either 16.72°/s (HSP16) or 33.44°/s (HSP33). In the vertical trial (vertical smooth pursuit, VSP), a single 8.66°/s maximum velocity was used. Each HSP task lasted 50,000 ms and the VSP task 30,000 ms. Subjects were instructed to follow the moving target as smoothly and as accurately as possible.

The gain of smooth pursuit was calculated as the ratio of the subject's V_{max} and the target V_{max} on the middle of the curve. If a saccade occurred during SP, the measure was shifted on the curve backward or forward.

Each trial was presented twice during the entire oculomotor session in the following order: vertical prosaccades, horizontal prosaccades, HSP16, HSP33, vertical smooth pursuit, horizontal antisaccades, vertical antisaccades and mixed antisaccades.

2.3. Recording, apparatus and analysis of data

Eye movements were recorded with a binocular video-based eye tracker (mobile eBT Eyebrain, Ivry-sur-Seine, France, www.eye-brain.com) with a 300 Hz sampling rate and 0.5° spatial resolution. The left eye trace was analysed by default, however the right eye was used if the left eye signal was contaminated by artefacts. Saccades were automatically detected according to a velocity

threshold (Eyebrain software) but were individually inspected and manually corrected by the experimenter if necessary. Saccades perturbed by blinks or other artefacts were discarded (less than 10% of the trials in all subjects). In the pro-and antisaccade tasks, we defined the SRT as the interval between target onset and saccade onset. SRT below 80 ms were considered anticipatory saccades and rejected, and SRT between 81 and 130 ms were considered "express saccades" (Delinte et al., 2002).

2.4. Statistical analysis

Analysis of variance (ANOVA) with post hoc Bonferroni adjustment was applied to assess differences between the general characteristics of metrics and age groups, as the variables were normally distributed (Kolmogorov–Smirnov test). The level of significance was set at $p < 0.05$. Subsequently, the Pearson analysis was used to examine the strength of the relationships between parameters. Due to the number of comparisons between age and metrics, the alpha level was adjusted to 0.0019 by dividing the customary alpha level of 0.05 by the number of correlations tested (27). Robust linear regression was used to obtain rate of increase/decrease per year for all significantly age-dependent variables.

3. Results

3.1. Group characteristics

We recruited 145 subjects aged 19–82 years (y) (mean age: 47.48; SD: 18.17), including 81 women (55.86%) and 64 men (44.13%). The majority of subjects were right handed (right handed: 136; left handed: 6). Laterality was not assessed in three subjects. The education level was determined by the number of years of education: 92 subjects had <13 years of education (primary and secondary school) and 49 subjects had a university degree (total years of education $\geqslant$17). Education level was not assessed in 4 volunteers.

3.2. Gender, laterality, education level and correlation to EM metrics

There were no statistically significant differences in gender or educational level across all EM metrics (Table 1). Due to the statistically incomparable sample size for right- and left-handed subjects (136 participants, of which only 6 were left-handed), we were not able to study the influence of laterality on EM metrics.

3.3. Age and EM metrics (Table 2)

Eye movement metrics were correlated with age for the entire series. Subjects were divided into six groups by decades (19–29, 30–39, 40–49, 50–59, 60–69, 70–82 years) to precisely determine in which group age differences in EM metrics were more significant. Each group was composed of nearly equally numbers of female and male subjects. The upper age limit was included in each group.

3.3.1. Prosaccades (Fig. 1)

3.3.1.1. Horizontal. Only the SRT of leftward saccades increased significantly (0.71 ms/y) and correlated with age. ANOVA revealed a significant main effect of group for the SRT of leftward saccades

Table 1
Influence of gender, education level and direction of target presentation on EM metrics. Group differences. SRT: saccade reaction time; V_{avg}: average velocity of saccades; *p*: *p* value; H: horizontal; V: vertical; R: right; L: left; U: up; D: down; r: target of antisaccades presented on the right, correct movement to the left; *l*: target of antisaccades presented on the left, correct movement to the right; u: target of antisaccades presented up, correct movement down; d: target of antisaccades presented down, correct movement up; Smooth pursuit 16°: V_{max} of the target 16,72°/s; smooth pursuit 33°: V_{max} of the target 33,44°/s; smooth pursuit 8°: V_{max} of the target 866°/s.

Paradigm	EM metric	Side/direction of presented target	Total *n*: 145 64 M/81 F (mean values)	Gender *p* value	Education level *p* value	Direction of target presentation R/L, U/D, r/*l*, u/d *p* value
Prosaccades H	SRT (ms)	R	187 ± 31	0.097	0.18	<0.001
		L	173 ± 30	0.71	0.70	
	V_{avg} (°/s)	R	239 ± 43	0.43	0.17	<0.05
		L	228 ± 48	0.75	0.49	
	V_{max} (°/s)	R	500 ± 106	0.43	0.05	<0.05
		L	473 ± 101	0.93	0.12	
	Gain	R	0.94 ± 0.07	0.45	0.37	0.21
		L	0.93 ± 0.06	0.15	0.64	
Prosaccades V	SRT (ms)	U	186 ± 32	0.88	0.69	0.61
		D	184 ± 32	0.38	0.82	
	V_{avg} (°/s)	U	174 ± 49	0.34	0.65	<0.001
		D	222 ± 57	0.16	0.84	
	V_{max} (°/s)	U	402 ± 109	0.16	0.73	<0.001
		D	491 ± 120	0.34	0.98	
	Gain	U	0.86 ± 0.09	0.48	0.85	<0.001
		D	0.99 ± 0.07	0.57	0.31	
Antisaccades H	SRT (ms)	*r*	218 ± 42	0.91	0.19	0.11
		l	227 ± 52	0.66	0.93	
	Errors (%)	*r*	33 ± 26	0.30	0.66	<0.05
		l	27 ± 23	0.05	0.73	
Antisaccades V	SRT (ms)	u	241 ± 53	0.99	0.70	0.24
		d	234 ± 48	0.91	0.58	
	Errors (%)	u	32 ± 25	0.51	0.68	0.98
		d	32 ± 23	0.12	0.97	
Smooth pursuit H 16°/s	Gain	R	1.06 ± 0.18	0.67	0.13	0.46
		L	1.04 ± 0.16	0.63	0.41	
Smooth pursuit H 33°/s	Gain	R	1.03 ± 0.16	0.34	0.13	<0.05
		L	0.99 ± 0.16	0.52	0.78	
Smooth pursuit V 8°/s	Gain	R	0.97 ± 0.24	0.31	0.67	0.98
		L	0.97 ± 0.22	0.05	0.97	

[$F(5, 139) = 8.35$, $p < 0.001$] with post hoc analysis indicating more prominent differences between subjects aged 19 and 39 years versus 70 and 82 years ($p < 0.001$). The velocity (V_{avg} and V_{max}) did not correlate with age. Only the gain of rightward prosaccades decreased significantly (−0.0015/y). This decrease was associated with a significant main effect of group [$F(5, 139) = 5.89$, $p < 0.001$] with post hoc analysis indicating differences mainly between subjects aged 19 and 29 years versus 70 and 82 years, ($p < 0.001$).

3.3.1.2. Vertical. The SRT increased for targets presented up (0.63 ms/y) and correlated with subject age. This increase was associated with a significant main effect of group [$F(5, 139) = 6.54$, $p < 0.001$] with post hoc analysis indicating differences mainly between participants aged 19 and 29 years vs. 50 and 82 years ($p < 0.001$). SRT also increased for downward prosaccades (0.84 ms/y), a significant main effect of group [$F(5, 139) = 7.33$, $p < 0.001$] with post hoc analysis indicating differences mainly between subjects aged between 19 and 29 years vs. 70 and 82 years ($p < 0.001$). Only upward saccades became slower [V_{avg} −0.44°/s/y; $F(5, 139) = 3.98$, $p < 0.01$] and hypometric [gain −0.0017/y; $F(5, 139) = 3.98$, $p < 0.001$] with senescence.

3.3.2. Antisaccades (Fig. 2)

3.3.2.1. Horizontal. Only the SRT of antisaccades made to a left presented target increased (0.96 ms/y) and correlated with age. This increase was associated with a significant main effect of group [$F(5, 139) = 6.04$, $p < 0.001$] with post hoc analysis indicating the greatest differences between subjects aged 19 and 29 years versus 60 and 69 years ($p < 0.001$). The error rate increased for targets presented right (0.54%/y) and left (0.37%/y) and correlated with senescence. For right targets, there was a significant main effect of group [$F(5, 139) = 6.62$, $p < 0.001$] with post hoc analysis indicating more prominent differences between subjects aged 19 and 29 years versus 60 and 82 years ($p < 0.001$). For left targets, there was a significant main effect of group [$F(5, 139) = 4.24$, $p < 0.01$] with post hoc analysis indicating differences between 19 and 29 years versus 70 and 82 years ($p < 0.001$). The rate of movement correction after an incorrect antisaccade in all age groups was 99.3–99.8%. During the interleaved antisaccade task, the SRT increased in both directions [target right: $F(5, 139) = 3.65$, $p < 0{,}01$; target left: $F(5, 139) = 3.69$, $p < 0.01$) as well as the error rate (right: $F(5, 139) = 2.44$, $p < 0.05$; left: $F(5, 139) = 6.78$, $p < 0.001$). In addition, post hoc analysis indicated differences between 19 and 39 years versus 70 and 82 years ($p < 0.001$) for left error rate.

3.3.2.2. Vertical. The SRT increased for targets presented up (1.01 ms/y) and down (1.10 ms/y) and correlated with age. For targets presented up, there was a significant main effect of group [$F(5, 139) = 5.05$, $p < 0.001$] with post hoc analysis indicating

Table 2
Correlation between age and EM metrics. y: years; *n*: number of subjects; M: male; F: female; *r*: *r* value, pearson product-moment correlation coefficient; *p*: *p* value; SRT: saccade reaction time; V_{avg}: average velocity of saccades; H: horizontal; V: vertical; R: right; L: left; U: up; D: down; r: target of antisaccades presented on the right, correct movement to the left; l: target of antisaccades presented on the left, correct movement to the right; u: target of antisaccades presented up, correct movement down; d: target of antisaccades presented down, correct movement up; smooth pursuit 16°: V_{max} of the target 16.72°/s; smooth pursuit 33°: V_{max} of the target 33.44°/s; smooth pursuit 8°: V_{max} of the target 8.66°/s. Due to the number of comparisons between age and metrics, the alpha level was adjusted to 0.0019 by dividing the customary alpha level of 0.05 by the number of correlations tested (27), (see Section 2).

Paradigm	EM metric	Side/direction of presented target	19–29 years *n*: 32 11 M/21 F	30–39 years *n*: 25 11 M/14 F	40–49 years *n*: 21 8 M/13 F	50–59 years *n*: 23 11 M/12 F	60–69 years *n*:24 11 M/9 F	70–82 years *n*: 20 11 M/9 F	*r*	*p*
Prosaccades H	SRT	R	183 ± 31	180 ± 22	177 ± 28	192 ± 35	188 ± 26	201 ± 38	0.21	0.011
	(ms)	L	158 ± 29	162 ± 20	166 ± 29	176 ± 21	187 ± 25	199 ± 33	0.47	**<0.001**
	V_{avg}	R	248 ± 42	240 ± 36	250 ± 38	234 ± 44	240 ± 53	215 ± 39	−0.18	0.027
	(°/s)	L	235 ± 38	242 ± 44	232 ± 34	226 ± 36	222 ± 69	202 ± 58	−0.22	0.007
	V_{max}	R	523 ± 88	524 ± 114	513 ± 93	490 ± 117	497 ± 104	432 ± 104	−0.24	0.003
	(°/s)	L	488 ± 92	490 ± 103	476 ± 65	495 ± 101	476 ± 131	394 ± 74	−0.22	0.009
	Gain	R	0.97 ± 0.06	0.96 ± 0.05	0.94 ± 0.06	0.94 ± 0.07	0.91 ± 0.07	0.89 ± 0.06	−0.40	**<0.001**
		L	0.95 ± 0.07	0.93 ± 0.07	0.93 ± 0.05	0.92 ± 0.05	0.93 ± 0.06	0.91 ± 0.07	−0.20	0.017
Prosaccades V	SRT	U	167 ± 25	179 ± 19	182 ± 37	203 ± 31	205 ± 36	192 ± 28	0.38	**<0.001**
	(ms)	D	166 ± 35	178 ± 24	177 ± 15	191 ± 31	196 ± 25	209 ± 35	0.47	**<0.001**
	V_{avg}	U	194 ± 68	187 ± 43	159 ± 46	185 ± 38	158 ± 35	153 ± 30	−0.27	**<0.001**
	(°/s)	D	218 ± 56	229 ± 56	216 ± 52	225 ± 63	239 ± 69	202 ± 42	−0.01	0.94
	V_{max}	U	418 ± 116	423 ± 108	374 ± 117	432 ± 130	370 ± 89	385 ± 77	−0.13	0.11
	(°/s)	D	480 ± 116	507 ± 115	481 ± 123	487 ± 128	505 ± 119	491 ± 130	0.02	0.81
	Gain	U	0.89 ± 0.06	0.90 ± 0.07	0.85 ± 0.06	0.84 ± 0.09	0.84 ± 0.11	0.81 ± 0.11	−0.36	**<0.001**
		D	0.99 ± 0.05	0.99 ± 0.06	1.01 ± 0.05	0.97 ± 0.07	0.98 ± 0.10	0.98 ± 0.08	−0.08	0.33
Antisaccades H	SRT	*r*	202 ± 36	215 ± 32	224 ± 33	215 ± 52	229 ± 43	232 ± 50	0.22	0.009
	(ms)	*l*	195 ± 42	218 ± 40	223 ± 33	233 ± 47	258 ± 58	250 ± 63	0.40	**<0.001**
	Errors	*r*	21 ± 18	21 ± 19	34 ± 20	47 ± 29	34 ± 25	51 ± 29	0.39	**<0.001**
	(%)	*l*	19 ± 16	20 ± 19	24 ± 17	33 ± 26	26 ± 25	44 ± 31	0.33	**<0.001**
Antisaccades V	SRT	u	227 ± 52	235 ± 50	242 ± 40	246 ± 62	272 ± 45	256 ± 57	0.36	**<0.001**
	(ms)	d	205 ± 43	227 ± 39	228 ± 33	228 ± 47	265 ± 44	264 ± 53	0.43	**<0.001**
	Errors	u	27 ± 23	23 ± 18	23 ± 20	38 ± 28	34 ± 22	50 ± 30	0.29	**<0.001**
	(%)	d	22 ± 16	27 ± 19	34 ± 21	38 ± 29	32 ± 23	43 ± 26	0.30	**<0.001**
Smooth pursuit H 16°/s	Gain	RL	1.02 ± 0.13	1.09 ± 0.14	1.00 ± 0.22	1.04 ± 0.15	1.10 ± 0.21	1.10 ± 0.22	0.11	0.18
Smooth pursuit H 33°/s		RL	1.03 ± 0.12	1.07 ± 0.17	1.02 ± 0.17	1.02 ± 0.12	1.06 ± 0.20	0.99 ± 0.18	0.07	0.39
Smooth pursuit V 8°/s		RL	0.98 ± 0.24	0.96 ± 0.27	0.95 ± 0.20	0.97 ± 0.25	0.96 ± 0.22	1.02 ± 0.25	0.03	0.76

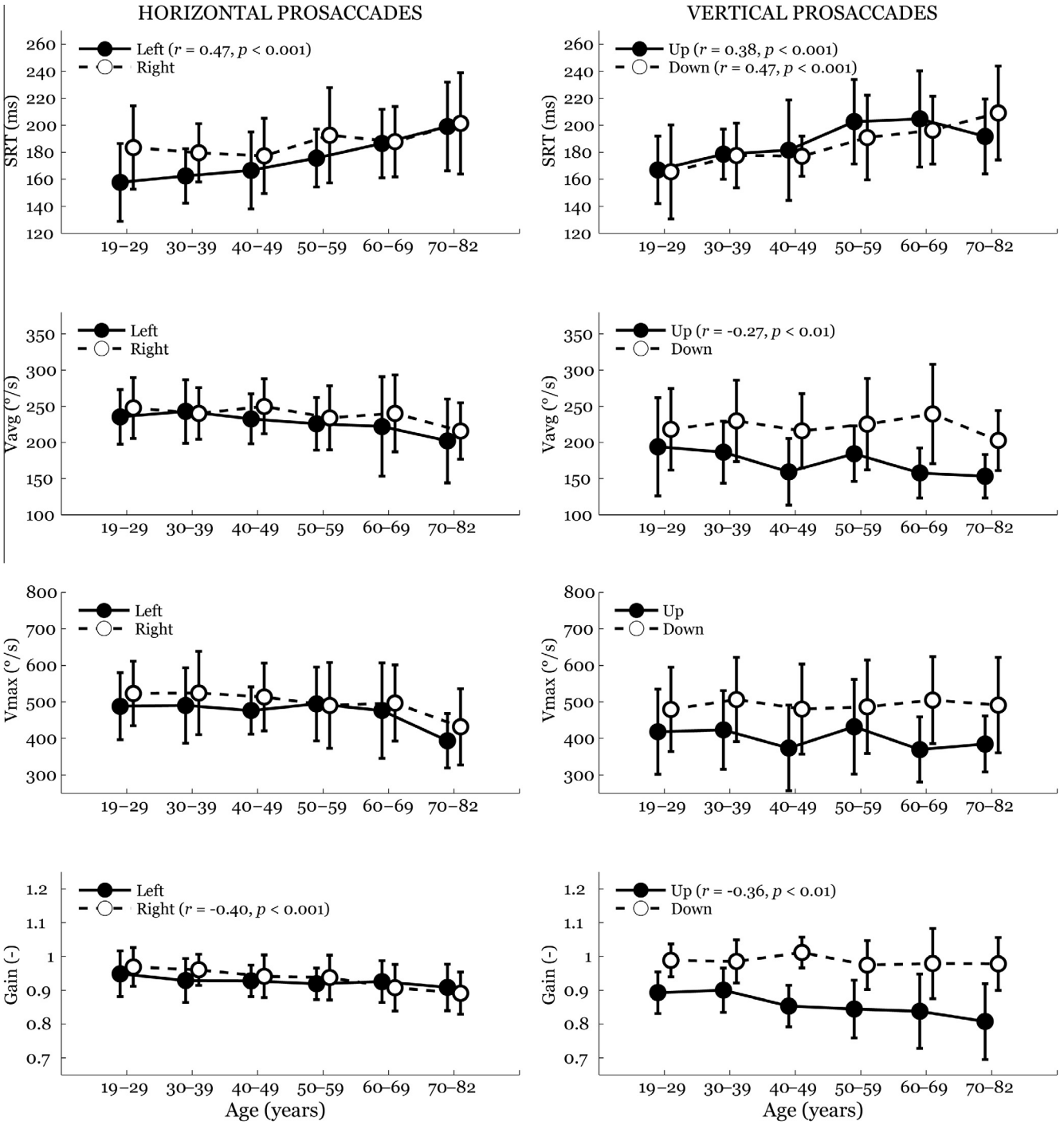

Fig. 1. Age-related changes of horizontal and vertical prosaccades. SRT: saccade reaction time; V_{avg}: average velocity; V_{max}: maximal velocity; left: target presented at the left side; right: target presented at the right side; up: target presented up; down: target presented down.

more prominent differences between subjects aged 19 and 29 years versus 70 and 82 years ($p < 0.001$). For targets presented down, there was a significant main effect of group [$F(5, 139) = 7.52$, $p < 0.001$] with post hoc analysis indicating differences between subjects aged 19 and 29 years versus 60 and 82 years ($p < 0.001$). The error rate increased in the simple and interleaved task, for targets presented up (0.40%/y; $F(5, 139) = 4.36$, $p < 0.01$) and down (0.35%/y; $F(5, 139) = 2.88$, $p < 0.05$), but subjects of all age groups still able to correct 99% of the errors made.

3.3.3. Smooth pursuit

We did not find any correlation between age and gain of horizontal slow, fast or for vertical smooth pursuit.

3.4. Influence of target presentation direction on EM metrics (Table 1)

3.4.1. Right–left

3.4.1.1. Prosaccades. (Fig. 3) Rightward showed longer SRT than leftward saccades [$F(1, 288) = 14.39$, $p < 0.001$]. The index comparing the SRT of rightward vs. leftward prosaccades decreased and

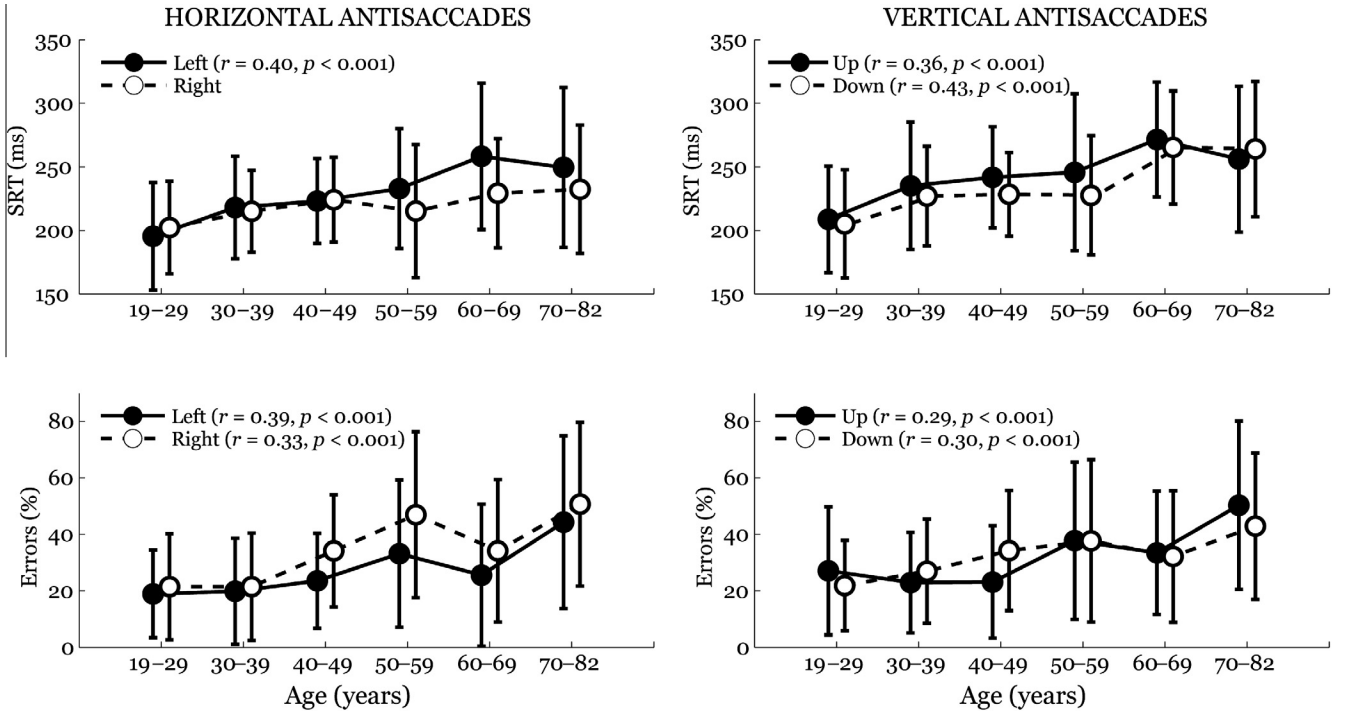

Fig. 2. Age-related changes of horizontal and vertical antisaccades. SRT: saccade reaction time; left: target presented at the left side, correct movement to the right; right: target presented at the right side, correct movement to the left; up: target presented up, correct movement downward; down: target presented down, correct movement upward.

Table 3
Index comparing SRT of pro- and antisaccades in the horizontal and vertical direction. Index of SRT resulting from the division of horizontal and vertical pro- and antisaccade SRT. PS: prosaccades; AS: antisaccades; *r*: *r* value, pearson product-moment correlation coefficient; *p*: *p* value; values in bold are significant *p* values ($p<0.001$), significant correlation with age $p < 0.001$. R: right; L: left; U: up; D: down; r: target presented on the right, correct movement to the left; *l*: target presented on the left, correct movement to the right; u: target presented up, correct movement down; d: target presented down.

	Horizontal prosaccades Index STR R/SRT L	Antisaccades Index SRT *r*/SRT *l*	Vertical prosaccades Index SRT U/SRT D	Antisaccades Index SRT u/SRT d
19–29 years	1.8	1.03	1.03	0.095
30–39 years	1.1	1.04	1.01	1
40–49 years	1.1	1.06	1.02	1.01
50–59 years	1.1	1.08	1.01	0.93
60–69 years	1.01	1.03	1.05	0.91
70–80 years	1.02	0.98	0.93	0.95
r, *p*	$r = -0.32$, $p <$ **0.001**	$r = -0.33$, $p <$ **0.001**	$r = -0.09$, $p = 0.27$	$r = -0.05$, $p = 0.52$

correlated with age (Table 3). This decrease was associated with a significant main effect of group [$F(5, 139) = 4.32$, $p = 0.001$] with post hoc analysis indicating differences mainly between subjects aged 19 and 29 years versus 60 and 82 years, ($p < 0.01$). Rightward saccades were faster (V_{avg} and V_{max}) than leftward [V_{avg}: $F(1, 288) = 4.13$, $p < 0.05$; V_{max}: $F(1, 288) = 4.91$, $p < 0.05$], but the gain was similar for both sides.

3.4.1.2. Antisaccades. Generally, horizontal antisaccades had longer SRT than horizontal prosaccades [$F(1, 288) = 54.11$, $p < 0.001$]. The SRT in the simple task was similar for both sides (Fig. 4). In the interleaved horizontal and vertical task, the SRT for horizontal antisaccades did not change significantly, being similar in both directions [$F(1, 288) = 0.17$, $p = 0.68$]. Similarly to prosaccades, the index of SRT antisaccades to both sides (SRT right/SRT left) decreased and correlated with age (Table 3). This decrease was associated with a significant main effect of group [$F(5, 139) = 4.24$, $p < 0.01$]. The error rate on the antisaccade simple task was modestly higher for saccades presented at the right side (movement to the left) [$F(1, 288) = 5.63$, $p < 0.05$], whereas in the mixed task no difference in laterality was detected [$F(1, 288) = 1.66$, $p = 0.20$].

The SRT of prosaccades and antisaccades to one determined side, correlated for targets presented at the right ($r = 0.52$, $p < 0.001$) and at the left ($r = 0.51$, $p < 0.001$) side.

3.4.1.3. Smooth pursuit. Gain for slow horizontal SP was similar in both directions, while in faster SP the gain was modestly higher for the rightward direction [$F(1, 288) = 5.54$, $p < 0.05$].

3.4.2. Up–down

3.4.2.1. Prosaccades. (Fig. 3) Vertical prosaccades had similar SRT in both directions. The index comparing the latency of both directions (Table 3) did not significantly correlate with age. Upward saccades were slower (V_{avg} and V_{max}) and had lower gain than downward prosaccades [V_{avg}: $F(1, 288) = 57.32$, $p < 0.001$; V_{max}: $F(1, 288) = 43.41$, $p < 0.001$]

3.4.2.2. Antisaccades. The SRT for vertical antisaccades was longer than for prosaccades [$F(1, 288) = 111.84$, $p < 0.001$]. The SRT in

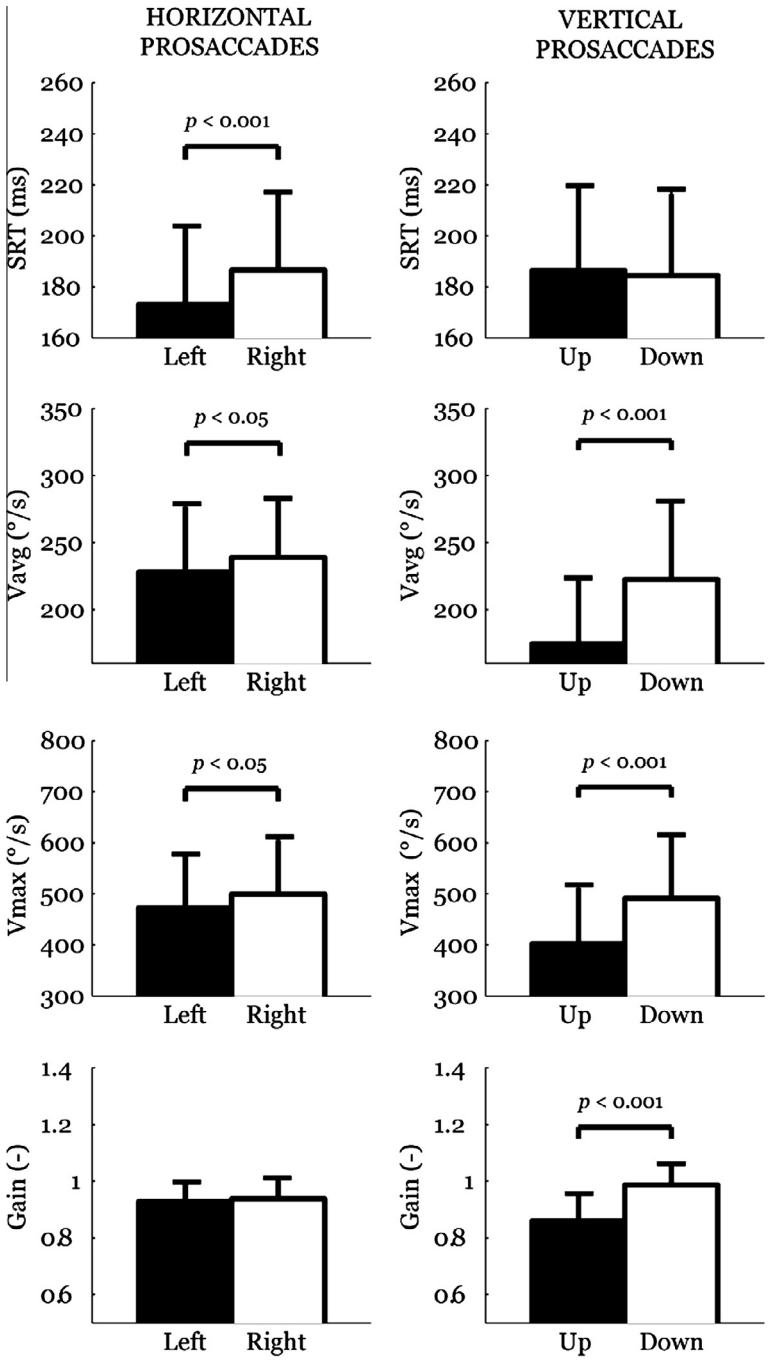

Fig. 3. Side/direction differences in prosaccades. SRT: saccade reaction time; V_{avg}: average velocity; V_{max}: maximal velocity; left: target presented at the left side; right: target presented at the right side; up: target presented up; down: target presented down.

the simple (Fig. 4) antisaccade task was similar in both directions [$F(1, 288) = 0.17$, $p < 0.68$]. The same was noted in the interleaved [$F(1, 288) = 0.02$, $p = 0.88$] task. The index comparing the latency of antisaccades to both directions (SRT up/down) was not significant (Table 3) and did not correlate with age. The error rate in the simple [$F(1, 288) = 0$, $p = 0.98$] and interleaved [$F(1, 288) = 1.44$, $p = 0.23$] antisaccade was similar in both directions.

The SRT of prosaccades and antisaccades correlated with targets presented up ($r = 0.51$, $p < 0.001$) and down ($r = 0.60$, $p < 0.001$).

3.4.2.2. Smooth pursuit. Gain for vertical SP was similar in both directions.

3.5. Skewness of horizontal pro- and antisaccades

Table 4 summarizes the mean value of this metric for horizontal pro- and antisaccades, and its correlation with age and other EM metrics. Skewness did not correlate significantly with age in horizontal prosaccades or antisaccades. Skewness of horizontal prosaccades on overlap between 5° and 20° and gap 13° did not correlate with duration, amplitude, latency, velocity or gain. Antisaccades have a more skewed velocity profile than prosaccades [$F(1, 288) = 156.97$, $p < 0.001$]. Skewness of horizontal antisaccades correlated significantly only with duration and amplitude for both sides.

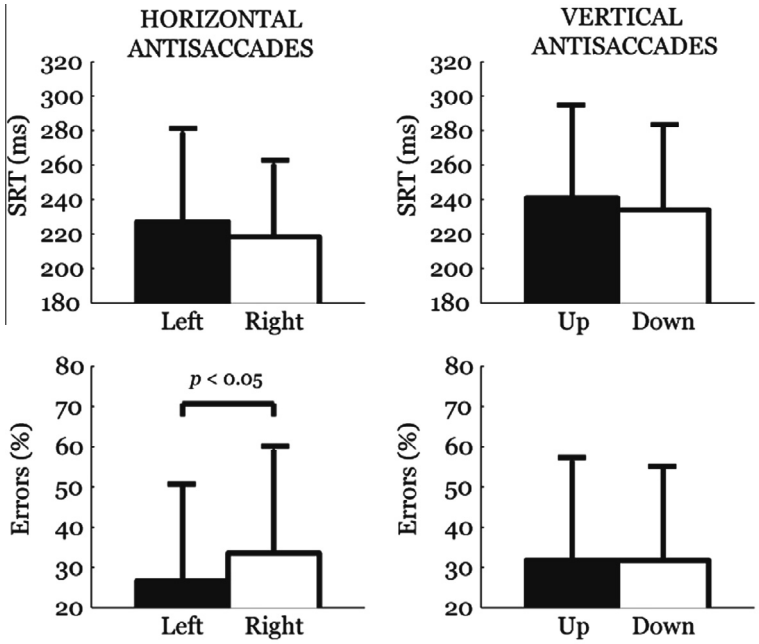

Fig. 4. Side/direction differences in antisaccades. SRT: saccade reaction time; left: target presented at the left side, correct movement to the right; right: target presented at the right side, correct movement to the left; up: target presented up, correct movement downward; down: target presented down, correct movement upward.

Table 4
Skewness and its correlation to age and other eye movement metrics. PS: prosaccades; AS: antisaccades; GAP: gap task; OT: overlap task; R: right; L: left; r: target of antisaccades presented on the right, correct movement to the left; *l*: target of antisaccades presented on theleft; V_{avg}: average velocity; V_{max}: maximal velocity; *r*: *r* value, pearson product-moment correlation coefficient; *p*: *p* value; $^{*}p < 0.05$; $^{**}p < 0.01$ and $^{***}p < 0.001$. Values in bold are significant *p* values.

		Skewness	Age		Duration		Amplitude		Latency		V_{avg}		V_{max}		Gain	
		Main value ± SD	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
PS																
GAP 13°	R	0.44 ± 0.08	−0.10	0.25	−0.18*	0.04*	−0.02	0.82	0.08	0.35	0.13	0.14	0.09	0.28	0.04	0.68
	L	0.43 ± 0.06	−0.14	0.10	−0.06	0.50	−0.11	0.20	−0.09	0.28	−0.13	0.13	−0.18*	0.03*	−0.19*	0.03*
OT 5°	R	0.56 ± 0.10	0.01	0.87	−0.01	0.91	0.07	0.40	−0.09	0.31	0.07	0.42	0.14	0.12	0.15	0.09
	L	0.49 ± 0.16	0.02	0.73	−0.11	0.20	0.01	0.95	−0.13	0.14	−0.03	0.69	0	0.99	0.13	0.13
OT 10°	R	0.56 ± 0.11	0.02	0.74	−0.10	0.23	−0.06	0.49	−0.09	0.31	0.11	0.19	0.26**	0.0023**	0.19*	0.03*
	L	0.50 ± 0.11	0.08	0.35	−0.07	0.41	−0.25**	0.0029**	−0.04	0.62	−0.21*	0.01*	−0.15	0.09	0.08	0.33
OT 15°	R	0.48 ± 0.17	−0.15	0.08	0.03	0.75	0.25**	0.0039**	−0.27**	0.0016**	0.23**	0.0066**	0.22*	0.01*	0.16	0.06
	L	0.42 ± 0.11	−0.09	0.30	−0.22*	0.01*	0.07	0.42	−0.12	0.16	−0.04	0.65	−0.04	0.61	0	0.96
OT 20°	R	0.48 ± 0.12	−0.14	0.11	0	0.98	−0.01	0.95	−0.20*	0.02*	−0.05	0.60	0.22	0.11	0.10	0.24
	L	0.44 ± 0.09	−0.08	0.39	−0.23**	0.0076**	−0.04	0.65	−0.29**	0.0006**	−0.16	0.07	−0.19*	0.03*	−0.17	0.06
AS																
13°	*r*	0.38 ± 0.12	−0.15	0.08	**−0.40****	**<0.0001****	**0.25****	**0.0046****	0.04	0.67						
	l	0.41 ± 0.12	−0.21*	0.019*	**−0.62****	**<0.0001****	**−0.35****	**<0.0001****	−0.06	0.52						

3.6. Inter subject variability in EM metrics according to subject age

There was a constant standard deviation across the six groups, indicating that there was no higher intersubject variability with respect to age.

4. Discussion

The present study examines ocular movements in a large number of healthy subjects using two standard saccade paradigms and smooth pursuit in the horizontal and vertical planes, and provides important clues for new oculomotor laboratories. The most relevant criterion in the constitution of control groups was age, whereas gender and education level did not influence the ocular motor performance. Age correlates with the latency of leftward and vertical pro- and antisaccades, velocity of upward prosaccades, gain of rightward and upward prosaccades and error rate of antisaccades. Eye movements should be investigated in the horizontal and vertical planes. The direction of the target affects mainly the SRT and velocity of horizontal prosaccades, velocity and gain of vertical prosaccades, and the error rate of horizontal antisaccades.

4.1. Constitution of subject group

Age influences several EM metrics. Our decision to group subjects by intervals of 10 years was rather arbitrary. Subjects may be grouped by 5 years (Peltsch et al., 2011), 10 years (present study) (Munoz et al., 1998), 15 years (Bono et al., 1996) or 20 years of age. There is no clear division of EM metrics between the different groups. Some metrics differ between the 2nd, 3rd and 7th decade, while others differ between the 2nd, 3rd and 5th decade. Although the effect of age on EM metrics is linear, grouping subjects by 20 years or more would hinder the accurate detection of several results. We chose to enrol more than 20 subjects per group, in an effort to obtain significant results. The intersubject variability

of EM metrics in the different age groups was large, revealed by high standard deviation (SD). However, intersubject variablity did not increase with age, so that there is no additional interest to increase the number of subjects in advanced decades.

We did not observe any differences by gender or education level in EM metrics, even though some values were coincidentally on the boundary of statistical significance. We were unable to investigate laterality, considering the high rate of right-handed participants. However, the proportion of right- to left-handed subjects in the present study is reflective of the worldwide population (9:1) (Frayer et al., 2012). Future studies with equal proportions of left- and right-handed participants are needed to shed light on the influence of laterality on EM performance. Intelligence (Evdokimidis et al., 2002; Haishi et al., 2011) and performance of executive functions (Mirsky et al., 2011) have also been reported to correlate with EM metrics. However the required neuropsychological test battery to asses both is rather complex and not used in clinical practice.

4.2. Paradigms and analysis

Eye movements should be studied separately in the horizontal and vertical plane. Clinical and basic science studies have demonstrated some anatomical segregation in the motor control of both kinds of movements. The caudal pons is important for horizontal saccades, and the rostral mesencephalon for vertical saccades (Leigh and Zee, 2006). Selective slowing of horizontal or vertical saccades is the hallmark of different neurodegenerative diseases. All more upward prosaccades are faster (Goldring and Fischer, 1997; Honda and Findlay, 1992; Zhou and King, 2002) than horizontal, and only the velocity of upward prosaccades declines with age. Vertical antisaccades have longer SRT than horizontal antisaccades, whereas this directional difference is not noted for prosaccades. There is no advantage in mixing antisaccades in the horizontal and vertical plane, as the latency and error rate did not differ between the simple and the interleaved task, suggesting that while task instruction is still the same, response switching (switching of direction) does not influence the oculomotor program (Cherkasova et al., 2002; Reuter et al., 2006).

To be reliable the paradigm to analyse eye movements must be simple, applicable to a large number of subjects of all ages, and feasible to perform within a period of 20 and 30 min. We have chosen two common saccade paradigms and smooth pursuit used in clinical practice aiming to investigate the function of large brain areas. The targets were presented with the same gap, same angle but varying directions, to efficiently compare the obtained metrics. As for other types of saccades (predicted or self-placed), their neural bases and contribution to clinical practice are not well known, we did not include them in the present study (Leigh and Kennard, 2004). Memory-guided saccades, an interesting paradigm used to investigate spatial memory (Leigh and Kennard, 2004; Pierrot-Deseilligny et al., 2003b), has not been included as it requires a learning phase, which would exceed our fixed examination time. Additionally, this task requires absolute darkness in the examination room, which would interfere with typical examination conditions, where the investigator's screen slightly illuminates the space. Furthermore, in this task, a significant number of trials are frequently invalid (e.g., saccades towards the flash, before the end of the delay) and must therefore be rejected.

Two main variables influence EM metrics and need to be taken in account in the analysis; the age of the subject and the direction of stimulus presentation:

4.3. Age and eye movements

Age induces changes in the following metrics: (i) SRT increase for horizontal but only leftward prosaccades, for antisaccades when the target is presented at the left side, for vertical prosaccades and for antisaccades in both directions; (ii) velocity decrease (V_{avg}) for vertical upward prosaccades; (iii) gain decrease for rightward and upward prosaccades; (iv) error rate increase: for horizontal and vertical antisaccades.

Other EM metrics remain stable during the lifespan: (i) SRT of pro- and antisaccades for targets presented at the right side; (ii) velocity, V_{avg} and V_{max} of horizontal prosaccades.V_{max} of vertical prosaccades and V_{avg} of downward prosaccades; (iii) gain of leftward and downward prosaccades; (iv) smooth pursuit gain in the horizontal and vertical direction.

The increase of SRT for horizontal prosaccades (Bono et al., 1996; Fischer et al., 1997a; Moschner and Baloh, 1994; Pratt et al., 1997; Sharpe and Zackon, 1987; Spooner et al., 1980; Warabi et al., 1984), vertical prosaccades (Yang and Kapoula, 2006), and antisaccades in both directions reported in the literature (Abel and Douglas, 2007; Klein et al., 2000; Munoz et al., 1998; Olincy et al., 1997; Shafiq-Antonacci et al., 1999) has been related to reduction of brain volume (Folstein and Folstein, 2010; Kochunov et al., 2008) and global cortical brain atrophy (Creasey and Rapoport, 1985; Nyberg et al., 2010; Salat et al., 2001). We noted three important characteristics regarding this change. First, the SRT length of pro- and antisaccades is similar, without difference in favour of pro- or antisaccades in the horizontal (Klein et al., 2000) or vertical direction, pointing to a common cortical control. Second, we observed significant age-related changes for pro- and antisaccades for left and vertical targets, but not for targets presented at the right side. This last phenomenon may be explained by some studies suggesting that the left hemisphere undergoes less age-related changes than the right (Albert, 1988; Bonilha et al., 2009; Brown and Jaffe, 1975; Vallesi et al., 2010; Dolcos et al., 2002). The right hemisphere is involved in the processing of pictorial/spatial information (Nebes, 1974; Sergent et al., 1992) and according to the right hemi-aging model, it seems to be the principal cause of age related changes on SRT. Third, the concordance in the increase of SRT of pro- and antisaccades suggests that the position of the target (sensorial posterior parietal and/or occipital right cortices) rather than the direction of movement is the relevant parameter.

Velocity and gain of prosaccades are less affected by senescence and this may be explained by studies observing that structures responsible for their function, such as the brainstem and cerebellum, remain relatively unchanged with age (Henson et al., 2003; Raz et al., 2001; Walhovd et al., 2011). We confirm that the velocity of horizontal prosaccades does not change (Munoz et al., 1998) and that upward prosaccades become slower (Wennmo et al., 1984; Yang and Kapoula, 2006) with advancing age. This is not surprising as the horizontal and vertical gaze centers are segregated in the brainstem, so that they could age in a differing manner. Conversely, upward saccades became not only slower but also hypometric with advancing age (Huaman and Sharpe, 1993), probably due to biomechanical changes in the orbital fascia, extraocular muscles (Clark and Demer, 2002; Clark and Isenberg, 2001; Oguro et al., 2004) and degeneration of the lateral rectus–superior rectus band (Rutar and Demer, 2009). These changes seem to not affect downward saccades, which remain stable throughout the lifespan. However, the fact that the gain of horizontal saccades decreased significantly only for rightward prosaccades and not bilaterally as previously described (Huaman and Sharpe, 1993; Irving et al., 2006; Olincy et al., 1997; Sharpe and Zackon, 1987; Tedeschi et al., 1989), warrants further investigation.

We have demonstrated that the error rate of antisaccades may reach up to 80% in advanced age (70–80 years), much higher than described in the literature (<30%) (Abel et al., 1983; Butler et al., 1999; Everling and Fischer, 1998; Klein et al., 2000; Leigh and Zee, 2006; Olincy et al., 1997; Peltsch et al., 2011; Shafiq-Antonacci

et al.,1999; Sweeney et al., 2001). This has been related to deterioration of the saccadic inhibition system (Butler and Zacks, 2006; Davis et al., 2008; Nieuwenhuis et al., 2000; Nyberg et al., 2010; Persson and Nyberg, 2006; Persson et al., 2006; Rajah and D'Esposito, 2005). Moreover, subjects of all age groups are continuously able to correct over 99% of the errors made (Fiehler et al., 2004, 2005; Taylor and Hutton, 2009, 2011), even in the interleaved antisaccade task. Thus, older subjects did not forget the instruction during the task. The neural mechanism underlying the monitoring, detection and correction of errors has been related to the anterior cingulate cortex and lateral prefrontal cortex (Carter et al., 1998; Gehring and Knight, 2000; Hester et al., 2005; Kiehl et al., 2000). Our findings suggest that this last mechanism is the more preserved of age-related changes (Eenshuistra et al., 2004) and the ability to correct errors should be included regularly in the analysis of antisaccades. Furthermore, it remains to be clarified how patients presenting a high error rate on the AS task, such as those diagnosed with progressive supranuclear palsy, Huntington's disease or schizophrenia (Garbutt et al., 2008; Leigh and Zee, 2006; Rivaud-Pechoux et al., 2007, 2000; Rivaud et al. 1994; Vidailhet and Rivaud-Pechoux, 2000) or developmental dyslexia (Biscaldi et al., 2000; Leigh and Zee, 2006) correct their errors.

The stability of smooth pursuit gain during the entire lifespan shown in the present study may be explained by the paradigm used. We used relatively slow moving targets and the analysis was simplified to the gain of pursuit. Previous studies have shown that the gain of smooth pursuit with predictable sinusoidal moving targets is stable with advancing age (Kerber et al., 2006). This has been attributed to the ability of the oculomotor system to compensate the age-related decline of velocity, acceleration, and latency due to the preservation of anticipation and the prediction of target motion continuation (Sprenger et al., 2011).

4.4. Direction of stimulus presentation and eye movements

The direction of stimulus presentation influences the SRT and the velocity of horizontal prosaccades (not gain), as well as the error rate of antisaccades. The SRT is longer for targets presented at the right side, and the V_{avg} and V_{max} higher for rightward saccades. The error rate of antisaccades is higher for targets presented at the right side (movement to the left) (Dafoe et al., 2007; Fischer et al., 1997a; Munoz et al., 1998). This correlates with the side that has shorter SRT for antisaccades. The direction of stimulus presentation influences velocity and gain of vertical eye movements, not the SRT. The V_{avg} is slower (Dafoe et al., 2007) and the gain lower for upward saccades.

Several questions concerning the differences between rightward and leftward, or upward and downward saccades remain open. We believe that our normative study does not allow one to draw strong conclusions about brain physiology, assuming, for example, an asymmetry of the cerebral cortex because an asymmetry was detected on the SRT of the horizontal saccades. Functional magnetic resonance imaging performed during the horizontal visually guided saccades showed that the brain network involved in their execution, irrespective of the direction, presented specific right and left asymmetries that were not related to anatomical differences in gray matter or sulci positions (Petit et al., 2009). The visual–spatial attention system also modulates the SRT of eye movements. This results in a left/right asymmetry, which is specific to individual subjects and to the dynamic modulations of the target (Klein, 1980; Posner, 1980; Shepherd et al., 1986; Weber and Fischer, 1995). Vertical eye movements, unlike the horizontal movements, have similar SRT. This may be explained by the proposed symmetric neural representations of the upper and lower visual fields in the FEF, parietal eye fields and SEF (Felleman and Van Essen, 1991).

Metrics independent of the direction of stimulus presentation are: (i) SRT of vertical prosaccades (Yang and Kapoula, 2008); (ii) SRT of horizontal and vertical antisaccades; (iii) gain of horizontal prosaccades; (iv) error rate vertical antisaccades; (iv) smooth pursuit gain horizontal (Bono et al., 1996) and vertical.

The index comparing the SRT of horizontal pro- and antisaccades is an interesting parameter for the clinical practice. For prosaccades (SRT rightward vs. leftward prosaccades), this index is larger than one and correlates negatively with age. For antisaccades (SRT rightward vs. leftward antisaccades), even if the SRT is similar for both sides, the index reveals an asymmetry and is also correlated with age. We believe that both indices may be useful for the diagnosis of diseases with asymmetric SRT, as the corticobasal syndrome. There is no interest to compute this index for vertical saccades.

4.5. Skewness

The shape of the velocity profile of horizontal pro- and antisaccades is stable during the entire lifespan. Within the range of reflexive horizontal prosaccades analyzed in the present study, it is not correlated to direction, amplitude, latency, velocity or gain. However, there is a strong correlation between the skewness and the amplitude and direction of horizontal antisaccades, regardless of the side of target presentation.

4.6. Conclusions

We conclude that the most important criterion for the control group of healthy subjects is age and that some metrics must be separated by the direction of movement, others according to the age of the subject, while others may be pooled. If only one measure of velocity for horizontal and vertical saccades should be chosen, we recommend the V_{avg}, as only this is significantly correlated to age for vertical saccades. The index of SRT of horizontal pro/antisaccades and the rate of error correction in the antisaccade task should be taken into account in the diagnosis of patients with eye movement abnormalities.

In our study, the major findings concerning aging and eye movements, and their possible physiological meaning are: (i) The age-related changes concern mainly the latency of saccades and the error rate of antisaccades. (ii) The latency of horizontal pro- and antisaccades lengthens with age only for targets presented on the left side, possibly reflecting an asymmetrical hemispheric aging. (iii) The error rate of antisaccades may reach up to 80% by the seventh decade of life, nevertheless, subjects of all age groups are continuously able to correct over 99% of the errors made. This suggests a deterioration of the saccadic inhibition system with a preservation of the monitoring, detection and correction of errors. (iv) The relative preservation of velocity and gain of horizontal prosaccades points to the stability of the brainstem and cerebellar occulomor systems. By contrast, the age-related changes in the velocity and accuracy of vertical prosaccades are probably due to biomechanical changes in the eye muscles and adjacent structures. (v) The skewness of horizontal saccades and the gain of slow horizontal and vertical smooth pursuit are stable throughout a person's lifespan.

The limitations of this study are the absence of skewness analysis for vertical eye movements, and that the smooth pursuit analysis did not include velocity, acceleration and latency of movement initiation. To the best of our knowledge, this remains the first large study of horizontal and vertical eye movements conducted in healthy subjects. We have summarized in Table 5 how the results of a routine eye movement examination can be presented.

Table 5
Example video-oculography assessment report. SRT: saccade reaction time; V_{avg}: average velocity of saccades; H: horizontal; V: vertical; antisaccades: right target presented on the right, correct movement to the left. Left target of antisaccades presented on the left, correct movement to the right. Up target for antisaccades presented up, correct movement down. Down target of antisaccades presented down, correct movement up; Smooth pursuit 16°: V_{max} of the target 16.72°/s; smooth pursuit 8°: V_{max} of the target 8.66°/s.

Name:		Date of birth:	Date of examination:
	Horizontal prosaccades		**Vertical prosaccades**
SRT (ms)	Right (187 ± 31) Left (Table 1)	Index SRT right/SRT left (Table 2)	SRT up/SRT down/2 (Table 1)
V_{avg} (°/s)	Right (239 ± 43) Left (228 ± 48)		up (Table 1) down (222 ± 57)
Gain	Right (Table 1) Left (0.93 ± 0.06)		up (Table 1) down (0.99 ± 0.07)
	Horizontal Antisaccades		**Vertical Antisaccades**
SRT (ms)	Right (218 ± 42) Left (Table 1)	Index SRT right/SRT left (Table 2)	SRT up/SRT down/2 (Table 1)
Error rate (%)	Right (Table 1) Left (Table 1)		% ER up + % ER down/2 (Table 1)
	Horizontal Smooth Pursuit		**Vertical Smooth Pursuit**
	(16 deg/s): Gain right + Gain left/2: (1.06 ± 0.18)		(8 deg/s): Gain up + Gain down/2: (0.97 ± 0.23)

Disclosure statement

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2.3 Exploring a non-negligible non-motor symptom: Vergence eye movements in Parkinson's disease and other parkinsonian syndromes

Fast vergence eye movements are disrupted in Parkinson's disease: A video oculography study. *Hanuška J, Bonnet C, Rusz J, Sieger T, Jech R, Rivaud-Péchoux S, Vidailhet M, Gaymard B, Růžička E. Parkinsonism Relat Disord. 2015 Jul;21(7):797-9.*[43]

Vergence eye movements (VEM) are slow, disjunctive movements of the eyes necessary to read and track objects moving in depth, maintaining a fused and single percept.[20, 21] They are divided into a convergent and a divergent movement. Convergence insufficiency may cause important difficulties in every-day life and is a frequent cause of visual discomfort in patients with Parkinson's disease (PD).[44-46] Until now characterization of VEM has been limited to clinical reports or to small patient series and to the best of our knowledge, never been characterized in PD.

In progressive supranuclear palsy (PSP) one study has shown decreased amplitude/velocity ratio of VEM,[47] and in multiple system atrophy (MSA) diplopia due to convergence insufficiency has been reported in two patients.[48] A recently described parkinsonian syndrome, the Ephedrone-induced parkinsonian syndrome (EP) secondary to ephedrone abuse, is characterized by severe, rapid progressive, irreversible Parkinsonism and dystonia.[49-56] Patients with EP, in the large majority young ephedrone abusers, complain often about visual discomfort during reading (Bonnet, personal communication), while VEM have also never been characterized. Moreover in PD and other parkinsonian syndromes convergent and divergent VEM have not been studied separately.

The aim of this study was to refine the description of vergence movements in PD and parkinsonian syndromes focusing two questions: i) Is it possible to record vergence eye movements in patients with parkinsonian syndromes in the clinical practice using videooculography? ii) Are VEM metrics altered in PD and are they different from MSA, PSP and EP? To address this issue, we have examined a series of patients and age-matched healthy control subjects.

We finally decided only to publish the results of PD patients because of the inhomogeneity of the patients groups. Nevertheless we would like to expose here our results, because we believe they are a good base for further research. We compared PD with EP patients and MSA and PSP patients, with a respective control group.

Methods

Subjects Patients with PD, MSA, PSP and all healthy controls were examined at the Department of Neurology of Charles University in Prague. Patients with EP were examined by the same team using the same videooculography device at the Neurology department of S. Khechinashvili University Clinic in Tbilisi, Rep. of Georgia. All participants signed the informed consent. The study was approved by the local ethics committees and was in compliance with the Declaration of Helsinki.

Eighteen PD patients (Table 1) diagnosed according to UK Parkinson's Disease Society Brain Bank criteria,[57] were clinically evaluated with the part III of the MDS-UPDRS.[58]

Pat.	Gender/Age M-F /years	Disease duration years	MDS UPDRS I /52	MDS UPDRS II /52	MDS UPDRS III /72	MDS UPDRS IV /28	Levodopa equiv. mg
1	M49	6	5	12	36	0	300
2	M64	21	6	7	29	0	480
3	M52	13	8	12	14	7	2535
4	M60	7	3	11	27	0	300
5	M50	12	12	15	35	0	300
6	M66	3	2	3	21	0	400
7	F40	4	5	9	16	0	360
8	F43	19	5	21	31	9	1120
9	M44	7	2	2	38	0	870
10	F70	11	5	6	25	3	1050
11	M54	12	1	3	8	0	900
12	F42	4	2	4	47	0	480
13	F71	1	6	6	2	0	0
14	M53	12	9	11	36	3	320
15	F63	11	4	10	17	0	320
16	M56	15	12	12	26	9	2620
17	F42	6	4	0	11	0	100
18	F43	4	14	7	24	0	100
mean	**8F/10M 53.44**	**9,33**	**5,83**	**8,39**	**24,61**	**1,72**	**697,50**

Table 1. Characteristics of Parkinson´s disease patients'
Pat: patient; M: Male; F: Female; Levodopa equiv.: Levodopa or equivalent of dopamine agonist per day, (0, 7 mg pramipexol = 100 mg levodopa; 5 mg ropirinol = 100 mg levodopa). Amantadine was used in Pat. 4 (300 mg), Pat 5 (200 mg) and Pat 15 (300 mg). Patient 12 and 17 receive additionally 2 and 3 mg of biperiden respectively.

All patients were examined with their usual medication in the "levodopa on" condition. Four patients with probable MSA according to Gillman,[59] six patients with probable PSP according to the Litvan criteria,[60] and 27 EP patients with a firm diagnosis of EP, based on history of former ephedrone consumption and ephedrone-induced parkinsonian syndrome, were enrolled. Patients with MSA, PSP and EP (Table 2), were clinically evaluated with the NNIPPS-Parkinson plus scale.[61] EP patients affirmed to have stopped the use of ephedrone between three months and 12 years (mean 3.9 years) before examination day. We have additionally examined 42 control subjects age- and gender-matched to the different patients. All patients and control subjects denied visual discomfort during near vision.

Patient	Gender	DD	Medication	NNIPPS														
	Age			mental	Bulbar	ADL	Tremor	Rigidity	Myoclonus	Bradyki-nesia		Dystonia		Pyramidal	Cerebellar	Orthostatic	Urinary	**Total**
	M/F years	years	Levodopa mg	(0-38)	(0-24)	(0-32)	(0-28)	(0-20)	(0-12)	(0-32)	axial (0-24)	(0-12)	limb (0-16)	(0-4)	(0-24)	(0-12)	(0-10)	
E1	M42	3	71	3	7	6	0	1	0	4	6	0	0	2	0	0	0	29
E2	M44	1	0	0	6	4	0	7	0	10	6	0	1	1	0	0	0	35
E3	M32	3	0	8	1	4	3	3	0	5	7	0	0	2	2	0	0	35
E4	M44	6	571	3	9	12	0	2	0	7	12	0	5	1	0	0	0	51
E5	M39	4	0	9	8	20	2	5	0	8	13	0	0	0	4	0	0	69
E6	M44	5	0	2	0	9	2	0	0	3	5	0	2	0	0	0	0	23
E7	M38	5	0	7	6	11	2	2	0	17	4	0	6	2	0	0	0	57
E8	M44	4	750	3	8	7	0	1	0	6	5	0	0	1	0	0	0	31
E9	M42	3	0	5	6	5	0	0	0	8	4	1	0	2	5	0	0	36
E10	M46	3	0	9	11	9	3	0	0	2	6	0	0	1	0	0	0	41
E11	F45	3	0	7	6	11	0	1	0	1	9	0	1	1	0	0	0	37
E12	M43	3	0	4	3	6	0	4	0	9	9	0	0	2	0	0	0	37
E13	M35	5	0	0	3	7	0	2	0	5	8	0	5	2	0	0	0	32
E14	M40	6	0	2	5	1	0	0	0	2	4	0	0	2	0	0	0	16
E15	M28	3	0	0	4	7	2	4	0	8	9	0	0	0	0	0	0	34
E16	M39	5	0	10	4	3	5	4	2	10	5	0	9	4	0	0	0	56
E17	M36	2	0	2	4	4	0	2	0	1	5	0	0	1	0	0	0	19
E18	M32	6	0	9	15	19	0	0	0	22	6	4	11	2	0	0	0	88
E19	M37	3	0	1	2	14	0	0	0	4	10	0	5	2	0	0	0	38
E20	M42	6	0	0	6	5	0	0	0	8	3	0	2	0	0	0	0	24
E21	M48	3	0	6	9	3	4	8	2	8	4	0	0	2	0	0	0	46
E22	M43	3	0	8	10	10	1	1	0	14	5	2	2	1	0	0	0	54
E23	M41	5	0	1	1	3	2	2	0	11	3	0	2	2	0	0	0	27
E24	M36	1	0	2	5	1	0	0	0	2	4	0	0	2	0	0	0	16
E25	M34	3	0	11	3	5	2	1	0	8	5	0	3	2	0	0	0	40
E26	M40	5	0	10	14	27	0	3	0	16	12	0	0	0	0	0	0	82
E27	M39	9	0	9	10	9	0	2	0	13	13	4	5	4	0	0	0	69
mean	**1F/26M 39,7**	**4,85**	**51,56**	**4,85**	**6,15**	**8,22**	**1,04**	**2,04**	**0,15**	**7,85**	**6,74**	**0,41**	**2,19**	**1,52**	**0,41**	**0**	**0**	**41,56**
M1	M56	3	0	10	11	34	0	4	0	17	22	0	0	0	22	0	1	121
M2	F55	4	750	3	6	10	2	3	0	16	8	0	0	3	10	0	1	62
M3	F56	6	0	8	7	13	3	5	0	16	13	0	0	3	7	12	6	93
M4	M61	3	0	8	7	11	9	11	0	16	5	0	0	2	0	7	3	79
mean	**2F/2M 57**	**4**	**187,5**	**7,25**	**7,75**	**17**	**3,5**	**5,75**	**0**	**16,25**	**12**	**0**	**0**	**2**	**9,75**	**4,75**	**2,75**	**88,75**

PSP1	F61	2	0	11	9	12	3	2	0	4	11	0	0	1	0	0	0	53
PSP2	F65	4	1250	2	11	9	4	3	1	18	15	0	2	0	0	0	0	65
PSP3	M62	6	1320	3	6	8	3	3	4	16	12	0	0	0	0	0	0	55
PSP4	M69	3	500	0	8	7	6	4	1	14	15	0	0	0	0	0	0	55
PSP5	M59	5	500	6	13	22	5	0	0	15	13	0	3	1	0	0	0	78
PSP6	M66	3	0	10	10	9	5	3	0	6	9	0	0	0	0	4	0	56
mean	**2F/4M 63,7**	**3,83**	**595**	**5,33**	**9,5**	**11,17**	**4,33**	**2,5**	**1**	**12,17**	**12,5**	**0**	**0,833**	**0,33**	**0**	**0,67**	**0**	**60,33**

Table 2. Characteristics of EP, PSP and MSA patients:
Pat: patient; M: Male; F: Female; DD: Disease duration; Levodopa equiv.: Levodopa or equivalent equivalent of dopamine agonist per day, (0,7 mg pramipexol = 100 mg levodopa ; 5 mg ropirinol = 100 mg levodopa. Amantadine was used in Pat. M3 (300 mg), Pat M4 (200 mg), Pat PSP 2 (200 mg), PSP 4 (100 mg) and PSP6 (200 mg).

Experimental paradigm, recording apparatus and vergence metrics are described in the published paper.

Statistical analysis in PD and EP

Parkinson´s disease patients and EP patients were compared with their respective control group using a statistical analysis. Since the Kolmogorov-Smirnov test for independent samples showed that oculomotor variables were widely normally distributed, analysis of variance (ANOVA) with post hoc Bonferroni adjustment was used to assess differences between the PD and appropriate control group as well as between EP and appropriate control group across the data. The Pearson analysis was used to examine the strength of the relationships between eye metrics and clinical data. The adjusted level of significance was set as $p < 0.05$.

Analysis of data in MSA and PSP

We did not provide statistical analysis for MSA and PSP groups due to a low number of patients included. Since the MSA and PSP patients span in same age range as PD patients, Mean and SD for each their eye movement metric was compared with the metrics of PD control group. We additionally calculated the rate of subjects with affected metrics (which do not span into interval Mean±SD of PD control group) and highlighted only metrics that were affected in more than 50% of the cases.

Results

Parkinson´s disease and EP

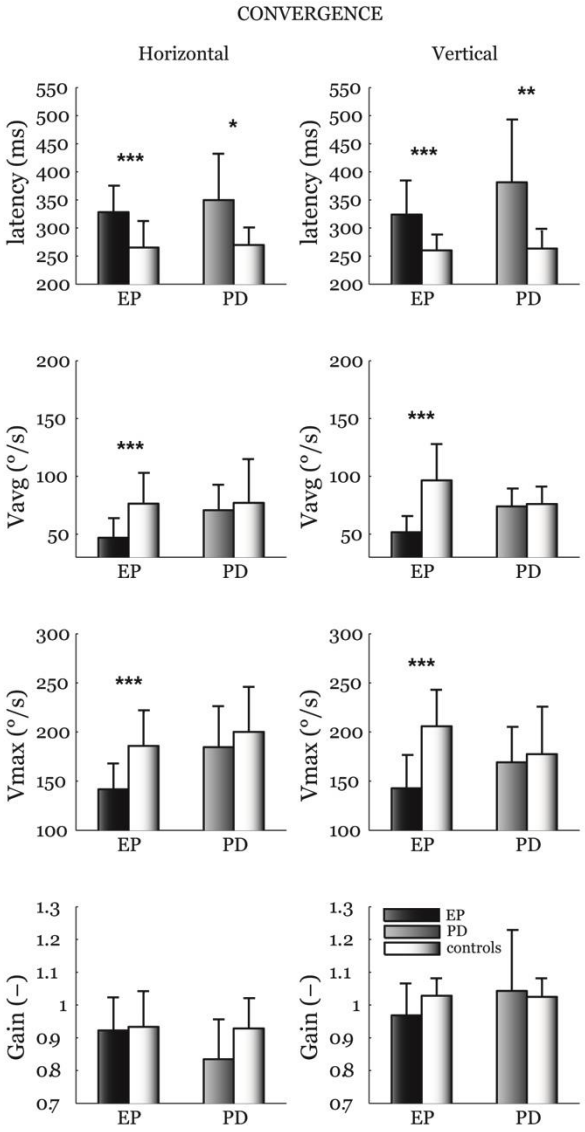

Figure 7 represents the results for convergence. The SRT was significantly increased in both groups when compared to controls ($p < 0.001$), but only EP patients showed slower velocities (Vavg and Vmax) ($p < 0.001$). Skewness was found to be increased in both PD and EP groups, while PD patients showed more affected horizontal saccades (PD: $p = 0.008$; EP: $p = 0.04$) and EP vertical saccades (PD: $p = 0.003$; EP: $p = 0.0005$). We have also found decreased gain for horizontal saccades and in PD group ($p = 0.01$) and vertical saccades for EP group ($p = 0.006$).

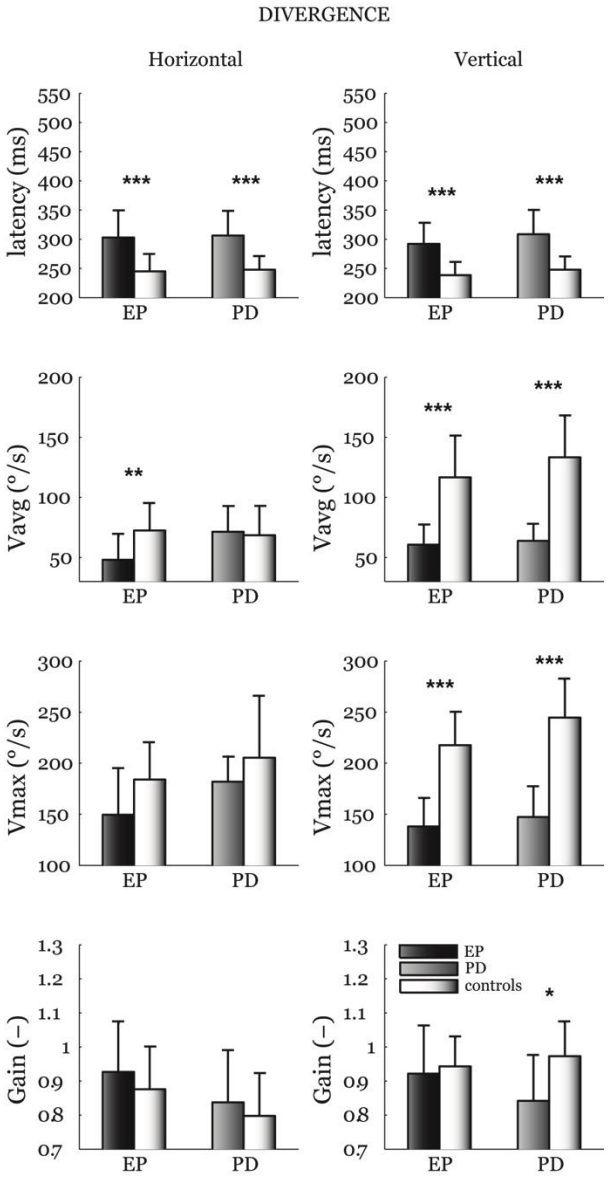

Figure 8 shows the results for divergence. Both PD and EP patient groups showed longer SRT when compared to controls ($p < 0.001$). While both groups manifested slower velocities (Vavg and Vmax) for vertical saccades ($p < 0.001$), velocity for horizontal was affected only for EP group (Vavg: $p = 0.0001$; Vmax: $p = 0.006$). Conversely increased skewness and decreased gain was found only in vertical saccades of PD patients (skewness: $p = 0.04$; gain: $p = 0.002$).

MSA and PSP patients

Table 3 shows the rate of patients with VEM metrics different from their control group. More than 50% of our MSA patients have longer SRT of both convergence and divergence. Additionally, the gain of VEM was lower and the velocity slower and skewed for divergence. Similar abnormalities were found in more than 50 % of our PSP patients. These abnormalities seem to be similar to those found in PD but not in EP patients.

					Number of affected patients			
		Controls		Condition	**MSA**		**PSP**	
		Mean	SD	(Mean±SD)	n/4	%	n/6	%
Convergence								
Horizontal	SRT	269,6	31,2	> 300	3	**75**	4	**67**
	Avg.Velocity	77,04	36,8	< 41	0	0	0	0
	Max.Velocity	200,1	45,8	< 154	0	0	2	33
	Skewness	0,35	0,11	> 0.46	1	25	4	**67**
	Gain	0,93	0,09	< 0.84	2	**50**	1	17
Vertical	SRT	247,7	23,5	> 271	2	**50**	1	17
	Avg.Velocity	68,39	24,5	< 44	0	0	0	0
	Max.Velocity	205,4	60,7	< 144	0	0	0	0
	Skewness	0,40	0,11	> 0.51	0	0	1	17
	Gain	0,8	0,13	< 0.67	1	25	0	0
Divergence								
Horizontal	SRT	263,6	30,5	> 295	3	**75**	5	**83**
	Avg.Velocity	76,08	14,6	< 61	0	0	0	0
	Max.Velocity	177,5	48,3	< 129	1	25	0	0
	Skewness	0,39	0,07	> 0.46	4	**100**	4	**67**
	Gain	1,025	0,05	< 0.98	2	**50**	1	17
Vertical	SRT	247,9	22,7	> 271	2	**50**	1	17
	Avg.Velocity	133,3	33,9	< 99	2	**50**	4	**67**
	Max.Velocity	244,6	36,9	< 208	2	**50**	3	**50**
	Skewness	0,39	0,09	> 0.49	1	25	4	**67**
	Gain	0,97	0,1	< 0.87	1	25	2	33

Table 3. Vergence eye movements in MSA and PSP compared with their control group: VM: Vergence movement; MSA: multisystem atrophy; PSP: progressive supranuclear palsy; SRT: saccade reaction time; Vavg: average velocity; Vmax: maximal velocity; SD: standard deviation; Condition: value over the mean ± SD from control group; n: number of patients completing the condition; %: rate of patients completing the condition. Values in bold represent > than 50% of affected subjects in their respective group.

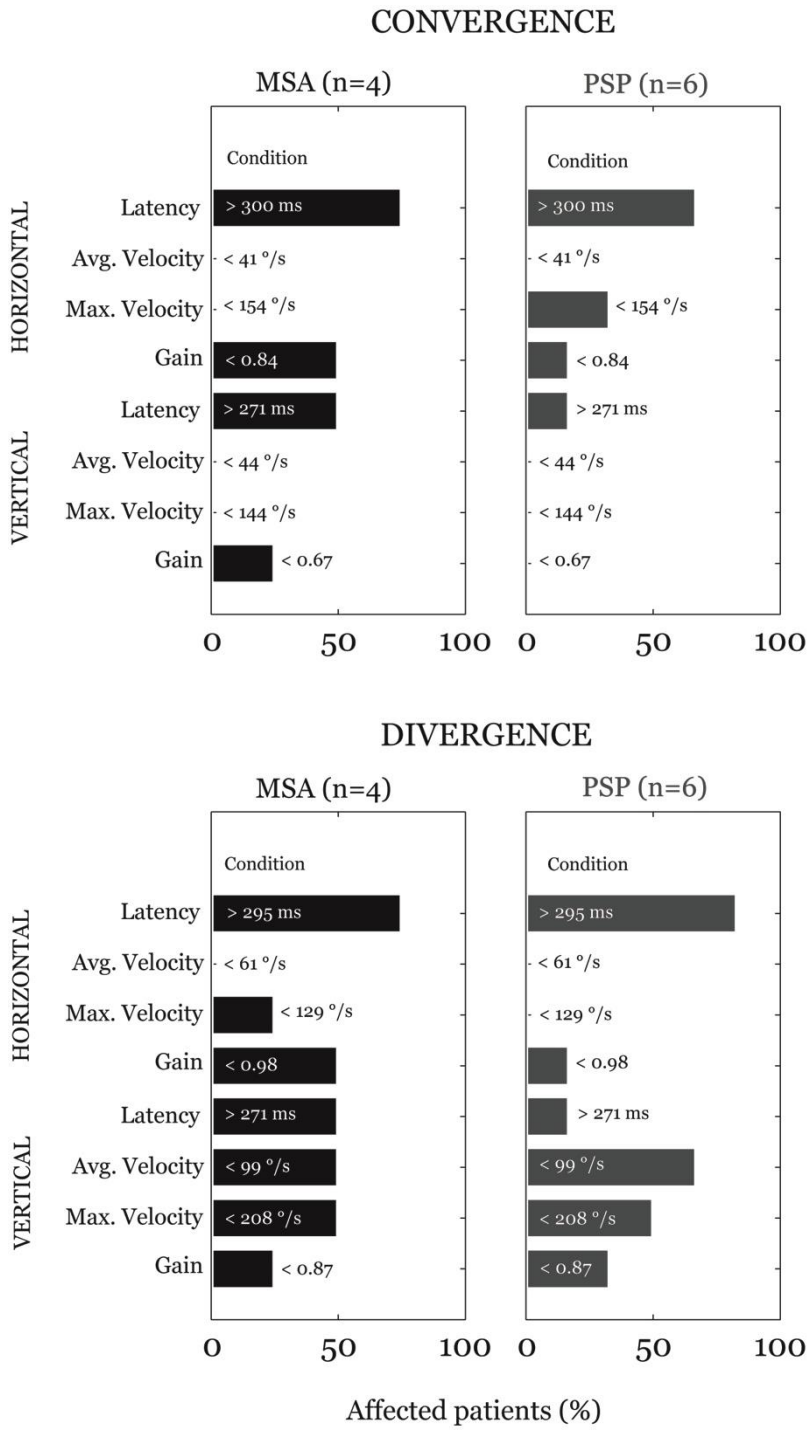

Figure 9 resumes the results: both MSA and PSP patients have longer latencies for convergence and divergence. Additionally MSA patients have lower gain of VEM, slower and skewed velocity of divergence. Similar abnormalities were found in the majority of our PSP patients.

Discussion

The present study shows that several VEM metrics were distorted in PD, MSA, PSP and EP and differently for convergence and divergence.

The SRT for convergence and divergence was longer in all patient groups. The SRT of VEM is mainly commanded by the frontal eye field (FEF),[62-64] the posterior parietal,[65] extra striate and primary visual cortices.[29, 66] Longer SRT in all patients reflect longer cortical processing time for target selection and decision making to initiate the movement and point to dysfunctional cortical processing in the before mentioned areas. In PD the degeneration of the dopamine neurons at the substantia nigra pars compacta and their projections to the striatum, result in disrupted functional connectivity at the thalamus, brainstem, cerebellum and cerebral cortices. According to the Braak staging schema of PD pathology, the pathological process initially occur at the dorsal nuclei thereafter, cortical areas gradually become affected,[67] and patients may show widespread cortical hypo metabolism specially at the frontal and parietal regions.[68, 69] In MSA the substantia nigra, striatum, locus coeruleus, pontine nuclei, inferior olives, cerebellum and spinal cord are predominantly affected by alpha-Synuclein inclusion pathology.[70] However a significant hypometabolism at the frontoparietal cortex of MSA patients has been shown with functional imagery.[71] Pathologically, PSP is defined by the accumulation of tau protein and neuropil threads, mainly in the basal ganglia, pontine tegmentum, oculomotor nucleus, medulla, and dentate nucleus.[72] Although several recently described clinicopathological variants of PSP point to different clinical features, due to different regions of pathology. These differences are mainly found at the cerebral cortex, pons, caudate, cerebellar dentate nucleus, and cerebellar white matter.[72] Manganese (Mn) induces neurotoxicity in EP and lesions in the frontal white matter and cortical structures have been reported in Mn overexposed non-human primates.[54, 73]

EP shows the largest abnormalities on VEM, despite their young age and short disease duration. They have very slow convergence and divergence. Ephedrone induced parkinsonism affects predominantly the pallidum and substantia nigra pars reticulata, but may damage also the brainstem and cerebellum.[74] Patients with PD MSA and PSP had decreased velocity of divergence not for convergence. The mesencephalic reticular formation, situated dorsolateral to the oculomotor nucleus,[75, 76] the medial longitudinal fasciculus (MLF)[62] and the nucleus reticularis tegmenti pontis (NRTP)[77] play an important role in influencing the velocity of

VEM. However according to our results it is probable that the control of velocity of convergence and divergence is segregated depending on the VEM. On the other hand, it is important to highlight that all our PD patients and some patients with PSP and MSA were treated with levodopa. Convergence insufficiency in PD has been described to improve with levodopa therapy,[78] and deep brain stimulation.[45] We cannot exclude that levodopa have improved at least in PD the oculomotor performances. Therefore, future studies investigating the VEM before and after therapy are required.

Parkinson´s disease, EP and MSA patients exhibit reduced gain of VEM, while this was not observed in PSP. The colliculus superior and cerebellum [79, 80] particularly cerebellar flocculus influence the vergence angle and the dorsal vermis ocular alignment with orbital position.[81] Increasing evidence suggests that the cerebellum may have certain roles in the pathophysiology of PD, and in MSA the systems most consistently and severely affected include the olivopontocerebellar (OPC) and striatonigral (StrN) systems.[81]

We have also found that almost all patients show skewed shape of the velocity profile of VEM. Skewness has been related to the amplitude [82-84] and duration [85, 86] of saccades and is known to be stable during the entire lifespan.[42] Several neurophysiological studies have suggested that the skewness of saccades is also related to the cerebellum.[87, 88] In PSP lesions at the superior cerebellar peduncle and cerebellar dentate nucleus in PSP, may explain the skewed velocity profiles found in these patients. Varlibas has additionally demonstrated that chronic ephedrone abusers may have bilateral symmetric hyper-intensities on T1-weighted-MRI images at the dentate nucleus and cerebellar hemispheres.[89]

Our study proposes several changes in VEM in patients with Parkinsonism, and open new ways for further research.

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Short communication

Fast vergence eye movements are disrupted in Parkinson's disease: A video-oculography study

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ABSTRACT

Background: Blurred near vision is a common non-motor symptom in patients with Parkinson's disease (PD), however detailed characterization of vergence eye movements (VEM) is lacking.
Methods: Convergence and divergence were examined in 18 patients with PD and 18 control subjects using infrared video-oculography. VEM metrics analyzed included latency, velocity and accuracy, in vertical and horizontal planes.
Results: The latency of convergence and divergence was significantly increased in PD subjects. Additionally, divergence was slow and hypometric, while other convergence metrics were similar to controls.
Conclusion: We provide evidence in favor of disrupted VEM in PD.

1. Introduction

Vergence eye movements (VEM) are disjunctive eye movements necessary for reading and tracking objects moving in depth, maintaining a fused and singular percept [1]. They consist of convergent and divergent movements that may be divided into fast vergence (to step targets) and slow vergence (to track sinusoidal targets).

Patients with Parkinson's disease (PD) sometimes complain about blurred near vision, which may be related to convergence deficits. Diplopia is one of the non-motor signs found in approximately 20% of PD patients and may be caused by convergence insufficiency [2]. Visual discomfort in PD has been studied with several ophthalmological devices and questionaries revealing decreased convergence amplitude, convergence insufficiency, heterophoria and divergent strabism [3]. Video-based infrared eye tracking is an objective method increasingly used in clinical neurology for oculomotor testing in the diagnosis of some neurodegenerative (e.g., parkinsonian syndromes), hereditary or metabolic disorders [4].

The aim of the present study was thus to describe fast VEM in PD using video-oculography.

2. Materials and methods

2.1. Subjects

Patients and healthy controls were examined at the Department of Neurology, First Faculty of Medicine, Charles University in Prague. All participants provided signed, informed consent. The study was approved by the local ethics committee and was in compliance with the Declaration of Helsinki. Eighteen PD patients (8 female, 10 males; age 40–71 (mean 53.4, SD 10.2) years diagnosed according to UK Parkinson's Disease Society Brain Bank criteria and followed at the movement disorders clinic were included. PD duration ranged from 1 to 21 (mean 9.3, SD 5.6) years, with UPDRS III score 8–47 (mean 25.2, SD 10.8) and Hoehn & Yahr [5] score 1–3 (mean 1.9, SD 0.6). Sixteen of eighteen patients were treated with dopamine agonists (10 ropinirol, 6 pramipexol), 10 of which were treated in combination with levodopa, while 1 patient received levodopa monotherapy and one patient was

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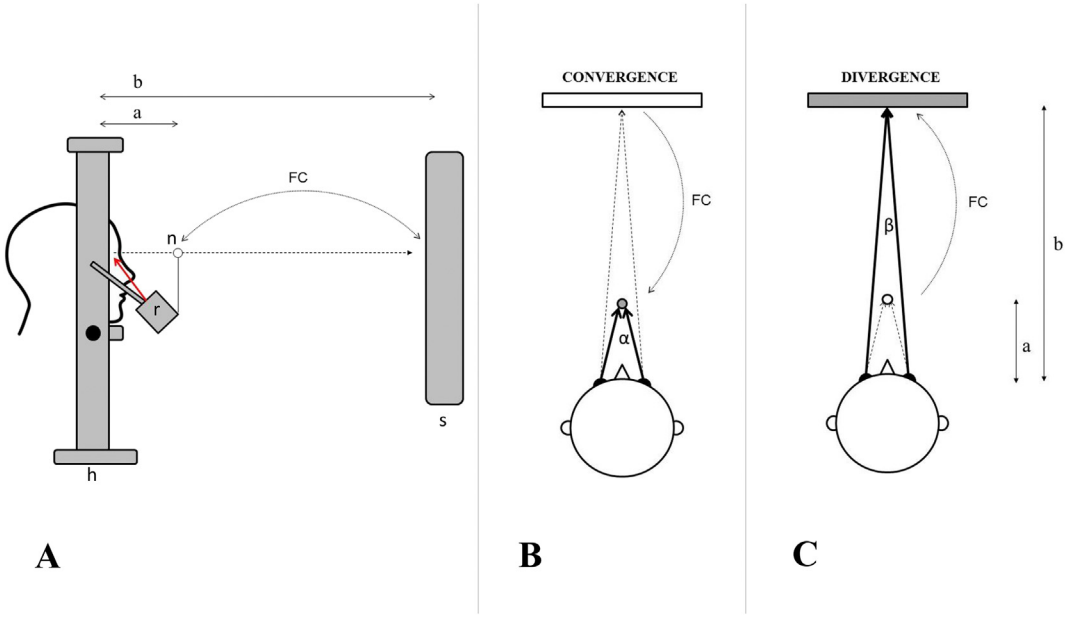

Fig. 1. Eye movement task employed. A: patient position; B: examination of convergence; C: examination of divergence; h: head support construction; r: eye movement recorder; n: near fixation point; s: screen; a = 10 cm; b = 60 cm; $\alpha = 43.6°$; $\beta = 7.6°$; FC: focus change.

untreated at the time of examination. The levodopa equivalent daily dose was mean 822, range 0–2890 mg [6]. All medicated patients were examined in the "on" condition, following a regular dose of their dopaminergic medication. Exclusion criteria were cognitive impairment with an MMSE <26 points, any other neuropsychiatric or eye disease, or a history of brain surgery including deep brain stimulation. In addition, 18 volunteers (10 men, 8 women; age 31–72 (mean 53.9, SD 11.6) years) with no history of neurological or eye disease and not using medication affecting central nervous system were included as healthy controls.

2.2. Experimental paradigm

Subjects were seated in a calm, dark room with their chin resting on a chin strap and their forehead placed against a frontal support and with a screen located 60 cm in front of their eyes. Eye movements were initially calibrated following the illumination of 16 consecutive targets covering the entire visual field. Both patients and controls were examined with the same device and the same paradigm. The trial began with the appearance of a distant fixation point (25 × 25 pixels oval, luminance 240 cd/m^2), located in the middle of the screen, 60 cm from the subject's eyes. The near fixation point was a white plastic ball 1 cm in diameter, positioned 10 cm in front of the subject's eyes. The subject was instructed to gaze at the distant fixation point until its extinction (go signal 1), then to change gaze focus as quickly as possible to the near point and continue looking at it until the distant point lights up again (go signal 2). The timing between each go signal was fixed, regular and periodic, characterized by turning on and off of the distant target for 2000 ms Fig. 1(B,C). One trial consisted of 6 divergent and 6 convergent movements. Three trials were performed in each subject, with a total of 18 convergent and 18 divergent fast VEM within 10 min. Every subject was asked to report double vision or any other problem with near vision. During the examination, all subjects were able to clearly see both fixation points without visual discomfort.

2.3. Recording apparatus and vergence metrics

Eye movements were recorded with a video-based binocular pupil tracker (mobile eBT Eyebrain, Ivry-sur-Seine, France), with an acquisition frequency of 300 Hz, and precision of 0.5° horizontally and 0.5° vertically. In analysis of vergence metrics, preference was given to the left eye. VEM are composed of horizontal, vertical and cyclovergent components [7]. As our pupil tracker allowed us to analyze only horizontal and vertical movement components, we chose to describe both. We defined the following parameters for all experimental conditions: latency *(ms)*, gain (−), and velocity *(°/sec)* divided into average (Vavg) and maximal velocity (Vmax). Latency was defined as the reaction time from the divergent target onset/offset to the beginning of the VEM. Latencies below 80 ms were considered premature anticipatory movements and were rejected. VEM containing directional errors were discarded as well. Gain was defined as the ratio between VEM amplitude and target location.

2.4. Statistical analysis

Statistical analyses were performed in Matlab© (Mathworks, Massachusetts, USA). First, the average of all saccades for each metric was calculated for each subject. Subsequently, a *t* test for independent samples was used for intergroup comparison. The Bonferroni adjustment was applied to correct for the number of tests performed according to each paradigm (convergence, divergence). The Pearson correlation was applied to evaluate the relation between vergence metrics and the UPDRS III. The level of significance after adjustment was set at $p < 0.05$.

3. Results

3.1. Convergence

in comparison to controls, latency was significantly increased in the PD group in the horizontal ($t(34) = 3.9$, $p = 0.003$) and vertical ($t(34) = 4.3$, $p < 0.001$) planes. There were no significant differences between the PD and control groups for velocity and gain (Fig. 2).

3.2. Divergence

increased latencies were found in PD patients in comparison to controls in both planes ($t(34) = 5.3$, $p < 0.001$). In addition, PD patients showed slower velocities Vavg ($t(34) = -6.7$, $p < 0.001$) and Vmax ($t(34) = -6.8$, $p < 0.001$), but only in the vertical plane. Furthermore, decreased gain in PD patients was also found only in the vertical plane ($t(34) = -3.3$, $p = 0.02$) (Fig. 2).

No significant correlations were seen between VEM metrics and the UPDRS III, disease duration or levodopa equivalent daily dose.

4. Discussion

In the present study, we describe distorted VEM metrics in PD using video-oculography. One of the more significant results of this study concerns the prolongation of latencies for convergence and divergence. The latency of VEM reflects the function of several areas of the brain including the frontal eye field (FEF), the posterior parietal, extrastriate and primary visual cortices [7]. Previous studies in PD patients have demonstrated widespread

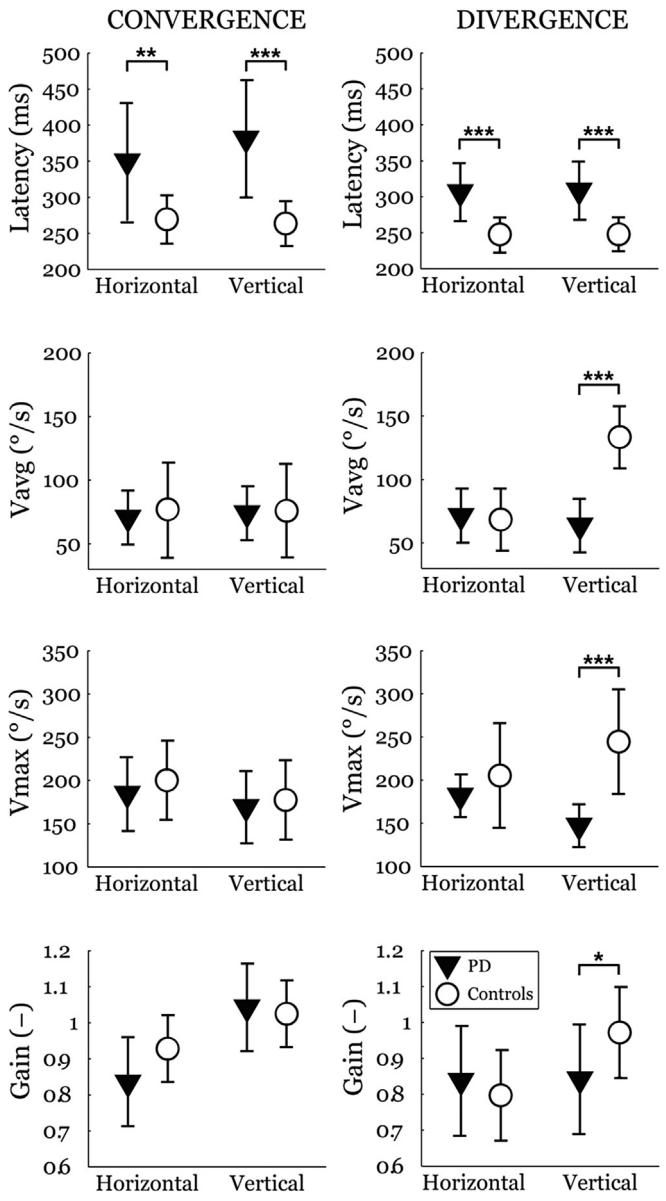

Fig. 2. Latencies, average velocities (Vavg), maximal velocities (Vmax), and gains for convergence and divergence. Comparison between PD and healthy control groups after Bonferroni adjustment: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

cortical hypometabolism and disrupted sensorimotor connectivity in these areas, especially in the frontal and parietal regions [8,9].

Surprisingly, the velocity and gain of convergence were similar to controls, whereas PD patients showed slower velocities and slightly lower gain for divergence. The mesencephalic reticular formation, situated dorsolateral to the oculomotor nucleus, the medial longitudinal fasciculus (MLF) and the nucleus reticularis tegmenti pontis (NRTP) play an important role in influencing the velocity of VEM. Our findings are in agreement with previous observations that velocities of convergent and divergent VEM in primates may be under separate neural control, with convergence burst cells in another more dorsal mesencephalic region, rostral to the superior colliculus [10].

One admitted limitation of our study is that patients were investigated under medication. Previous studies have shown that both dopaminergic and extradopaminergic mechanisms may affect eye movements [11]. In particular, convergence insufficiency in PD has been described to improve with levodopa therapy [12], and deep brain stimulation [3]. However, the present results demonstrate VEM abnormalities in PD patients, despite the fact that dopaminergic treatment may have normalized eye-movement dysfunction related to dopamine deficit.

In summary, we show that VEM may be used in the assessment of PD in clinical practice using a simple paradigm. We found that PD patients have longer latencies in all VEM as well as slower and hypometric divergence, even in the absence of complaints of visual discomfort in near vision. We believe that further studies of VEM may provide further insight into the pathophysiology of PD, particularly in regard to possible involvement of the upper brainstem. Further studies on early-stage and presymptomatic PD subjects are needed to determine if VEM disturbance can serve as a biomarker of the disease.

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2.4 An early diagnostic marker of Parkinson's disease

Impairment of ocular saccades as possible early sign of neurodegeneration in REM sleep behaviour disorder

Cecilia Bonnet, Bertrand Gaymard, Jan Rusz, Lenka Plchová, Tomáš Sieger, Jitka Bušková, Jaromír Hanuška, Sophie Rivaud-Péchoux, Evžen Růžička and Karel Šonka. (unpublished)

Background

Rapid eye movement (REM) sleep behavior disorder (RBD) is a parasomnia and movement disorder characterized by violent movements and behaviours during REM sleep. Among the neurodegenerative diseases increasing evidence show that 50-80% of patients with idiopathic RBD convert to synucleopathies specially Parkinson's disease (PD). We aimed to asses saccades in a group of patients diagnosed as idiopathic RBD (iRBD). Thirteen subjects aged 47-83 (mean 64.5) years, 10 males and 3 females, diagnosed as iRBD using clinical and polysomnographic criteria, were prospectively enrolled. In addition, they were assessed with UPDRS part III and Montreal Cognitive Assessment (MOCA) test. Then patients were divided in two groups, one group with PD signs and another without. The two patient groups were compared to 14 matched healthy subjects. Horizontal and vertical pro and antisaccades were examined with infrared videooculography. We obtained *(*i) iRBD: Five Patients with RBD without parkinsonian signs, or idiopathic RBD. These patients had similar eye movements as controls. (ii) Eight RBD PD: composed by patients with possible PD, diagnosed accordingly to the UK Brain Bank criteria. Significant differences were found in latency for vertical saccades (χ^2 = 9.0, p < 0.01) and increased errors for horizontal antisaccades (χ^2 = 5.5, p < 0.05) when compared to controls. Both metrics were correlated to bad performance on MOCA test.

We conclude that saccades are normal in patients with iRBD however in asymptomatic patients with possible PD they are disrupted. Eye movement abnormalities could be considered as an additional early diagnostic marker of PD.

Introduction

Rapid eye movement (REM) sleep behaviour disorder (RBD) is a parasomnia and movement disorder characterized by violent movements and behaviours during REM sleep.[90, 91] The main suspected mechanism of RBD is a lesion of the REM sleep atonic system, located at the pontomedullary brain stem.[92] If RBD is not associated with neurological disorders, it is termed idiopathic (iRBD). Among the neurodegenerative diseases increasing evidence show that 50-80% of patients with idiopathic RBD convert to synucleopathies specially Parkinson's disease (PD), but also to dementia with Lewy bodies (DLB) [93] and multiple system atrophy (MSA).[91] Consequently, RBD have been considered to be a sensitive prodromal marker for PD, which can appear up to 5-15 years before disease onset.[94, 95]

The study of the dynamic properties of eye movements provides an interesting and non-invasive tool to understand brain function. In the last decades, it has been possible to identify several, distinct brain areas involved in control of eye movements, from the brainstem and cerebellum to the cortex.[29] Brainstem structures are involved in the motor control of saccades mainly the pons for horizontal and midbrain for vertical saccades. Cortical areas have been related to the cognitive control of eye movements (e.g., visuospatial attention, decision making or inhibition).

We raised the hypothesis that accordingly to the rostrocaudal progression theory of the synucleopathy in PD proposed by Braak,[67], patients with RBD who will probably develop a PD should present some eye movement abnormalities. We aimed to characterize eye movements in a series of iRBD patients, in order to identify sub-clinical dysfunction of meso-pontine structures.

Methods

Subjects

Thirteen patients, age range 47-83 (mean 64. ± SD 9.4 years), 10 males and 3 females, with suspicion of iRBD were enrolled. Inclusion criteria were age > 18, anamnestic and

polysomnographic confirmed RBD, absence of other neurological or psychiatric illness. Daytime sleepiness was evaluated through the Epworth Sleepiness Scale and cognitive impairment with Montreal Cognitive Assessment (MoCA) assessment battery. Therapy and MRI results were taken from the medical charts of the patients. None of the patients complained about other symptoms than sleep disturbances. All were drug naïve and asymptomatic, except Pat 2 who suffered from a symptomatic epilepsy after stroke (see Table 5).

A movement disorder specialist performed clinical evaluation with the part III of the MDS-UPDRS.[58] All patients were asymptomatic for parkinsonian symptoms. Possible Parkinson's disease (PD) was diagnosed according to the UK Parkinson's disease society brain bank clinical criteria.[96] None of them had received a diagnosis of PD before and none of them where treated with levodopa.

Fourteen healthy controls (11 Males and 3 Females), age range 50 – 80 years (mean 63.2 ± 7.9 years), free of any neurological or psychiatric illness and denying the intake of any medication acting on the central nervous system, were enrolled.

All participants provided signed, informed consent. The study was approved by the local ethics committee and was in compliance with the Declaration of Helsinki.

Recording, apparatus, experimental paradigm and analysis of data

Saccades were recorded with the binocular video-based eye tracker (mobile eBT Eyebrain, Ivry-sur-Seine, France, www.eye-brain.com, 300 Hz sampling rate and 0.5° spatial resolution) using a standardized protocol. Three different task were performed in the same order, in one session lasting for 30-min: i) Prosaccades horizontal and vertical GAP 13°; ii) Single antisaccades horizontal and vertical; iii) Mixed horizontal and vertical antisaccades. Subjects were facing a screen located 60 cm before their eyes, with their chin on a chinstrap. A green central fixation point (15 x 15 pixels; luminance: 120 cd/m2) was presented for a pseudorandom duration.

i) Single Prosaccades horizontal and vertical: The fixation point was turned off and 200 ms later (gap), a red peripheral target (15 x 15 square, luminance 120 cd/m²) appeared during 1000 ms at 11,86°, in a random order right or left, up or down. Twenty-eight saccades

were recorded. Latency (or saccade reaction time SRT), velocity and gain were analysed. Mean values were obtained for each subject for each side and direction.

ii) Simple antisaccades horizontal and vertical: In this task the colour of the central fixation point was red. Target locations were presented in a random order at 11,86° in the horizontal and vertical direction. Subjects were instructed to look as fast as possible in the direction opposite to the peripheral target. Thirty-two saccades were recorded. Latency, error rate and rate of corrected errors were extracted for each direction and then for each subject. Saccades with a latency below 80 ms or above 1000 ms, or an amplitude below 1° were rejected, but this represented <1% of all trials. Mean latency was determined only for correct antisaccades. Directional errors were defined as saccades initially directed towards the hemi field away from the target following a prosaccade instruction, or towards the target following an antisaccade instruction.

iii) Mixed task of horizontal and vertical antisaccades: The task design was the same as in the simple antisaccade task, with the exception that targets were presented in a random order, 8 times in the horizontal and 8 times in the vertical direction.

Statistical analysis

For further analysis, all values were separately averaged for each participant across individual eye movement metrics. All metrics were first compared across all three groups using a Kruskal-Wallis test. Significant results were further analysed by multiple comparisons using a Mann-Whitney test. The significance level was set as $p < 0.05$.

Results

Two groups of patients were identified after clinical examination: (i) iRBD composed by 5 patients, free from any parkinsonian sign; (ii) RBD with possible PD (RBD PD) composed by 8 patients. Table 4 resumes the principal clinical characteristics of both groups.

Pat.Nr.	Age	Gender	DD	Epworth	MRI	Treatment	MoCA	MDS UPDRS III
	years	M/F	years	/24		mg	/30	/52
RBD Idiopathic								
1	66	M	4	12	ND	0	27	2
2	63	F	1	12	ND	Valpr. Lamotrigine	26	0
3	53	M	7	13	Leucariosis	0	29	9
4	58	M	3	8	ND	0	29	2
5	59	M	4	10	Leucariosis	0	27	0
	60	4M/1F	*6*	*11*		*0*	*27,6*	*2.66*
RDB Park								
1	84	M	34	11	ND	0	25	19
2	69	M	8	5	ND	Clonazepam	20	9
3	75	M	8	12	ND	0	ND	11
4	48	M	3	13	normal	0	28	2
5	64	F	4	7	Leucariosis	0	26	10
6	72	F	13	6	ND	0	ND	3
7	65	M	11	3	Leucariosis	Clonazepam	ND	7
8	61	M	9	6	Leucariosis	Clonazepam	26	8
	67	6M/2F	*11*	*8*			*25*	*9.42*

Table 4: Clinical characteristics of patients and abnormal eye movement metrics. Pat.Nr.: Patient number; DD: RBD duration; MRI: Magnetic resonance imagery;

Statistically significant differences between the two patient groups and controls was found only in latency for vertical saccades ($\chi^2 = 9.0$, $p = 0.01$) which was associated mainly with increased latencies for RBD PD patients when compared to controls ($p < 0.01$). Furthermore, the increased errors for horizontal antisaccades ($\chi^2 = 5.5$, $p < 0.05$) associated with increased errors in RBD PD patients ($p < 0.05$) was also significant (Figure 10). We have found no differences between groups for other metrics.

Considering performances of individual patients, six of eight RBD PD patients (75%) showed longer latencies than 220 ms for vertical prosaccades whereas such phenomenon was observed only for one iRBD patient, (pat. iRBD 2) and none of control subjects. Taking into account error metrics, 5 RBD PD patients (63%) showed increased error rate for horizontal antisaccades. In the iRBD only 2 patients (pat. iRBD 1 and 2) (40%) presented a high error rate for horizontal antisaccades.

Eye movement metrics did not correlate to disease duration, nor to the MDS UPDRS III score. However we found a negative correlation between latency for vertical prosacades and MOCA score (r = - 0.85, p = 0.002) as well as positive correlation between the high error rate on antisaccades and MOCA score (r = -0.67, $p < 0.05$).

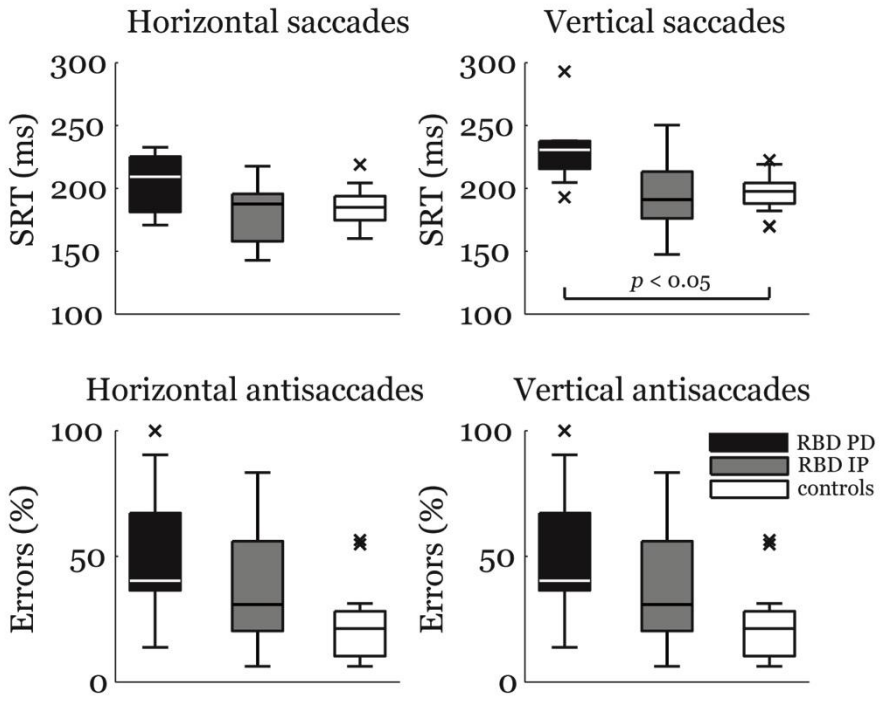

Figure 10: Horizontal and vertical saccades in RBD patient's compared to controls.

Discussion

This is the first study investigating eye movement performance in patients with RBD. We show that eye movements in patients with iRBD did not differ from controls, while

patients with possible RBD PD present long latencies for vertical prosaccades and a high error rate in the antisaccade task.

Eye movements in PD have been well described, even if some results are controversial. Horizontal prosaccades use to be normal,[97, 98] but vertical prosaccades may be hypometric.[97-100] The error rate has been described as normal,[63, 80, 101-106] or increased [107-110] depending of the stage of disease progression. Interestingly, in our Lab, we have found in 20 PD patients, similar to RBD PD patients, long latencies for vertical saccades and a high error rate (Bonnet unpublished data). Moreover Kapoula has shown that patients with dementia with Lewy Bodies may have long latencies of vertical saccades.[111] We suggest that the saccade abnormalities in patients with PD and perhaps also patients with other synucleiopathies, begin in early, sometimes asymptomatic stages of the disease.

The SRT of vertical prosaccades have been related in humans to bilateral activation of parietal and frontal cortices,[29, 112] and the control of antisaccades to the dorsolateral prefrontal cortex (DLPFC).[113, 114] Recent studies in non-human primates relieve the importance of other subcortical structures in the control of both metrics such as the nucleus reticularis tegmenti pontis in the control of the latency of vertical saccades,[115] and the globus pallidus in the control of antisaccades.[116]

We observed that the eye movement abnormalities present in RBD PD patients were correlated with bad performance in the MOCA test. Cognitive impairment has been described in 30% to 40% of PD patients. Moreover PD patients with cognitive impairment and RBD are affected in similar brainstem structures and their ascending projections to the cerebral cortex, probably preceding involvement of the neocortex.[117]

Limitations of this study were the low number of patients included and the fact that the diagnosis of possible PD was made by one movement disorders expert in one session. Future larger longitudinal studies of eye movements in patients with RBD are necessary. We believe that saccades may be associated to other early markers of PD as olfaction, RBD, autonomic symptoms, depression, constipation, visual abnormalities and cognitive impairment and presynaptic dopamine depletion.[95]

2.5 A physiological approach, investigating scanning eye movements in PD with microelectrode recording

Basal Ganglia Neuronal Activity during Scanning Eye Movements in Parkinson's Disease. *Tomáš Sieger, Cecilia Bonnet, Tereza Serranová, Jiří Wild, Daniel Novák, Filip Růžička, Dušan Urgošík, Evžen Růžička, Bertrand Gaymard, Robert Jech*. Plos One. 2013 Nov 6;8(11):e78581.*[118]

Stereotactic, microelectrode-guided implantation of deep brain stimulation (DBS) electrodes at the globus pallidus internus (GPi) and the subthalamic nucleus (STN) is part of the routine surgical treatment in Parkinson's disease (PD), when the medication fails and treatment complications appear. During the implantation of the stimulator intraoperative microelectrode single unit recordings (MER) are used to identify the basal ganglia structures based on their electrophysiological response, to localize the target for DBS electrode implantation,[119] and offers researchers a unique opportunity to investigate brain behavior related to single-unit responses.

The pattern of EM carried out while exploring an image, also called scanning EM, is composed of a succession of saccades and fixations, and results from successive re-allocation of attention from one detail to another.[120, 121] Scanning EM involve planning, visuospatial attention, and spatial working memory.[122]

Saccades made during scanning EM can be considered as internally triggered, as the subject moves the gaze around a complex visual image actively searching for information relevant to current motivations and goals.

The basal ganglia are considered to drive voluntary movements, and their role in the control of eye movements has been supported by extensive evidence derived from

experimental studies in monkeys,[123-125] and in humans through clinical [105, 126-128] and electrophysiological studies.[128, 129] Although most of them have focused on behaviour such as reflexive visually guided, memory guided or predictive saccades and their role in scanning EM have received little attention and is, to our knowledge, not known. One study in humans investigating regional cerebral blood flow (rCBF) using N-isopropyl-p-[123I] iodoamphetamine (123I-IMP) and single photon emission computer tomography in healthy and schizophrenic patients with altered scanning EM, suggest an involvement of the superior frontal area and left basal ganglia in this kind of EM.[130]

The main goal of our study was to determine whether basal ganglia are involved in SEM through simultaneous intraoperative microelectrode recordings in a homogeneous group of PD patients undergoing implantation of deep brain stimulation electrodes.

We identified for the first time a significant number of neurons related to scanning eye movements in human STN, GP and SNr from awake and alert PD patients. A major finding is that neurons of the STN related to SEM were to a great extent, not related to reflexive prosaccades and vice versa. This allowed us to strongly support the hypothesis of a functional and anatomical segregation between internally and externally generated EM, as it has been suggested at different cortical and subcortical levels over the past years.

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Basal Ganglia Neuronal Activity during Scanning Eye Movements in Parkinson's Disease

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Abstract

The oculomotor role of the basal ganglia has been supported by extensive evidence, although their role in scanning eye movements is poorly understood. Nineteen Parkinsons disease patients, which underwent implantation of deep brain stimulation electrodes, were investigated with simultaneous intraoperative microelectrode recordings and single channel electrooculography in a scanning eye movement task by viewing a series of colored pictures selected from the International Affective Picture System. Four patients additionally underwent a visually guided saccade task. Microelectrode recordings were analyzed selectively from the subthalamic nucleus, substantia nigra pars reticulata and from the globus pallidus by the WaveClus program which allowed for detection and sorting of individual neurons. The relationship between neuronal firing rate and eye movements was studied by crosscorrelation analysis. Out of 183 neurons that were detected, 130 were found in the subthalamic nucleus, 30 in the substantia nigra and 23 in the globus pallidus. Twenty percent of the neurons in each of these structures showed eye movement-related activity. Neurons related to scanning eye movements were mostly unrelated to the visually guided saccades. We conclude that a relatively large number of basal ganglia neurons are involved in eye motion control. Surprisingly, neurons related to scanning eye movements differed from neurons activated during saccades suggesting functional specialization and segregation of both systems for eye movement control.

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Introduction

In everyday life we scan the environment with a series of eye movements, pointing the fovea towards objects of interest and the most salient areas of the scene. The pattern of such eye movements (EM) carried out while exploring an image, also called scanning EM, is composed of a succession of small saccades and fixations, corresponding to successive re-allocation of attention from one detail to another [1,2]. Therefore, scanning EM can be considered as internally triggered EM, as the subject moves the gaze around a complex visual image actively searching for information relevant to current motivations and goals. The visual scanpath is generated by complex parallel strategies [3] and depends on planning, visuospatial attention, spatial working memory and emotional state [4,5]. Scanning EM have mostly been the domain of psychiatric research which has focused on the behavioral aspects of the eye scanning path rather than to pathophysiological origin and scanning EM control [6].

The structures and mechanisms involved in scanning EM are still poorly understood. At the subcortical level, an involvement of the basal ganglia during scanning EM was suggested by early research using regional cerebral blood flow in healthy controls and schizophrenic patients [7]. The importance of the basal ganglia in EM control was further confirmed by animal studies [8,9,10,11,12], which discovered neurons co-activated during EM by single cell recordings in several regions of the basal ganglia and brainstem [9,11,13]. However, subcortical neuronal activity during scanning EM is still unknown and has never been studied in animals or in humans before. Several human studies supported the participation of the basal ganglia in EM control but just with results based on reflexive and voluntary saccades analyzed from oculographic recordings [14,15,16,17,18,19] or local field potentials [20]. The only evidence of human EM-related neurons was obtained from the subthalamic nucleus during saccade tasks and smooth pursuit movements in patients with Parkinson's disease [21].

In our study, we systematically searched for basal ganglia neurons participating in scanning EM. We took advantage of intraoperative microelectrode recordings of single neuronal activity routinely used to identify the basal ganglia based on specific electrophysiological pattern [22]. We have focused on the subthalamic nucleus (STN), substantia nigra pars reticulata (SNr) and globus pallidus (GP) – i.e. nuclei in which EM-related activity was previously reported [11,13] and which are easily accessible

during the implantation procedure for deep brain stimulation in Parkinsons disease (PD).

Besides EM-related neurons firing selectively when a specific position, velocity or acceleration of the eyeballs is reached, we expected to find less specialized neurons with activity depending on two or more kinematic features simultaneously. This comes from the hypothesis of functional overlap based on neuronal convergence along the striato-pallido-thalamic projection and assuming compression of information when travelling from larger to smaller nuclei [23]. Findings of STN neurons showing co-activation during various eye movement tasks are in agreement with this theory [9,21]. On the other hand, there is a segregation hypothesis which expects different neuronal populations to selectively respond to specific kinematic parameters or to fire only during a specific kind of the EM. Indeed, functional and anatomical segregation between various EM tasks has been previously observed at different levels involving the cortex, basal ganglia or cerebellum [4,8,24]. Therefore, in a subgroup of patients, we additionally studied the basal ganglia neurons during externally triggered EM using a visually guided saccade task. To further elucidate the function of neurons related to EM, we explored temporal relations of EM kinematic parameters with respect to their preceding and following activity, which may suggest their involvement in execution or control processes.

Methods

Ethics statement

The study was approved by the Ethics Committee of the General University Hospital in Prague, Czech Republic and was conducted according to the Declaration of Helsinki.

Patients

Nineteen PD patients were enrolled consecutively from 2008 to 2011 (15 men, 4 women; mean age: 54.5, SD 9.8, range 28–69 years; mean PD duration: 13.8, SD 6.1, range 3–30 years; Hoehn-Yahr stage 2-4; mean motor score of the Unified Parkinsons Disease Rating scale – UPDRS III in OFF condition: mean 36.5, SD 13.6, range 10–65). All of them were suffering from motor fluctuations and/or disabling dyskinesias (demographic details in Table 1) and were indicated for treatment with deep brain stimulation due to motor fluctuations and dyskinesias. All of them met the UK Brain Bank Criteria for diagnosis of PD [25] and all gave their written informed consent for participation. Patients with dementia and/or depression had been excluded by a routine psychiatric examination and neuropsychological testing (Mini-mental state examination, Mattis dementia rating scale, Beck depression inventory). As a normal cognitive state was requested to fulfill the general indication criteria for implantation surgery, all patients understood the nature of the experiment. They had been informed that procedures related exclusively for study purposes could be skipped if desired. It had been emphasized that they were allowed to forego the experiment at any time before or during the surgery. Four days before surgery, dopamine agonists were substituted by equivalent doses of levodopa. Other anti-PD medication (amantadine, anticholinergics) was suspended earlier for the surgery preparation. Levodopa was withdrawn at least 12 hours before the surgery.

Surgery and intraoperative microrecording

Implantation of the deep brain stimulation system was performed separately in two steps: (i) stereotactic insertion of the permanent quadripolar electrode into the STN bilaterally and (ii) implantation of connection leads and the neurostimulator to the subclavial region. The Leksell frame and SurgiPlan software system (Elekta, Stockholm, Sweden) were employed in the stereotactic procedure. Pre-surgical planning was based on 1.5 T MRI with direct visualization of the target. The central trajectory was intentionally focused on the STN center near the anterior part of the red nucleus (15 patients) or to the posteroventrolateral portion of the GP interna (4 patients). The first surgery was performed while awake under local anesthesia. The extracellular neuronal activity was mapped by conventional microelectrode recordings (MER) using parallel insertion of five tungsten microelectrodes spaced 2 mm apart in a ''Ben-gun'' configuration to select sites for the macroelectrode intraoperative stimulation [26,27]. Four out of five channels of the Leadpoint recording system (Medtronic, MN) were used for the MER, filtered with 500 Hz high pass filter and 5 kHz low pass filter, sampled at 24 kHz and stored for off-line processing. As the firing pattern of the external globus pallidus could not always be distinguished from the internal globus pallidus, we classified both areas as one structure – GP. Up to six recording positions in the STN, SNr or GP were used for the EM tasks in each patient. The number of positions depended on the time course of the surgery, patients' clinical conditions and compliance. Tasks were not performed if patients demonstrated discomfort from being in the supine position or exhibited painful symptoms relating to the off-medication state as well as increased fatigue or sleepiness during surgery. Immediately after the procedure, the position of each permanent electrode was verified by two orthogonal X-ray images co-registered with a presurgical MRI plan. No dislocation larger than 1 mm was found in any patient.

Eye movement recording

Eye movements during scanning and visually guided EM tasks were recorded using electrooculography (EOG), a technique measuring the position of the eye in terms of the electric potential induced by the eye dipole. Technical constraints during surgery (limited space around the stereotactic frame and a limited number of recording channels) did not allow for more elaborate recordings than the use of one single-channel EOG. The signal was band-pass filtered in the range of 0.1–20 Hz and recorded using the Leadpoint recording system simultaneously with MER acquisition through a pair of surface electrodes attached near the outer canthus and the lower lid of the left eye. This setup enabled the orthogonal projection of the eye position on the axis connecting the two EOG electrodes. All eye movements except those which were orthogonal to the axis could be recorded with this technique.

Tasks

The EM tasks were presented on a 17''-computer screen placed approximately 55 cm in front of the eyes of patients lying in supine position.

The scanning EM task. The goal of this task was to induce self-initiated free-direction scanning EM. The task consisted of a presentation of a series of photographs selected from the International Affective Picture System (IAPS, Figure 1A) [28], depicting objects, persons, animals and landscapes. To avoid showing the same picture more than once, six unique variants of the test, each containing 24 pictures, were prepared. Each picture was presented for a period of 2 s and was preceded by a black screen for various durations (3500–5500 ms) with a white cross in the center. Patients were asked to fix their eyes on the cross on the black screen and then to simply watch the pictures presented. The MER and EOG signals were acquired in 2 s epoch intervals recorded both during the picture presentation and the black screen. The task lasted approximately for 2.5 minutes.

Table 1. Description of patients with Parkinson's disease.

patient	Age [years]	DD [years]	levodopa [mg]	UPDRS III	H-Y	DBS target	task	neurons
1	64	14	1375	31	2.0	STN	SEM	12
2	61	14	1200	37	2.5	STN	SEM	7
3	46	15	1000	40	3.0	STN	SEM	15
4	63	30	1250	50	3.0	STN	SEM	3
5	53	12	700	37	2.5	STN	SEM	14
6	69	9	750	47	3.0	STN	SEM	5
7	49	12	1550	65	4.0	STN	SEM	7
8	59	12	600	30	2.5	STN	SEM	8
9	63	14	1350	21	2.0	GPi	SEM	4
10	53	10	750	42	4.0	GPi	SEM	11
11	53	11	1663	45	2.5	STN	SEM	5
12	57	26	2000	59	4.0	STN	SEM	12
13	28	3	720	10	2.0	GPi	SEM	6
14	64	17	1500	31	2.5	STN	SEM	12
15	53	12	1000	40	4.0	STN	SEM	9
16	44	10	1130	23	2.0	GPi	SEM, VGS	2
17	42	9	740	33	3.0	STN	SEM, VGS	20
18	55	19	1980	35	2.0	STN	SEM, VGS	16
19	60	14	1060	18	2.0	STN	SEM, VGS	15

Age – age on the day of surgery; STN – subthalamic nucleus; GPi – globus pallidus interna; DD – Parkinsons disease duration; Levodopa – dose/day in mg including levodopa equivalent dosage of dopamine agonist; patient 4 was also treated with mianserin; patients 6, 7, 8, 9, 10 with citalopram and 16 with bupropion; UPDRS III – motor score of the Unified Parkinsons Disease Rating Scale in OFF medication condition; H-Y – Hoehn and Yahr stage in OFF medication condition; DBS target – nucleus chosen for bilateral deep brain stimulation; SEM – scanning eye movement task; VGS – visually guided saccade task; neurons – number of neurons identified in the basal ganglia.

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The visually guided saccade task. The goal of the task was to induce externally generated horizontal saccades (Figure 1B). Initially, a black screen with a central white cross was shown for a pseudorandom period of 2, 2.25, or 2.5 seconds. Subsequently, a peripheral target, a small white square, was presented for 1 s, 17 degrees laterally from the central fixation cross, pseudorandomly to the left (5 trials) or right (5 trials). Patients were instructed to initially fixate on the central cross and then to track the lateral target as fast as possible. The MER and EOG signals of 2 s durations were recorded during all 10 trials. The task lasted for 32.5 seconds.

Data analysis

Microelectrode recordings. WaveClus [29], an unsupervised spike detection and sorting tool, which performed reasonably well on the single channel MER [30], was used to extract the series of action potentials of individual neurons from MER signals (Figure 2). Instantaneous firing rate (IFR) of each neuron was estimated by convolving the series of action potentials with the causal kernel function $\alpha^2*t*exp(-\alpha*t)$ defined for positive time t, where $1/\alpha$ was empirically set to 20 ms.

Each neuron was then mapped relative to the border of the STN, GPi and SNr identified by intraoperative MER. One-dimensional positions along the dorso-ventral microelectrode trajectory were determined using this technique (Figure S1).

EM recordings. EOG signals were rated manually and those contaminated with technical or major blinking artifacts, usually represented by large amplitude changes oversaturating the recording channel, were excluded from further analyses. As we presumed that neuronal activity could be related not only to the position of the eye, but also to its motion and the dynamics of the motion [21,31] we characterized EM by: i) the eye position (POS), defined by the EOG signal itself, ii) the eye velocity (VELOC), defined as the derivative of POS, and iii) the acceleration of the eye (ACCEL), defined as the derivative of VELOC. The derivative of the signal was defined in terms of the differences between successive samples in a low-pass filtered signal computed using a sliding rectangular window with the cutoff frequency of 12.5 Hz. The maximum and typical amplitude of the EM was extracted in each recording position in each task for each patient. While the maximum amplitude was defined as the extreme value in VELOC, the typical amplitude was defined as the median peak exceeding ± 1 SD of the VELOC.

To identify neurons whose activity was associated with EM, the relationships between IFR and POS, IFR and VELOC, and IFR and ACCEL were assessed. A neuron was considered connected to EM if its IFR was related to at least one of POS, VELOC, and ACCEL at the Bonferroni-corrected significance level of $p<0.05$. The relationships between IFR and the EM characteristics were analyzed using cross-correlation, which could reveal not only the link between concurrent IFR and EM, but also the link of IFR to preceding and following EM (Figure 3A-C). The maximal cross-correlation lag considered was ± 500 ms with steps of 2.5 ms. Biased estimates of correlation coefficients were computed to diminish uncertainty in estimates of correlation coefficients over longer lags. The cross-correlation coefficient between two signals was defined as the extreme correlation coefficient between the signals over all the lags considered. The lag in which the extreme cross-correlation was reached was called the *optimal EM-to-IFR cross-correlation lag*. The statistical significance of the cross-correla-

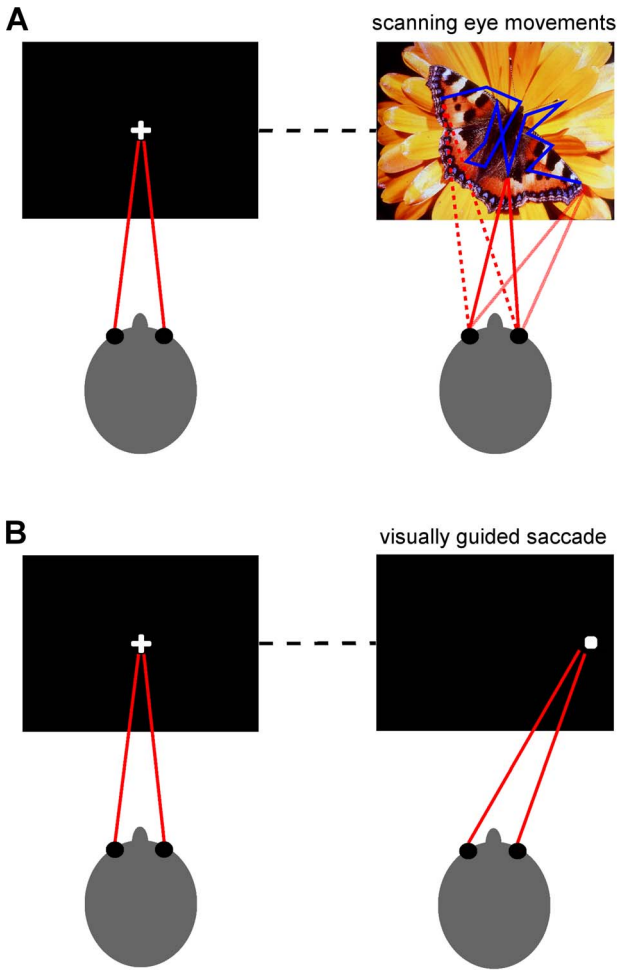

Figure 1. Eye movement (EM) tasks employed in the study. A - The scanning EM task. After the presentation of the black screen with a central cross, a photograph chosen from the International Affective Picture System was presented for 2 s. Patients were asked to initially fix their eyes on the cross (left picture) and then simply watch the photograph (right picture). In total, 24 pictures were consecutively used during the task. The blue line highlights a possible eye scanpath. **B - The visually guided saccade task** consisted of a presentation of 10 pairs of indifferent central (left picture) and lateral GO (right picture) targets positioned pseudorandomly on the left/right side of the screen. Patients were instructed to initially fixate the central cross and then track to the lateral targets as fast as possible.
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tion coefficient between two signals was assessed with Monte-Carlo simulations [32,33] using original and surrogate signals generated by randomly changing the phases of the spectral representation of the original signal.

The binomial test, Pearson's correlation coefficient test, Fisher exact test, two-sample proportion test, likelihood ratio test comparing Poisson regression models of dependence and independence in a 2-by-2-by-2 contingency table and paired t-test were used for statistical analysis. Data processing and analyses were performed in MATLAB (R2007b, The MathWorks, Natick, MA) and "R" software [34].

Results

We acquired 137 pairs of MER and EOG signals from 91 recording positions: 97 MERs were assigned to the STN, 21 to the GP and 19 to the SNr according to their firing pattern. In total, 183 neurons were detected using the spike sorting procedure, out of which 130 were located in the STN, 23 in the GP and 30 in the SNr (Table 2).

Neuronal activity related to scanning eye movements

Thirty seven (20%) out of 183 neurons identified in the basal ganglia during the scanning EM task were related to at least one of the EM kinematic parameters (POS, VELOC, ACCEL) (Table 3). Their proportion was higher than the expected false positive rate in each of the analyzed nuclei (binomial test, $p<0.001$): 26/130 neurons (20%) in the STN, 5/23 neurons (22%) in the GP and 6/30 neurons (20%) in the SNr. Locations of the EM-related neurons are depicted in the Figure S1. In the STN, the ratio of the EM-related neurons was higher in the ventral part (0 to 1 mm from the ventral STN border) compared to the rest of the nucleus (proportion test, $\chi^2 = 2.722$, df = 1, $P<0.05$).

The firing rate of the neurons relating to eye position (POS) significantly correlated with fluctuations of the EOG (Pearson's r = 0.89 (STN), 0.91 (GP), 0.86 (SNr); df = 18, $p<0.001$) (Figure 4). A relatively large number of neurons were related to more than one kinematic parameter (likelihood ratio test, D = 42.2 (STN), 19.8 (GP), 28.0 (SNr); df = 3, $p<0.001$).

As follows from cross-correlation analysis, the firing rate of the neurons was related either to concurrent, previous, or future EM (Figure 3). However, none of the nuclei predominantly contained any kind of the time-related neurons.

Neuronal activity related to visually guided saccades

There were 10/46 neurons (22%) whose activity was related to visually guided saccades in the STN, 1/2 of the neurons were in the GP and 2/5 were in the SNr. A description of neurons related to all EM kinematic parameters (POS, VELOC, ACCEL) is shown in Table 4.

Eye movements in the scanning and saccadic tasks

As both the scanning EM and visually guided saccades tasks were executed by only four patients, 19 relevant recording positions were analyzed. Neurons related to scanning EM were usually not activated in the visually guided saccades task and vice versa. Out of 46 STN neurons found in these patients, ten neurons related to scanning EM, ten neurons related to visually guided saccades and only two were activated during both tasks. These neuronal populations seemed to be independent in each of the two tasks as no evidence against the null hypothesis of independence was found (Fisher exact test, $p = 1.0$) although the test had enough power to reject the null hypothesis had the number of co-activated neurons been higher. In the GP and SNr, an insufficient number of neurons were detected for proper assessment of independence in neuronal activity between the two tasks. However, no GP or SNr neurons were co-activated during both tasks.

Descriptive analyses of the EM amplitude revealed that the maximal amplitude of the scanning EM and visually guided saccades were nearly identical. As requested by the visually guided task, patients executed large saccades, while small EM predominated in the scanning task where large EM occurred only rarely. The amplitude of the typical EM made during the visually guided saccades task was greater than during the scanning task (t = 5.7, df = 18, $p<0.001$). On average, the median saccade amplitude was 2.6 times larger in the visually guided task than in the scanning EM task.

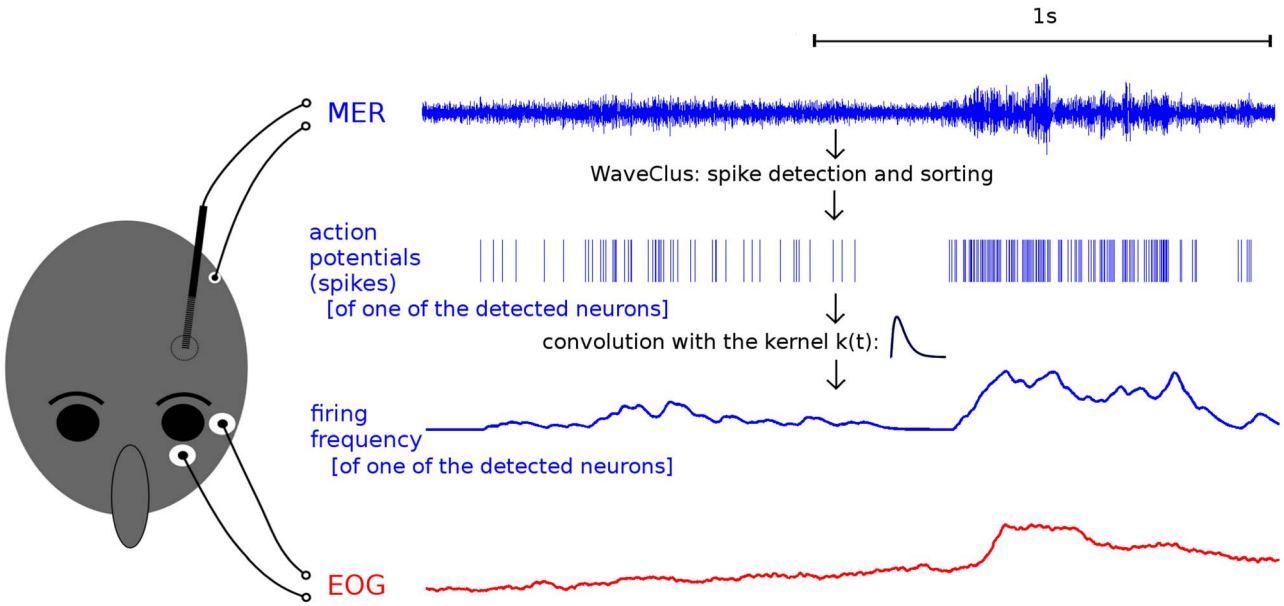

Figure 2. Microelectrode recording (MER) and electrooculography (EOG) signal acquisition and processing. Action potentials of individual neurons were identified using the WaveClus algorithm in the MER signal. The instantaneous firing rate (IFR) was then estimated by convolving a series of extracted action potentials generated by a single neuron with a causal kernel function. Finally, the IFR was correlated with the eye movement kinematic parameters derived from the EOG.
doi:10.1371/journal.pone.0078581.g002

Discussion

We showed that nuclei of the basal ganglia (namely, STN, GP and SNr) contain neurons whose firing rates correlated with eye movements during the scanning EM task. The proportion of EM-related neurons was relatively high reaching 20-22% in each of those nuclei (Table 3). Despite technical limitations due to the single-channel EOG recording we found relationships between different kinematic parameters of the EM and the firing rate in many neurons (Table 3, Figure 4). These findings point to the role of the basal ganglia in the static and dynamic representation of the EM, a role of importance for the maintenance of accuracy in goal-directed movements.

Eye movement activity in basal ganglia

Our single unit records from the STN showed that the proportion of EM-related neurons was higher in its ventral part (Figure S1). A 20% share of oculomotor neurons in the ventral part of the STN has already been noted in monkeys [10] and in humans [21]. However, those were solely neurons involved in saccadic EM. As suggested by our results, the SNr and GP are probably as equally important for control of voluntary scanning EM as the STN. We consider this as one of the major outcomes of our study because in both of these nuclei, the oculomotor activity had previously been noted during EM only in animals [11,13,35].

The role of the STN in EM has been largely explored in deep brain stimulation treated patients with Parkinson's disease. A high intensity STN neurostimulation resulted in contraversive eyeball deviations [36,37], similar to STN inactivation after locally injected GABA in animals [38] or after unilateral traumatic striato-subthalamic lesion [39]. An electrode penetration to the STN has an impact on the EM parameters as well. It causes a transitory microlesion [40] prolonging the latency of reflexive saccades [41] which are already prolonged due to Parkinson's disease [42]. Unlike microlesion, deep brain stimulation has an opposite effect on the STN as the latency of visually initiated reflexive saccades become shorter and normalized [41,43,44] while their gain is growing [45]. In addition, the STN deep brain stimulation improves some of the parameters of voluntary saccades [18,46] and suppresses interruptive saccades during fixation [47].

The significance of the STN in EM control is also well documented by other studies. The STN participates in the initiation of voluntary EM and in the inhibition of automatic EM [9,46], probably reflecting the influence of a hyperdirect pathway connecting the SMA and the motor cortex [48,49,50] including the supplementary eye field [51] with the STN, bypassing slower projections through the basal ganglia [52,53]. By the hyperdirect pathway, the motor plan can be rapidly implemented at the STN level and interfere with automatic EM [9,11]. The STN influence is then propagated by the following two main outputs [54,55]. The first is an excitatory glutamatergic projection to the SNr [56], whose activity is reduced or increased during saccades or smooth pursuit movements [12,13,35]. The SNr subsequently sends ipsi- as well as contralateral projections to the superior colliculus [57] which is an important nucleus involved in the control of automatic reflexive saccades [8]. The second glutamatergic output from the STN projects to the internal part of the GP [56] through which the oculomotor pattern can be further modified. The GP is more than just a skeletomotor structure as confirmed by several findings of EM-related neurons in its external and internal role during visually guided saccades [11] and anti-saccades in animals [58]. Moreover, bilateral pallidotomy affects the fixation [17] and reduces the velocity of self-initiated saccades [15]. On the other hand, deep brain stimulation of the GP interna modifies other parameters of automatic as well as voluntary saccades (Fawcett et al., 2005). Hence the fact that during the scanning EM task we found EM-related neurons in the STN, SNr and GP was not surprising.

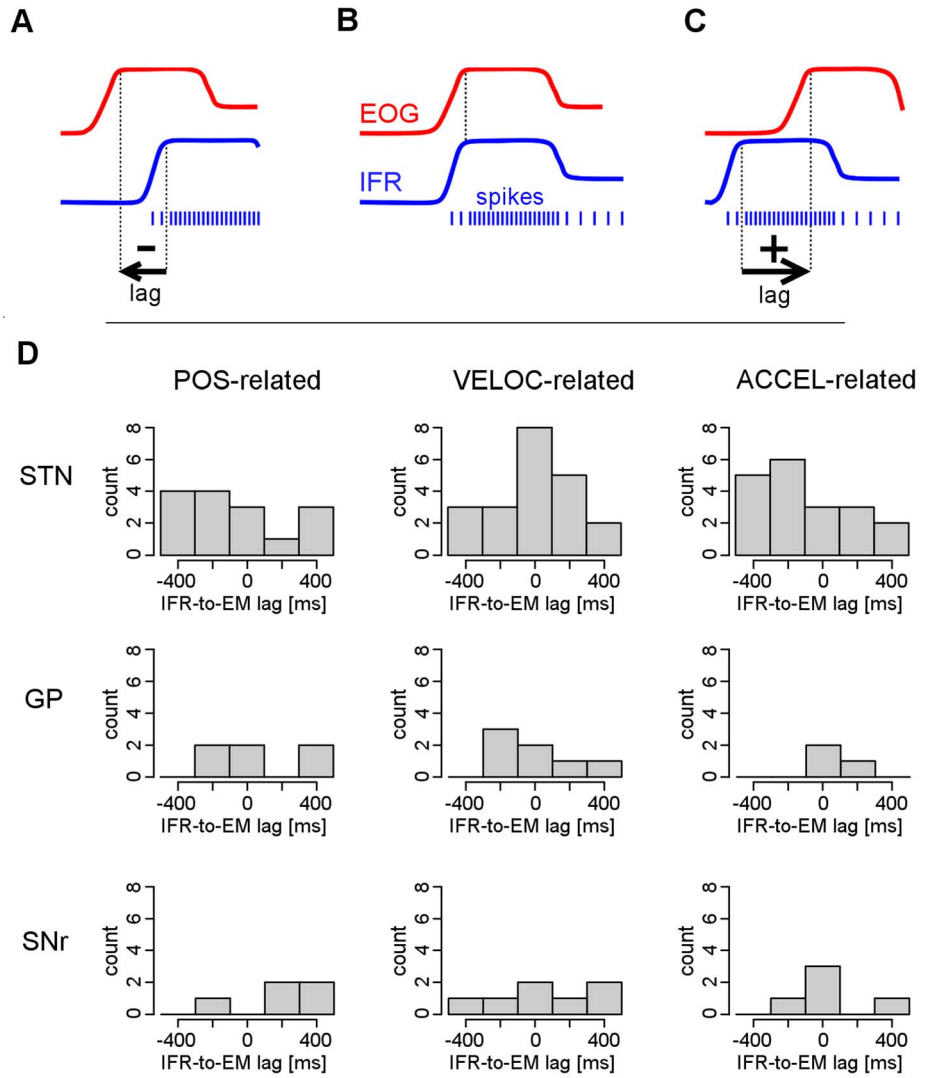

Figure 3. Time lag of neuronal activity with respect to electrooculography (EOG). A, B, C - Explanation of the cross-correlation procedure in three examples. Action potentials of three hypothetical neurons along with corresponding instantaneous firing rate (IFR) were correlated with the theoretical EOG signal. Figure A – the IFR correlates with the past EOG signal suggesting a sensory function of the neuron. Figure B – the IFR correlates with the concurrent EOG signal suggesting an executive function of the neuron. Figure C – the IFR correlates with the future EOG signal suggesting a preparatory function of the neuron. The time lag of the IFR in which the maximal (and significant) correlation with EM is reached is called the *optimal IFR-to-EM cross-correlation lag*. This lag is negative in A, zero in B and positive in C. Figure D - Frequency histograms of the optimal instantaneous firing rate (IFR) to eye movement cross-correlation lags in all eye movement-related neurons during the scanning eye movement task across the subthalamic nucleus (STN), globus pallidus (GP), and substantia nigra pars reticulata (SNr) considering kinematic parameters of the electrooculography (POS, VELOC, ACCEL, in columns). No significant differences in the locations of these distributions were found.
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Table 2. Numbers of microelectrode recordings and neurons detected.

	STN	GP	SNr	Total
MER count	97	21	19	137
neuron count (SEM task)	130	23	30	183
neuron count (SEM & VGS task)	46	2	5	53

MER count – number of microelectrode recordings obtained in each nucleus; SEM – scanning eye movement task; VGS – visually guided saccade task; neuron count – number of neurons identified in each nucleus during the SEM task (patients 1-19) and during both the SEM and VGS tasks (patients 16-19); STN – subthalamic nucleus; GP – globus pallidus; SNr – subtantia nigra pars reticulata.
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Segregation and convergence in eye movement control

Scanning EM are an important tool in the exploration of complex visual stimuli [6,59]. Their trajectory is made up of a sequence of variably large saccades and fixations with the visual field maintained for tens to hundreds of milliseconds. As a result, a certain detail is steadily projected on the fovea. This is followed by a saccade, a rapid voluntary movement, by means of which the fovea moves on to a new point of interest while information from the other parts of the retina is being concurrently assessed in search of another point of fixation. This distributed parallel processing has been recently confirmed by the sequential scanning task [60]. As expected, in four patients where both tasks were used, the median amplitude of scanning EM was smaller than that of the saccades in the visually guided task. At the same time, the

Table 3. Number of neurons related to eye movements in the scanning eye movement task.

	STN (130 neurons)	GP (23 neurons)	SNr (30 neurons)	Total (183 neurons)
EM-related neurons†	**26 (20%)*** **	**5 (22%)*** **	**6 (20%)*** **	**37 (20%)*** **
POS-related	15 (12%)**	6 (26%)***	5 (17%)*	26 (14%)***
VELOC-related	21 (16%)***	7 (30%)***	7 (23%)***	35 (19%)***
ACCEL-related	19 (15%)***	3 (13%)	5 (17%)*	27 (15%)***
POS & VELOC-related	10 (8%)	4 (17%)*	5 (17%)*	19 (10%)**
POS & ACCEL-related	7 (5%)	3 (13%)	3 (10%)	13 (7%)
VELOC & ACCEL-related	10 (8%)	3 (13%)	4 (13%)	17 (9%)*
POS & VELOC & ACCEL-related	7 (5%)	3 (13%)	3 (10%)	13 (7%)

EM-related neurons – the number of eye movement-related neurons associated with at least one kinematic parameter (†Bonferroni-corrected number of neurons for three kinematic parameters). Neurons functionally associated with one or more kinematic parameters (POS – eye position; VELOC – eye velocity; ACCEL – eye acceleration) are reported for each nucleus separately (STN – subthalamic nucleus; GP – globus pallidus; SNr – substantia nigra pars reticulata). Number of neurons significantly greater than expected 5% false positivity rate is denoted: *($p<0.05$), **($p<0.01$) ***($p<0.001$).
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amplitudes of largest EM executed in both tasks were similar. This is in agreement with previous studies, indicating that the amplitudes of scanning EM follow a heavily skewed distribution towards low values, with relatively rare movements of larger amplitude [61].

From what structures and in which way the scanning movements are controlled is still poorly understood. Since they are under voluntary control, they can be seen as a model with internally generated movements – unlike reflexive saccades which are initiated by external stimuli. Internally and externally triggered movements are generally subject to different control and executive mechanisms [62,63]. Hence, we assumed that both oculomotor systems are functionally segregated even at basal ganglia level. This hypothesis proved to be correct because in a subgroup of patients engaged in tasks which involved scanning as well as visually guided saccades, we observed that different EM-related neurons were involved in each of the tasks (Table 4). The principle of functional segregation in the control of voluntary and automatic EM had already been previously implied in connection with the interpretation of deep brain stimulation effects [16] or cerebellar lesions [24]. Animal studies have identified spatially segregated functional territories for the control of saccadic EM in the basal ganglia [64,65]. In primates, the majority of visuo-oculomotor neurons were found in the ventral part of the STN, one third of them being active during reflexive externally triggered saccades and another third being active predominantly during internally triggered (memory guided) saccades [10]. Our results go even further in terms of this specialization hierarchy. Apart from the segregation of populations of EM neurons for scanning movements and visually guided saccades, we identified a higher degree of segregation in all three nuclei neurons. In fact, some neurons responded exclusively to a specific kinematic parameter of the EM associated with an increasing or decreasing firing rate depending on whether or not the eye had reached a particular position, velocity or acceleration of movement (Figure 3).

Some of our results conform to the opposite principle arising from the convergence of cortico-striato-pallido-thalamic projection, i.e. from input nuclei which are larger, to output nuclei which are smaller [23,66] implying that initially complex information undergoes compression and simplification on its way to the output [67,68]. Indeed, a small percentage of the STN neurons showed the same neuronal activity in both types of tasks (Table 4). The convergence theory is supported by our observation of 5–8% of STN neurons, whose activity correlated with several kinematic parameters simultaneously (Table 3, 4) suggesting the presence of universal oculomotor neurons. This is in agreement with previous findings of STN neurons which become activated by switching from automatic to voluntary controlled EM [9], with the STN neurons activated from saccades and also during passive movements of the limb [21], with the SNr neurons activated during both pursuit and saccadic EM [13], or with anatomical connections documenting overlap between saccadic and pursuit oculomotor system at the brainstem level [69]. The functional convergence is further supported by the STN deep brain stimulation joint effect on the oculomotor and motor system of the neck and trunk in Parkinson's disease, marked by simultaneously improved orienting eye-head movements [45] or by improved oculomotor performance associated with body turning [70].

Time relation between EOG and neuronal activity

In our study, the eye-movement neurons in the STN, SNr or GP were not firing solely in a particular phase of the scanning EM task. In all three nuclei, these neurons became active 200–400 ms before EM, in its course and also 200–400 ms after its onset (Figure 3D). While STN neuronal activity expressed in saccade-related potentials already began 0.8–1.8 s before the saccade, suggesting the involvement of nonspecific readiness non-motor mechanisms [20], single unit neuronal STN and SNr activity culminated within 250 ms after the saccade onset [21] suggesting monitoring or sensory function. Our results are more in agreement with observations of the STN showing modified neuronal activity before, during and after the saccade [10]. This means that scanning EM-related neurons of the STN could be involved in all the preparatory, executive and monitoring phases of EM. This cannot be concluded for GP and SNr due to a relatively low amount of data.

Limitations

As there were several limitations we should interpret our results with caution. The main problem arised from the impossibility of using infra-red oculography or two-channel EOG during surgery. While their use would definitely have improved the accuracy of the kinematic parameters during EM, they would also have interfered with the established implantation procedure. The use of single-channel EOG, which failed to capture the full extent of free-

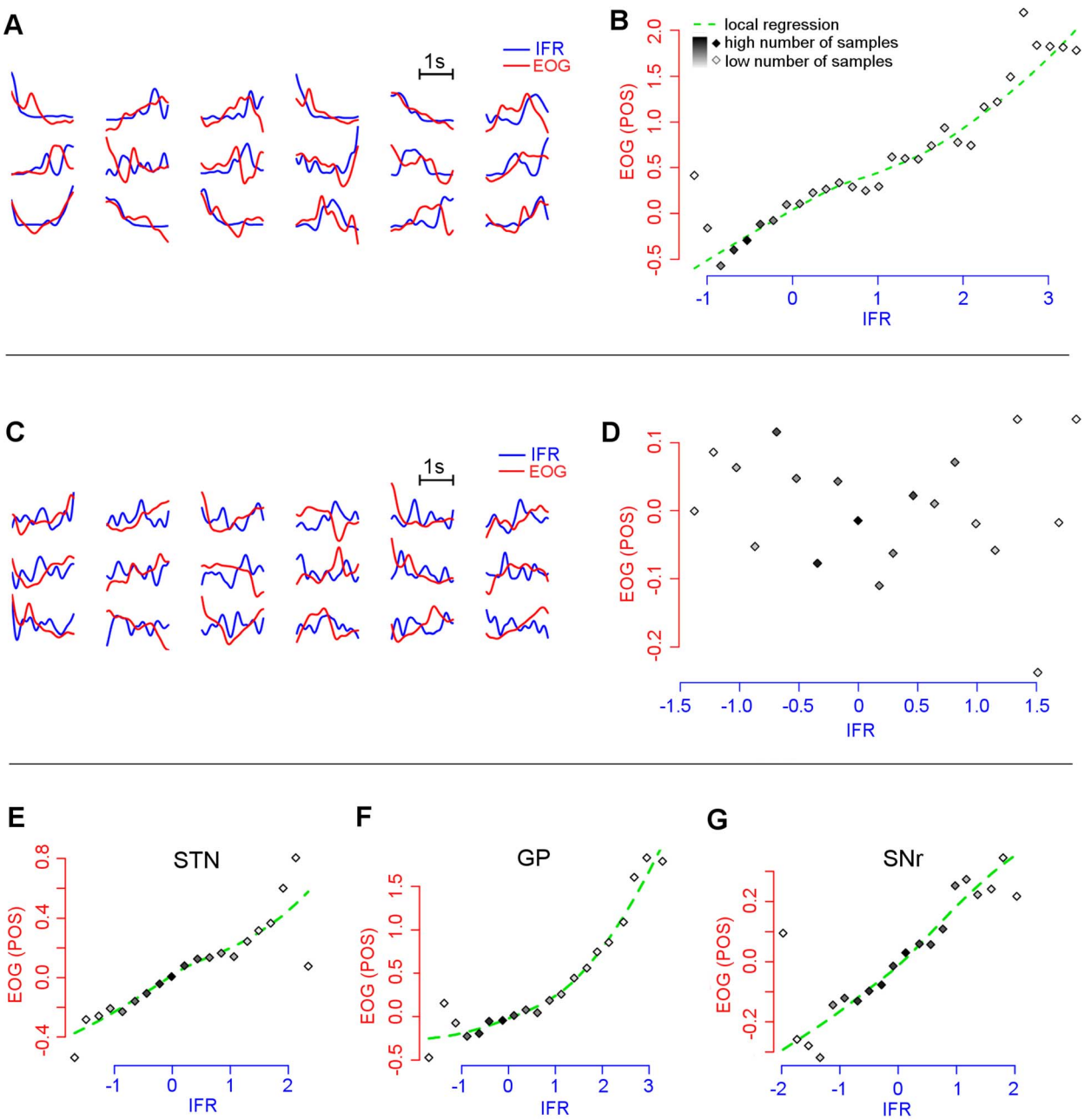

Figure 4. Neuronal activity during the scanning movement task. Example of neuron related (A, B) and unrelated (C, D) to eye movements based on correlation analysis of the instantaneous firing rate (IFR) and eye position (POS) derived from the electrooculography (EOG). All eye movement-related neuronal populations in the STN, GP and SNr are plotted in figures E, F, and G. Figures A, C show the IFR (blue) and EOG (red) pairs recorded during epochs of the task involving both the black screen and pictures presentations. Figures B, D, E, F, G show the dependency of the normalized eye position (POS) derived from the electrooculography (EOG) on the normalized, sorted and binned amplitude of the instantaneous firing rate (IFR). While the IFR from a single neuron was used on figures B and D; the IFR from all eye sensitive neurons were used on figures E, F, and G for each nucleus separately. The amplitudes of the POS signals which correlated negatively with the IFR signal were reversed. The number of signal samples in each bin is expressed by different shades of grey in the diamond glyphs.
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direction EM and yielded no more than EM projection into a one-dimensional space, is clearly a limitation which to some extent compromised the sensitivity of our study. Another limitation is connected with the assessment of neuronal activity during the oculomotor tasks based on just correlation analysis. Neuronal firing does not have to relate to EM activity alone but it may also reflect visual perception, planning, visuo-spatial attention or other cognitive processing which coincide with oculomotor activity. In addition, our results could be affected by the fact that our data was obtained from patients with Parkinson's disease in whom abnormal saccadic EM were repeatedly reported [42,44,46,71,72,73,74]. Whether any abnormalities exist in Parkinson's disease during scanning EM also is not clearly known since, with the exception of one study which showed a deficit in trans-saccadic working memory [75], no-one has systematically

Table 4. Eye movement-related neurons detected in the scanning eye movement task and/or visual guided saccade tasks.

	STN (46 neurons)			GP (2 neurons)			SNr (5 neurons)		
	SEM	VGS	Both	SEM	VGS	Both	SEM	VGS	Both
EM-related neurons[†]	**10**	**10**	**2**	**0**	**1**	**0**	**1**	**2**	**0**
POS-related	4	9	0	0	0	0	0	2	0
VELOC-related	9	4	1	0	0	0	1	0	0
ACCEL-related	8	11	3	0	1	0	2	0	0
POS & VELOC-related	3	4	0	0	0	0	0	0	0
POS & ACCEL-related	2	4	0	0	0	0	0	0	0
VELOC & ACCEL-related	4	2	0	0	0	0	1	0	0
POS & VELOC & ACCEL-related	2	2	0	0	0	0	0	0	0

EM-related neurons – the number of eye movement-related neurons associated with at least one kinematic parameter ([†]Bonferroni-corrected number of neurons for three kinematic parameters) identified from patients 16-19 which performed both the scanning eye movement task (SEM) and visual guided saccade task (VGS) in the subthalamic nucleus (STN), globus pallidus (GP) and substantia nigra pars reticulata (SNr). Neurons functionally associated with one or more kinematic parameters (POS – eye position; VELOC – eye velocity; ACCEL – eye acceleration) are reported for each nucleus separately.

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focused on scanpath or other parameters of complex exploratory EM in these patients.

Conclusions

As our results showed, the STN, SNr and GP contain neuronal populations related to scanning EM. Their representation reached about 20% in each of the three nuclei. Basal ganglia are thus not limited to previously described saccade control and perhaps play a more general role in EM circuitry. Oculomotor systems responsible for the execution and monitoring of scanning EM and visually guided saccades are mostly segregated as suggested by neurons involved exclusively in one of two EM tasks or by neurons selectively co-activated in association with a specific kinematic parameter. However, some functional overlap of the two oculomotor systems does exist, albeit confined to small groups of neurons conforming to the complementary convergence principle. Further studies combining clinical and electrophysiological approaches are needed to clarify the role of the basal ganglia in automatic and voluntary oculomotor behavior. We should emphasize, that the large representation of basal ganglia neurons showing activity during all phases of the EM is also an argument for taking them into account when designing new tasks using single unit microrecording. Many visual, ocular or motor experiments are potentially oculomotor in their nature which may compromise results if the EM-related neuronal activity was not considered.

Supporting Information

Figure S1 Positions of the eye movement-related neurons along dorso-ventral microelectrode trajectory within the basal ganglia. A – length of the subthalamic nucleus (STN), B – length of the globus pallidus (GP) and C – length of the substantia nigra pars reticulata (SNr) explored intraoperatively by the five microelectrodes in both the left and right hemispheres and projected to one-dimensional space aligned to the ventral border of the STN and GPi and to the dorsal border of the SNr. Position of each neuron along the dorso-ventral axis is shown in each subject. The proportion of eye movement-related neurons (EM) was significantly higher in the ventral part of the STN.
(TIFF)

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Author Contributions

Conceived and designed the experiments: RJ T. Serranová T. Sieger. Performed the experiments: RJ DU T. Sieger FR. Analyzed the data: T. Sieger RJ T. Serranová JW DN CB. Contributed reagents/materials/analysis tools: T. Sieger RJ DU ER T. Serranová JW DN CB BG. Wrote the paper: CB T. Sieger RJ. Critical revision of the manuscript: T. Sieger CB T. Serranová BG ER RJ DN.

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2.6 Secondary toxic parkinsonian syndrome: One interesting non-invasive tool to differentiate Ephedrone induced Parkinsonism from PD

Eye movements in Ephedrone-Induced Parkinsonism. *Cecilia Bonnet, Jan Rusz, Marika Megrelishvili, Tomáš Sieger, Olga Matoušková, Michael Okujava, Hana Brožová, Tomáš Nikolai, Jaromír Hanuška, Mariam Kapianidze, Nina Mikeladze, Nazi Botchorishvili, Irena Khatiashvili, Marina Janelidze , Tereza Serranová, Ondřej Fiala, Jan Roth, Jonas Bergquist, Sophie Rivaud-Péchoux, Bertrand Gaymard and Evžen Růžička. PLoS One. 2014 Aug 12;9(8):e104784.*[131]

This project was made in collaboration between our department and the Neurology Department S. Khechinashvili University Clinic, Tbilisi, Georgia. Paradigms in VOG are created in cooperation with the Pierre and Marie Curie University, Paris 6, 75013 Paris, France and CRICM UPMC/INSERM UMRS-975, 75013 Paris, France.

Methcathinone is a synthetic analog of cathinone, an alkaloid present in the leaves of the khat bush (Catha edulis) that grows in eastern Africa and southern Arabia. The inhabitants of these regions frequently chew it because of its stimulating properties. This natural amphetamine acts by releasing catecholamines from presynaptic storage sites, eliciting acute dose-dependent effects including euphoria, anxiety, agitation and hallucinations.[132]

As illicit drug, methcathinone is synthesized clandestinely by oxidation of ephedrine or pseudoephedrine in two different ways: i) In the USA, methcathinone is prepared by potassium or sodium dichromate in presence of sulfuric acid followed by purification, and is used by inhalation or by sniffing. Principal adverse effects in these subjects are depression, paranoia, hallucinations, anxiety, tremor, and insomnia.[49] ii) In the former USSR and Eastern Europe, methcathinone hydrochloride is used as an intravenously injected drug called "Russian cocktail, Ephedrone, Jeff" (Figure 11). It is prepared from over-the-counter cold tablets containing ephedrine or pseudoephedrine, by oxidation with potassium permanganate in presence of acetic acid, without any further purification.[50] In consequence, ephedrone

addicts may show extremely high manganese (Mn) blood concentrations (2,000–3,000 μg/L), that means up to 250 times higher than a normal subject (≤ 10–12 μg/L).[52, 89]

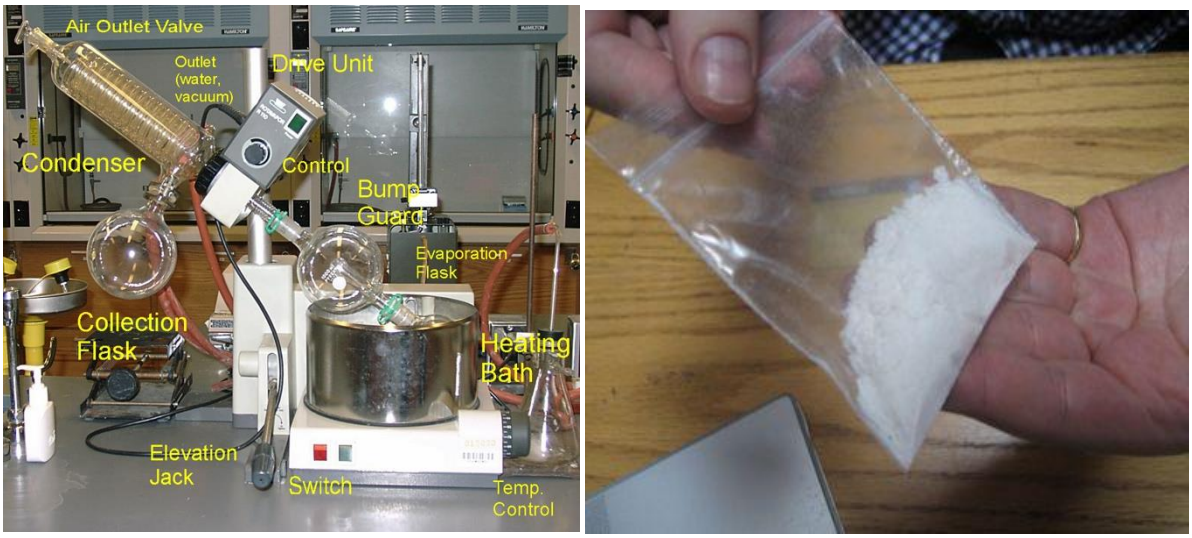

Figure 11. Addiction inbox. Mephedrone the new drug in town. 11.2010. Quality Ephedrone for sale. Available for sale on the Internet. 11.2011

Manganese toxicity

In humans, manganese toxicity has been reported after occupational exposure of workers from the manufacture of bleaching powder, in mining, welding and smelting,[53, 132-134] in subjects exposed to contaminated well water,[135] in some patients after long-term parenteral nutrition, and chronic liver failure.[134]

Recently, the clinical syndrome of manganese toxicity has been observed in methcathinone addicts using potassium permanganate as the oxidizing agent of ephedrine.[49, 51, 52, 54-56, 89] The reasons for attributing the development of neurological disorders in ephedrone abusers to the toxic effects of manganese include the consistency of the clinical syndrome with the occupational manganism, and the fact that no similar neurological disorders were described in methcathinone addicts using the drug prepared by chromate instead of permanganate oxidation.

Epidemiology of Ephedrone abuse and manganese-induced Parkinsonism

As no direct data are available on the scope of ephedrone abuse and on the prevalence of manganese-induced Parkinsonism we provide rough estimates. In Estonia, in a population of 1.4 million, there are approximately 13800 intravenous drug users (i.e. about 1 % prevalence), most of them living in Tallinn and its neighbouring areas.[136] 12% of intravenous drug users reported historical or current ephedrone use.[136] In Georgia, the number of inhabitants is about 4.4 million, 2.3 million of who are living in cities. If similar proportion of Georgian town population were drug users as in Estonia, we can assume that there are at least 20 thousand intravenous drug users in Georgia, 2 thousand of them having experience with ephedrone. About 40 000 i/v drug abusers are in Tbilisi. However, the proportion of ephedrone users among them is still unknown.

Ephedrone Parkinsonism

The clinical syndrome described in ephedrone abusers is characterized by a rapidly progressive, irreversible, and non-levodopa responsive atypical parkinsonian syndrome. Principal symptoms include early gait impairment and postural instability with falls, limb dystonia, facial dystonia and hypomimia, speech disorders and eye movement abnormalities. Other described symptoms include pseudobulbar syndrome, action tremor, multifocal myoclonus, ataxia, dystonia in upper limbs, micrographia, autonomic dysfunction, hypersomnia, lack of spontaneity, apathy, aboulia depression, cognitive and affective disorders, and frontal release signs.[49, 51] It seems that there is no correlation between the duration of drug abuse and the clinical severity [132], however the cumulative dose of ephedrone may play a role. On the other hand, some abusers do not develop neurological symptoms at all.[136]

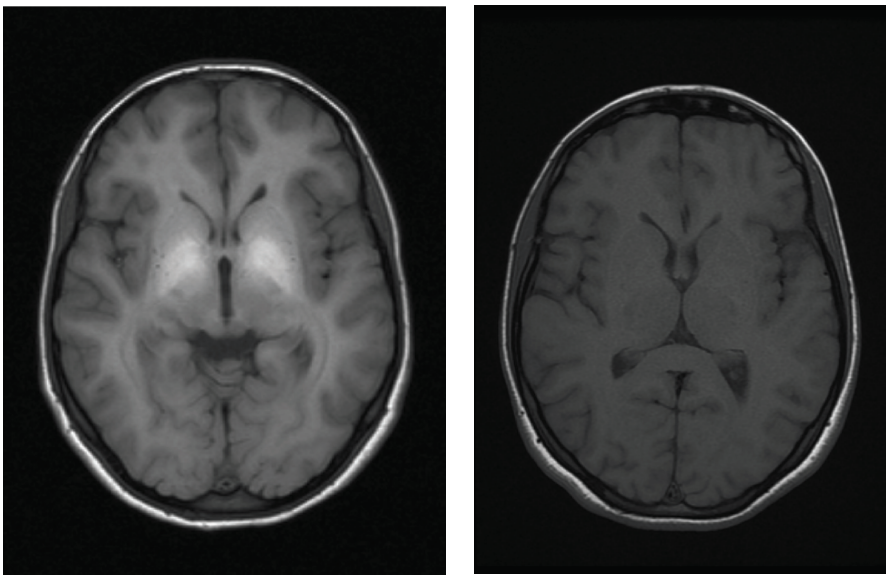

Figure 12 T1-weighted axial MRIs of the brain showing an increased signal in the globus pallidus in an active user and and two years after cessation of exposure. Sikk. Manganese-induced Parkinsonisme due to Ephedrone Abuse. Parkinson's Disease, Volume 2011, Article ID 865319

Eye movement abnormalities

Most case reports of ephedrone abusers noticed eye movement abnormalities, especially slowing and mild restriction of vertical saccadic eye movements, slow horizontal saccades and impaired vertical optokinetic nystagmus.[49, 55] In addition, apraxia of eyelid opening has been described. To our knowledge, characteristics of eye movements have not been assessed with objective methods like videooculography.

Neuropathology and pathophysiology

A very limited number of autopsy studies have been performed in conditions with increased manganese in the brain. No study has been reported in ephedrone abusers. The exact mechanism of manganese toxicity is unknown but a participation of oxidative stress is highly probable. The available evidence suggests that excessive levels of the toxic form of manganese (Mn^{3+}) accumulate in the brain leading to neurodegenerative changes reflected in numeric atrophy of neurons and gliosis in susceptible parts of the brain (mainly GPi and SNr). It is consistent with the clinical findings of levodopa non-responsive atypical Parkinsonism accompanied with dystonia and other features, corresponding to the position of the GPi/SNr

in the downstream of both direct and indirect pathways, conveying all parallel cortico-striato-thalamo-frontal circuits of the basal ganglia.

Comparison between manganese-induced Parkinsonism and Parkinson's disease

Parkinson's disease (PD) is a neurodegenerative disease related to a progressive loss of neurons in the substantia nigra, however it has been recently shown that in PD the neuropathology is distributed throughout the entire nervous system.[67] The three cardinal features of Parkinson's disease are rest tremor, rigidity, and bradykinesia. Postural instability is non-specific and is usually absent in early disease and in young patients. Non-motor features do typically develop with the progression of PD, including autonomic dysfunction, cognitive and psychiatric changes, sensory symptoms, and sleep disturbances. T1-weighted MRI is normal in PD patients, pre-synaptic dopaminergic SPECT and PET show asymmetric striatal deficits.

Axial disability including progressive deterioration of gait and postural instability leading to falls develops in advanced PD, mostly corresponding to a non-dopaminergic involvement. Freezing of gait can be related with both dopaminergic and non-dopaminergic lesions.

Parkinson patients have usually normal horizontal reflexive saccades[97] hypometric vertical prosaccades [98] and memory-guided saccades.[102, 110] Other saccade metrics in PD have shown more controversial results, for example the latency of saccades and the error rate of antisaccades. The latency of saccades may be normal, mildly increased or even decreased [77] and the error rate of antisaccades has been reported to be normal,[63, 80, 101-106] or increased [107-110] depending of the stage of disease progression.

In this project we aimed to characterize eye movement abnormalities in EP with videooculography (VOG). We assumed that vertical palsy of eye movements and early falls in Mn-induced Parkinsonism pointed to an involvement of midbrain and cortico-mescencephalic pathways and await a more precise description.

We investigated the function of the autonomic nervous system through Pupillometry. A imbalance of parasympathetic-sympathetic has been shown in welders with Mn-induced

toxicity in form of lower heart rate variation following deep breathing, immediate standing up and Valsalva manoeuvre.[137] Patients with EP may present autonomic features as impotence, hyper salivation and soborrehea.[49] The pupillometry provides a simple and non-invasive tool to study the autonomic system resulting in a dynamic equilibrium that is expressed in pupil diameter. The pupillary light reflex is driven primarily by increased parasympathetic activity, while the resting size of the pupil diameter in darkness and in response is determined primarily by changes in sympathetic activity.

Pupillometry:

Pupillometry was performed in 28 EP and in 19 PD using a monocular infrared pupillograph 'Compact Video Pupillometer'. The stimulation was performed with a 320 Lux light flash for a duration of 1s, duration of recording 4s. At each level, images were acquired by the system at 67 images per second. The measurements were conducted on the left eye of the subjects after at least 5 min of dark adaptation. The subjects were asked to look straight at the red fixation point. We extracted baseline pupil size, pupil reactivity (in %), constriction amplitude, pupil contraction speed and latency.

Pupil metrics are described in Table 5. Only the baseline pupil size in PD was larger, compared to the control group ($p < 0.05$). There was also approximately 20% increase of pupil contraction velocity in PD in comparison with the controls, while this difference has not reached the level of statistical significance. No significant difference in any pupillometry parameter has been statistically significant between ED and controls.

	EDp (n = 28) Value (SD)	**Controls (n = 24) Value (SD)**	***p***	**PDp (n = 19) Value (SD)**	**Controls (n = 14) Value (SD)**	***p***
Pupil size (mm)	5,43 (0,60)	5,48 (0,70)	0,8	5,08 (0,86)	4,40 (1,02)	0,05 *
Pupil reactivity (%)	42,41 (6,18)	43,89 (3,72)	0,31	45,28 (16,61)	42,34 (8,25)	0,55
Constriction amplitude (mm)	2,33 (0,41)	2,44 (0,40)	0,34	2,16 (0,48)	1,90 (0,57)	0,17
Pupil contraction speed (mm/s)	7,78 (9,06)	5,84 (0,88)	0,3	5,26 (1,93)	4,42 (1,44)	0,18
Latency (ms)	209,66 (39,97)	208,03 (29,39)	0,87	230,65 (38,71)	223,97 (48,71)	0,66

Table 5 Pupillometry in Patients and their control group. ED: Ephedrone patients; PD: Parkinson's disease patients; SD: standard deviation; p: *p* value

We did not show any abnormalities of the pupillometric analysis of EP. Besides, patients had no changes on heart rate and blood pressure after immediate and 2 minutes standing. Patients did not report any symptom of orthostatism nor urinary dysfunction. Further specific symptom oriented diagnostic studies may be necessary to gain insights about the function of the autonomic system in this disease.

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Eye Movements in Ephedrone-Induced Parkinsonism

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Abstract

Patients with ephedrone parkinsonism (EP) show a complex, rapidly progressive, irreversible, and levodopa non-responsive parkinsonian and dystonic syndrome due to manganese intoxication. Eye movements may help to differentiate parkinsonian syndromes providing insights into which brain networks are affected in the underlying disease, but they have never been systematically studied in EP. Horizontal and vertical eye movements were recorded in 28 EP and compared to 21 Parkinson's disease (PD) patients, and 27 age- and gender-matched healthy subjects using standardized oculomotor tasks with infrared videooculography. EP patients showed slow and hypometric horizontal saccades, an increased occurrence of square wave jerks, long latencies of vertical antisaccades, a high error rate in the horizontal antisaccade task, and made more errors than controls when pro- and antisaccades were mixed. Based on oculomotor performance, a direct differentiation between EP and PD was possible only by the velocity of horizontal saccades. All remaining metrics were similar between both patient groups. EP patients present extensive oculomotor disturbances probably due to manganese-induced damage to the basal ganglia, reflecting their role in oculomotor system.

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Introduction

Ephedrone is a home-made psychostimulant drug used frequently in the former Soviet Union. This drug is prepared from over-the-counter common cold tablets containing ephedrine or pseudoephedrine, by oxidation with potassium permanganate in presence of acetic acid, without any further purification [1], containing a high residual manganese in the final synthetic mixture [2]. As a consequence, ephedrone addicts may show extremely high manganese (Mn) blood concentrations [3] and develop a chronic manganic encephalopathy similar to the one seen in manganese ore miners and in welders. This so called ephedrone-induced parkinsonism (EP) consists of a severe, rapidly progressive, irreversible and non-levodopa responsive parkinsonian and dystonic syndrome characterized by speech disorder [4], early gait impairment and postural instability [1,3,5–10]. Several studies have shown that in EP, prominent lesions occur in the GPi and substantia nigra pars reticulata (SNr), but recent evidence suggests more widespread neuropathology. Investigations in chronic Mn-intoxicated monkeys and welders with Mn intoxication have shown lesions affecting the substantia nigra pars compacta [11], brainstem, cerebellum [12], frontal white matter and cortical structures [8,13].

Eye movements in EP have been reported to be slow and mildly restricted in the vertical and horizontal plane [1,5,9,14], however they have never been objectively studied with videooculography. The role of the basal ganglia in the control of eye movements has been supported by extensive evidence [15–17]. In EP, Mn is the most likely etiological agent for both clinical symptoms and MR image changes, which can be observed as hyperintensive signal in

T1-weighted MRI in the globus pallidus and in other basal ganglia (BG) structures such as the substantia nigra, caudate, and putamen [18]. With regard to the high representation of eye movement-related neurons in the BG [17], we hypothesized that BG damage due to Mn accumulation in EP can cause more serious dysfunction of eye movement control than in PD.

The aim of the present study was to analyse potential oculomotor abnormalities in EP patients by the use of video-oculography (VOG) and to compare these findings with VOG results in PD patients and healthy subjects.

Methods

Subjects

Patient characteristics are shown in Table 1.All participants signed the informed consent. The study was approved by the local ethics committees of the 1st Faculty of Medicine and General University Hospital, Prague, Czech Republic and of the S. Khechinashvili University Hospital, Tbilisi, Georgia and was in compliance with the Declaration of Helsinki.

EP patients: 28 patients (27 males, 1 female; mean age 39.9, SD 5.0, range 28.6–48.7 years) were examined at the department of neurology, S. Khechinashvili University Clinic, Tbilisi Georgia. The diagnosis of EP was based on a history of ephedrone use and subsequent development of a parkinsonian syndrome, with MRI showing pallidal hyperintensities on T1-weighted images in all patients. However, at the time of the present study, none of the patients were active consumers of ephedrone or other illicit drugs. The study was performed after the patients had stopped ephedrone consumption in average 3.9 years before the examination (range, 3 months to 12 years from stopping the drug use). A new 3T MRI was performed 2–3 weeks prior to the clinical examination (Magnetom Verio, SIEMENS) at the Research Institute of Clinical Medicine, Tbilisi, Georgia. Standard T1 (se), T2 (tse), FLAIR, T2*, and MPRAGE sequences were used for structural imaging. Only one patient (EP 27), who stopped ephedrone consumption 3 months before inclusion, showed typical bilateral diffuse hyperintensity on T1-weighted images in the globus pallidus (GP) and partially in the substantia nigra (SN). In all other cases, no pathological T1-hyperintensity was observed. Manganese concentration was measured in body and scalp hair at Uppsala University, Sweden (JB). Mean Mn concentration in our patients (0.50, SD 0.50 ppm) was well under the values obtained in the same laboratory for Estonian (0.82, SD 1.01 ppm Mn) and Swedish controls (0.83, SD 1.22 ppm Mn), confirming the absence of ongoing ephedrone use in EP patients. Patients were examined with the Natural History and Neuroprotection in Parkinson Plus Syndromes–Parkinson plus scale (NNIPPS) [19] to objectively assess parkinsonian-dystonic features and eye movement abnormalities. Neuropsychological testing consisted of the mini-mental state examination (MMSE) (mean 27.3/30), Beck Depression Inventory (BDI) (mean 19.1/64) and Frontal Assessment Battery (FAB) (mean 14.8/18).

PD control group: The group consisted of 21 patients (13 males, 8 females; mean age 54.8, SD 9.6, range 40–71 years) diagnosed according to the UK Parkinson's Disease Society Brain Bank criteria [20]. Patients younger than 40 years were genetically tested for the parkin (PARK2) mutation, and no carriers were found. All patients were examined at the Department of Neurology and Centre of Clinical Neuroscience, Charles University in Prague. The part III of the MDS-UPDRS [21] and Hoehn & Yahr [22] scales were used for clinical evaluation. Additionally eye movements were examined using the oculomotor part of the NNIPPS-Parkinson plus scale. Neuropsychological testing included the MMSE (mean 27.6/30), BDI (mean 10.3/64) and FAB (mean 16/18).

Healthy control group: The control group was included to establish a normal baseline and consisted of 27 participants (25 males, 2 females; mean age 36.2, SD 6.0, range 26–45 years), MMSE (mean 28.9/30), BDI (mean 4.9/64), FAB (mean 17.7/18). A questionnaire was used to determine that all controls were free of any neurological or psychiatric illness, and all controls denied the intake of any medication acting on the central nervous system.

Oculomotor examination

Eye movements were examined in all subjects by the same investigator (CB) using a binocular video-based eye tracker (mobile eBT Eye brain, Ivry-sur-Seine, France, www.eye-brain.com, 300 Hz sampling rate and 0.5° spatial resolution).Saccades were automatically detected according to a velocity threshold (Eye brain software) but were individually inspected and manually corrected by the experimenter if necessary. The left eye trace was analyzed by default, however the right eye was used if the left eye signal was contaminated by artifacts. Saccades perturbed by blinks or other artifacts were discarded (less than 10% of the trials in all subjects). Saccades with a latency below 80 ms were considered anticipatory saccades and rejected, and SRT between 81 and 130 ms were considered "express saccades" [23].

Three different tasks were performed in the same order in one session of 30 minutes duration: i) Simple prosaccades horizontal and vertical; ii) Simple antisaccades horizontal and vertical; iii) Mixed horizontal pro- and antisaccades. Subjects were seated in a calm, dark room with their chin supported by a chin strap and their forehead in contact with a frontal support. They faced a flat, 26 in. LCD screen (ProLite, Iiyama model PL 2600, size 550 mmx344 mm) located 60 cm in front of them at eye level.

i) Simple horizontal and vertical prosaccades: This task started with the onset of a green central fixation point(size: 15×15 pixels; luminance: 120 cd/m2) that was presented for a pseudorandom duration of 2800, 3200, 3500, 3800, 4000 or4100 ms. The fixation point was then turned off and 200 ms later, a red peripheral target (15×15 square, luminance 120 cd/m2) appeared during 1000 ms at a 13°right or left location, or at a 13°up or down location. Twenty-eight saccades were recorded. Latency, velocity [average (Vavg) and maximal (Vmax)] and gain were analyzed for each saccade. Then an average of all saccades for each metric was performed in each patient. Latency was defined as the reaction time from the target onset to begin of the saccade. Gain was defined as the ratio between saccade amplitude and target location. The number and amplitude of square wave jerks (SWJs) were measured during the period when the fixation point was on, lasting for 56 seconds. Square-wave jerks are small, inappropriate saccades that intrude on steady fixation by taking the eye away from the target and then returning it to the fixation position [24]. Only horizontal SWJs between 1–10° were considered for analysis, because SWJ over 10° are considered macro SWJ [25].

ii) Simple horizontal and vertical antisaccades: The task design was the same as in the prosaccade task, with the exception that the color of the central fixation point was red. Subjects were instructed to look as fast as possible in the direction opposite to the peripheral target. A total number of 32 saccades were recorded. Latency, error rate and rate of corrected errors were extracted. Saccades perturbed by blinks or other artefacts were discarded (less than10% of the

Table 1. Clinical characteristics of EP and PD patients.

Pat	Gender/Age	DD	Treatment	NNIPPS T	NNIPPS OM	Pat	Gender/Age	DD	Treatment levodopa	Park2	UPDRS III	H&Y	NNIPPS OM
EP	M-F/years	Years	mg	/332	/21	PD	M-F/years	years	mg		/72	/5	/21
1	M44	6	-	23	0	1	M48	6	300	-	36	2	0
2	M48	4	-	52	6	2	M53	10	300	-	28	2	0
3	M40	6	-	62	6	3	M64	21	480	-	29	2	1
4	M28	4	-	37	3	4	M52	13	2535	normal gene	14	2	0
5	M44	7	-	59	8	5	M60	7	300	-	27	2	1
6	M41	6	-	29	2	6	M49	12	300	Polymorp.V380L	35	2	0
7	M39	10	-	72	3	7	M66	3	400	-	21	1	0
8	M42	4	-	38	2	8	F40	4	360	normal gene	16	1	1
9	M43	4	Levodopa 750	63	9	9	M44	7	870	normal gene	38	2	1
10	M35	6	-	33	1	10	F70	11	1050	-	25	1	1
11	M42	7	-	28	4	11	M54	12	900	-	8	2	0
12	M38	6	-	62	5	12	M58	7	400	-	20	1	0
13	M32	7	Trihexyphenidyl 19	90	2	13	F42	4	480	normal gene	47	3	0
14	M40	5	Levodopa 571	75	6	14	F48	10	450	normal gene	39	3	0
15	M42	5	Levodopa 71	34	5	15	M65	26	600	Polymorph. D394N	35	3	0
16	M32	4	-	36	1	16	F71	1	0	-	12	2	0
17	M46	4	-	47	6	17	M53	12	320	-	36	2	1
18	M44	2	-	43	8	18	M56	15	2620	-	26	2	0
19	M35	4	-	45	5	19	F63	11	320	-	17	2	1
20	M43	4	-	41	4	20	F42	6	100	normal gene	11	1	0
21	M31	12	-	83	4	21	F43	4	100	normal gene	24	3	0
22	M44	44	-	37	6								
23	F45	4	-	80	11								
24	M36	3	-	27	8								
25	M40	7	-	41	4								
26	M37	4	-	42	4								
27	M37	2	EDTA 20	21	5								
28	M40	6	-	88	6								
	27M-1F/40	6,68		49,57	4,79		13M-8F/54	9,62	627,86		25,9	1,95	0,33

Levodopa treatment indicates the dose in mg of levodopa or equivalent of dopamine agonist per day (0.7 mg pramipexole = 100 mg levodopa; 5 mg ropinirole = 100 mg levodopa). Patients treated with levodopa were examined in the ''on'' condition. The Park2 gene was evaluated for mutation if the age at disease onset was less than 40 years. Pat: patient number; EP: ephedrone parkinsonism; PD: Parkinson's disease; Age: age at examination in years; F: female; M: male; DD: disease duration; NNIPPS: neuroprotection and natural history in Parkinson plus syndromes; OM score: oculomotor score; MDS-UPDRS: movement disorder society-sponsored revision of the unified Parkinson's disease rating scale; H&Y: Hoehn and Yahr scale; EDTA: ethylenediaminetetraacetic acid.

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trials in all subjects). In the pro-and antisaccade tasks, we defined the latency as the interval between target onset and saccade onset. Latency below 80 ms were considered anticipatory saccades and rejected [23]. Mean latency was determined only for correct antisaccades. Directional errors were defined as saccades initially directed towards the target. The rate of corrected errors (%) was extracted for the horizontal antisaccade task.

iii) Mixed task of pro- and antisaccades: This paradigm, performed according to Rivaud-Pechoux [26], was used to evaluate the ability to perform a task in which two task sets, rather than one, must be handled simultaneously, thereby demanding an increased cognitive load, increased demands on working memory, vigilance, sustained attention, motivation and response selection [26]. The central fixation point initially consisted of two vertically aligned and contiguous red and green points, with the same size and luminance as in the two previous task. After 3500–4200 ms, one of the two points (red or green) was turned off. The remaining point stayed on for 500 ms, and subjects were instructed that the color of the fixation point was to be used for selecting the appropriate response to the lateral target: a green point required a prosaccade and a red point anantisaccade. A 200 ms gap between the fixation point and the lateral target was used as in the previous tasks. We confirmed verbally that the instructions had been correctly understood. Seven prosaccades and six antisaccades were presented with an angle of 24°. In each subject, we calculated mean pro- and antisaccade latencies and error rates in the antisaccade task. Then we selectively analyzed saccades repeated in the same direction. Repeated trials were analyzed to provide a mixing cost for latencies and error rates, defined as performance. The performance in repeated trials was subtracted from the performance in the simple tasks of horizontal pro and antisaccades. We employed the restrictive method of analysis of Rivaud-Pechoux [26], taking into account only N-1 trials executed correctly with the same instructions. We first analyzed results separately to the right and left direction, and then as there were no differences between both sides, we elected to pool right and left pro/antisaccades.

Statistical analysis

Matlab© (Mathworks, Massachusetts, USA) was used for statistical analyses. As the Kolmogorov-Smirnov test for independent samples did not detect abnormal distribution of oculomotor variables, analysis of variance (ANOVA) was used to assess differences between the EP and healthy control group. Since the PD patients were generally older when compared to EP subjects, analysis of covariance (ANCOVA) was used to calculate differences between EP and PD groups with age as a covariate. The Pearson correlation analysis was used to examine the relationships between eye metrics and clinical and neuropsychological data. Post-hoc Bonferroni adjustment was applied to correct for the number of all tests performed according to the each paradigm. The level of significance after Bonferroni adjustment was set to $p<0.05$.

Results

The clinical data of EP as well as PD patients can be seen in Table 1.

i) Simple prosaccades (Figure 1.): In horizontal prosaccades, EP patients showed significantly decreased Vmax ($F_{1,54}=13.3$, $p=0.005$, $\eta^2=0.20$), significantly lower gain ($F_{1,54}=16.0$, $p=0.002$, $\eta^2=0.24$), a trend toward decrease Vavg ($F_{1,54}=8.0$, corrected $p=0.06$, uncorrected $p=0.007$, $\eta^2=0.13$), and normal latency ($F_{1,54}=0.09$, $p=1.00$, $\eta^2=0$) as compared to controls. In addition, EP patients showed decreased Vmax ($F_{1,44}=10.2$, $p=0.02$, $\eta^2=0.23$) in comparison to PD subjects. There were no differences between PD and EP patients regarding latency ($F_{1,44}=4.1$, $p=0.43$, $\eta^2=0.09$), Vavg ($F_{1,44}=4.5$, $p=0.36$, $\eta^2=0.10$), and gain ($F_{1,44}=0.2$, $p=1.00$, $\eta^2=0.01$).

In vertical prosaccades, when compared to controls, EP patients showed a trend toward longer latency ($F_{1,54}=7.8$, corrected $p=0.07$, uncorrected $p=0.007$, $\eta^2=0.13$) whereas other eye movement metrics including Vavg ($F_{1,54}=3.3$, $p=0.66$, $\eta^2=0.06$), Vmax ($F_{1,54}=3.5$, $p=0.60$, $\eta^2=0.06$), and gain ($F_{1,54}=2.6$, $p=1.00$, $\eta^2=0.05$) remained normal. In comparison to PD subjects, EP patients manifested significantly shorter latency ($F_{1,44}=13.8$, $p=0.005$, $\eta^2=0.31$) whereas no differences in Vavg ($F_{1,44}=0.1$, $p=1.00$, $\eta^2=0$), Vmax ($F_{1,44}=0.3$, $p=1.00$, $\eta^2=0.01$), and gain ($F_{1,44}=1.4$, $p=1.00$, $\eta^2=0.03$) were observed.

Considering square wave jerks, EP patients produced more SWJs (EP mean number 6.79, SD 6.72, controls mean number 2.26, SD 3.98; $F_{1,54}=9.2$, $p=0.03$, $\eta^2=0.15$) than controls but no difference in SWJ between EP and PD groups were observed (PD mean number 6.38, SD7.34; $F_{1,44}=0.3$, $p=1.00$, $\eta^2=0.01$).

ii) Simple antisaccades (Figure 2): In horizontal direction, EP patients produced more errors than controls ($F_{1,54}=17.8$, $p<0.001$, $\eta^2=0.25$) while there was no significant difference for latency ($F_{1,54}=0.3$, $p=1.00$, $\eta^2=0.01$). No significant differences were noted between PD and EP groups for both latencies ($F_{1,44}=0.6$, $p=1.00$, $\eta^2=0.01$) and errors ($F_{1,44}=0.9$, $p=1.00$, $\eta^2=0.02$).

In vertical direction, latency was found to be longer for EP group when compared to controls ($F_{1,54}=16.5$, $p=0.01$, $\eta^2=0.15$) whereas error rate remained unaffected ($F_{1,54}=3.6$, $p=0.25$, $\eta^2=0.06$). Interestingly, EP patients manifested significantly shorter latencies when compared to PD subjects ($F_{1,44}=10.1$, $p=0.01$, $\eta^2=0.22$). There was no difference between EP and PD group for error rate ($F_{1,44}=0.1$, $p=1.00$, $\eta^2=0$). EP patients showed a rate of movement correction after an incorrect antisaccade of 93%.

iii) Mixed task of pro- and antisaccades (Figure 3 details the results of mixing cost for the latency and error rate of antisaccades): There was increased error rate in EP group when compared to controls ($F_{1,54}=15.6$, $p<0.001$, $\eta^2=0.23$), whereas no differences were found for latency ($F_{1,54}=1.3$, $p=0.50$, $\eta^2=0.03$). No differences between EP and PD groups were seen for both latency ($F_{1,44}=0.2$, $p=1.00$, $\eta^2=0.01$) and error rate ($F_{1,44}=0$, $p=1.00$, $\eta^2=0$).

No correlations were found between the neuropsychological assessment scores and eye movement metrics in EP patients.

Discussion

Ephedrone patients, in comparison to healthy controls, had slow and hypometric horizontal saccades, long latencies of vertical antisaccades, a high error rate in the horizontal antisaccade task,

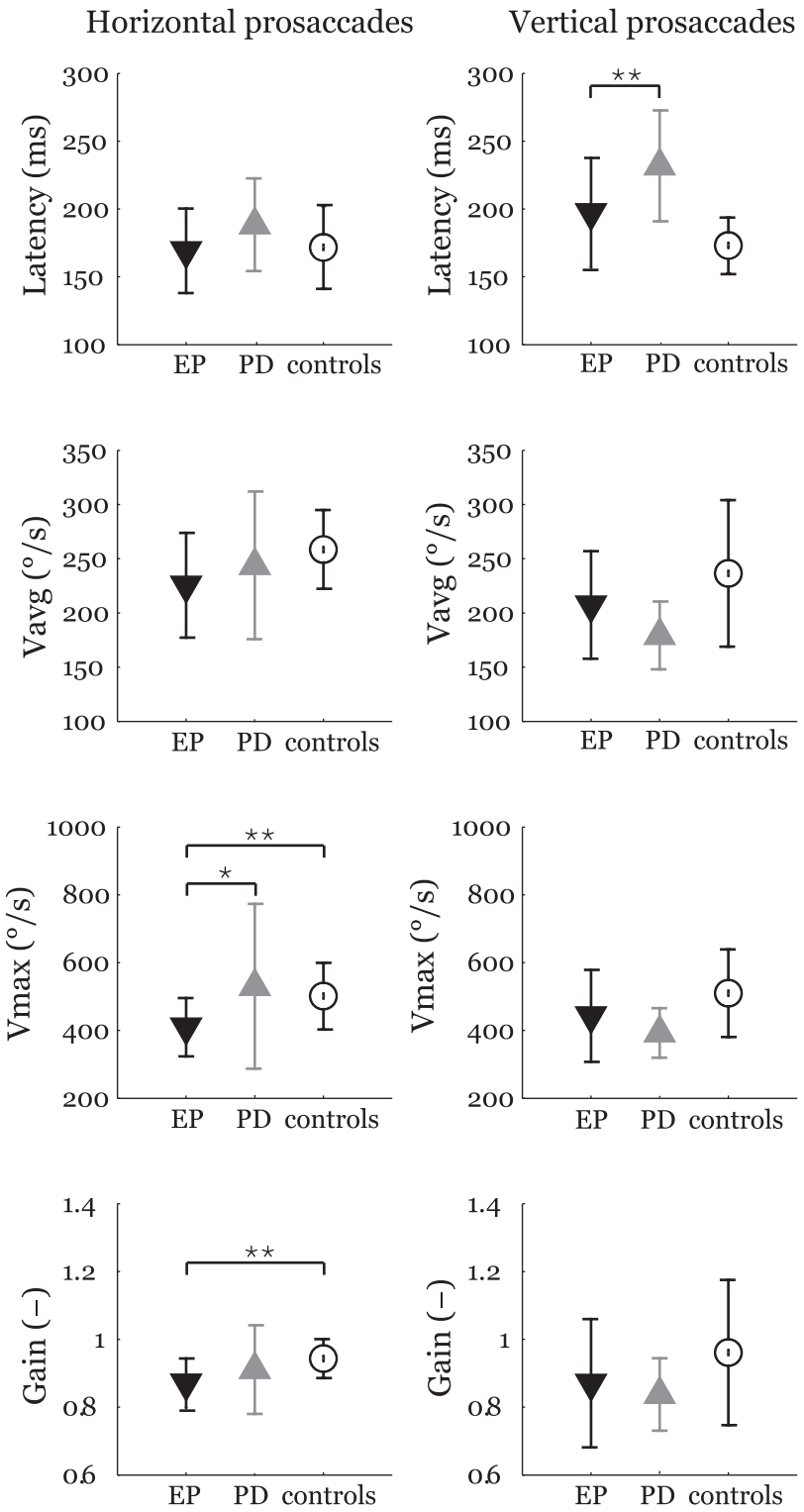

Figure 1. Latencies, average velocities (Vavg), maximal velocities (Vmax), and gains for horizontal (left) and vertical (right) prosaccades. Comparison of EP patients with PD and healthy control groups after Bonferroni adjustment: *$p<0.05$; **$p<0.01$; ***$p<0.001$. The symbols represent mean values and error bars standard deviations. EP = ephedrone parkinsonism; PD = Parkinson's disease.
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more errors than controls when pro- and antisaccades were mixed, and an increased occurrence of square wave jerks. The only direct significant difference between EP and PD concerned a slower peak velocity of horizontal saccades in EP. Yet, the latency for both vertical prosaccades and antisaccades was prolonged in EP when compared to healthy controls. In particular, an isolated prolon-

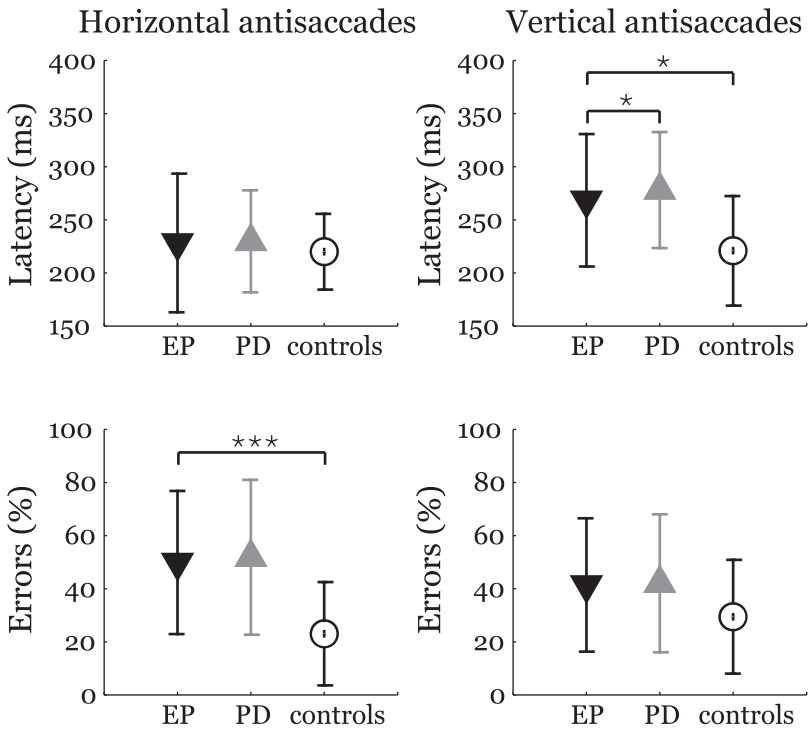

Figure 2. Latencies and error rates for horizontal (left) and vertical (right) antisaccades. Comparison of EP patients with PD and healthy control groups after Bonferroni adjustment: *$p<0.05$; **$p<0.01$; ***$p<0.001$. The symbols represent mean values and error bars standard deviations. EP = ephedrone parkinsonism; PD = Parkinson's disease.
doi:10.1371/journal.pone.0104784.g002

gation of latency of vertical, but not horizontal saccades, has to the best of our knowledge, not been observed previously. This difference suggests that the saccade reaction time may be driven independently in the horizontal and vertical plane, and highlights again the importance of studying EM in both directions [27]. In general terms the latency of saccades has been related to bilateral [28] activation of the posterior parietal and frontal cortices [29]. Nevertheless a study by Kaneko implicates also subcortical structures in the control of this metric, showing in the pharmacologically-inactivated nucleus reticularis tegmenti pontis of the monkey brain, unusually long latency of vertical saccades [30].

Horizontal prosaccades were slower and hypometric when comparing EP patients with controls, while the latency was preserved [29,31]. Slow and hypometric prosaccades are also hallmarks of patients with hereditary ataxias, vascular lesions at the pons and cerebellum, Gaucher's disease Type 3 and Tay-Sachs disease [29,32]. However, in those disorders, saccades seem to be considerably slower, clinically and in recordings. The velocity of horizontal saccades has been related to the prepontine reticular formation [31], while the accuracy, a less specific eye movement measure, may be distorted in disorders of the cerebellum, brainstem and peripheral oculomotor pathways [29].

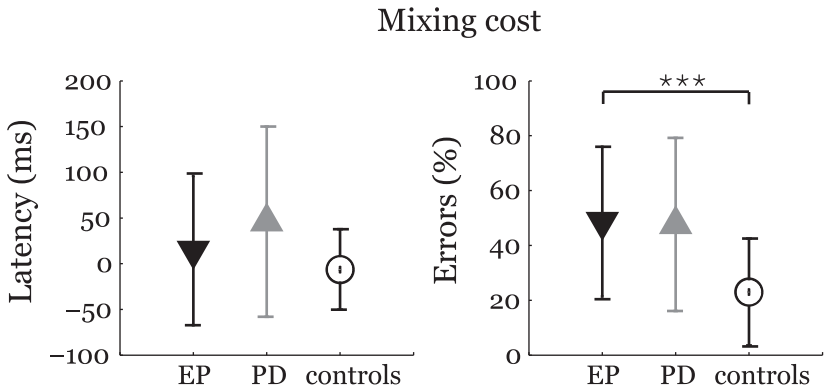

Figure 3. Latency and error rate for mixing cost. Comparison of EP patients with PD and healthy control groups after Bonferroni adjustment: *$p<0.05$; **$p<0.01$; ***$p<0.001$. The symbols represent mean values and error bars standard deviations. EP = ephedrone parkinsonism; PD = Parkinson's disease.
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EP patients presented an increased number of SWJ during saccade tasks. The pathophysiology of SWJs is unknown, but they have been related to disruption of cerebral, cerebellar, basal ganglia function [33,34] and specifically in lesions of the GP [16,33,35]. High number of SWJ has been previously reported in PD [36], after unilateral pallidotomy [16,37], or stimulation of the nucleus subthalamicus [38], and they have also been found in progressive supranuclear palsy (PSP) [39]. Similar to PSP, EP patients show gait and speech disturbances, and a non levodopa responsive parkinsonian syndrome. However, in PSP the predominant eye movement defects concern slow and hypometric vertical saccades [40], while those metrics were mostly preserved in our EP group.

Both in our EP and PD patients, the antisaccade error rate was increased for horizontal, but not for vertical antisaccades. To our knowledge, such dissociation between high error rates in the horizontal and not in the vertical plane has not been described before. These changes are not related to age since we demonstrated in a previous study that both metrics increase with age but not in a dissociated manner [27]. In humans and non-human primates, the dorsolateral prefrontal cortex (DLPFC) has been related to inhibition of reflexive saccades [41]. Impaired inhibition of reflexive horizontal saccades has been described in PSP patients associated with the involvement of the DLPFC in the degenerative process [42]. Recent non-human primate studies suggest that the GP might regulate eye movements through the nigro-collicular descending circuitry, via the basal ganglia thalamocortical pathways, playing an important role in suppressing inadequate antisaccades [43]. Consequently, a specific involvement of the GP might underlie the increased antisaccade error rate in EP patients [44] but it does not explain the dissociation between horizontal and vertical antisaccade direction.

In addition, our EP patients exhibit an increased error rate when pro and antisaccades were mixed. Mixing costs for pro- and antisaccade error rates were low in our control group, in agreement with previous studies [45,46] whereas it was increased in PD as previously described [47], without significant difference to EP patients. The increased mixing cost has been associated to recruitment of additional cerebral structures as the supplementary eye field [48], leading to the hypothesis that its activation may partially reflect task shifting [49,50].

As already mentioned, the only significant difference in oculomotor performance between EP and PD concerned peak horizontal saccade velocity. It may reflect a distinct impairment of specific neural networks underlying the pathology of EP.

A homozygous mutation of the Mn transporter SLC30A10 causing severe hypermanganesemia, dystonia, parkinsonism, polycythemia, and chronic hepatic disease has recently been described [51]. SLC30A10 is highly expressed in the GP, subthalamic nucleus, putamen, deep cerebellar nuclei, and other diencephalic and cortical areas [51]. At the annual meeting of the American Academy of Neurology in 2013, Pretegiani and Rufa [52] presented two cases of SLC3A10 mutations with eye movement abnormalities similar to those found in our EP patients, including slow and hypometric horizontal saccades, but also a high error rate in the antisaccade task. This suggests that manganese toxicity may be the determining factor in the pathogenesis of eye movement abnormalities in EP.

There were no correlations found in our data set between the VOG metrics and severity of eye movement abnormalities as rated by the oculomotor part of the NNIPPS. We chose the NNIPPS as it is the only available clinical scale that includes eye movement evaluation in patients with atypical parkinsonian syndromes. However, NNIPPS allows to semiquantitatively rate only amplitude and speed of voluntary horizontal and vertical saccades. Therefore it may not be sufficiently sensitive to reliably capture distinct but discrete oculomotor abnormalities observed using VOG in our EP group. In particular, latencies and error rates of antisaccades were clearly abnormal in EP but their evaluation is not contained in the NNIPPS. Anyhow, this highlights the importance of incorporating VOG examination, as a sensitive non-invasive tool to reveal slight oculomotor changes. Furthermore, although eye movement performance has been shown to be correlated with UPDRS subscores [53], cognitive function in PD [54,55] and/or verbal fluency [56], we did not reveal any correlation between the severity of neuropsychological impairment assessed with MMSE, BDI and FAB and EM metrics in our EP group. One possible explanation is that our EP patients manifested only very mild cognitive impairment and therefore a more specific neuropsychological assessment would be needed to reveal possible relationships between cognitive and eye movement functions.

In summary, the present study shows that eye movement abnormalities due to ephedrone abuse share similar features but also exhibit certain differences from PD. Similarly to PD patients, subjects with ephedrone-induced parkinsonism demonstrate decreased gain for horizontal prosaccades, increased occurrence of square wave jerks, long latencies of vertical antisaccades as well as a high error rate in the horizontal antisaccade task and when mixing pro- and antisaccades. On the other hand, aspects such as decreased peak velocity of horizontal saccades and affection of latencies only in vertical direction can correspond to pathogenic mechanisms of ephedrone-induced parkinsonism reflecting a specific involvement of globus pallidus and other brain structures due to manganese intoxication.

Acknowledgments

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Author Contributions

Conceived and designed the experiments: CB J. Rusz ER. Performed the experiments: CB J. Rusz MM OM MO HB TN JH MK NM NB IK T. Serranová OF J. Roth ER. Analyzed the data: CB J. Rusz T. Sieger JB. Contributed reagents/materials/analysis tools: SRP BG. Contributed to the writing of the manuscript: CB J. Rusz. Provided review and critique: T. Sieger MM OM MO HB TN JH MK NM NB IK MJ T. Serranová OF J. Roth JB RJ SRP BG ER.

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2.7. The atypical Parkinson syndrome progressive supranuclear palsy: The study of eye movements correlating a symptom with a brain neurotransmitter.

GABA Spectra and Remote Distractor Effect in Patients with Progressive Supranuclear Palsy: A pilot study

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Introduction

Progressive supranuclear palsy (PSP) is an atypical parkinsonian syndrome characterized by supranuclear ophthalmoplegia, axial dystonia, pseudobulbar palsy, early falls and subcortical dementia.[138] Cerebral cortical hypometabolism due to a combination of loss of interneurons containing benzodiazepine receptors and differentiation of the cerebral cortex from distant brain regions have been related to the pathophysiology of PSP.[139] Previous studies have shown that PSP patients may improve fine motor skills, dexterity, and voluntary saccadic eye movements after zolpidem (an agonist of the benzodiazepine subtype receptor BZ1).[140-144] To test the hypothesis that alterations in GABAergic transmission underlie the motor symptoms of PSP, we intended to analyse oculomotor performance in relation to frontal cortical concentrations of GABA in patients with PSP.

A method widely used for GABA determination is magnetic resonance spectroscopy (MRS), which allows the detection of metabolites in humans.[145] GABA levels have been correlated with people's susceptibility to distraction examined with an eye movement paradigm, the remote distractor effect (RDE).[146] The RDE consists of the delay of saccades to simple visual targets, when an irrelevant stimulus appears elsewhere in the visual field.[147] The RDE involves cell populations coding for visually guided saccades and inhibition of distractor, either at the level of the superior colliculus or within the cortical eye fields.[148] Summer has shown that healthy subjects with higher GABA levels, have more efficient suppression of distractors, so lower RDE.[146]

Based on these observations, we assumed that PSP patients will show higher RDE and lower GABA spectra at the frontal cortex, including the frontal eye field. To assess this issue we measured GABA levels with 3T MRS and investigated the RDE in 7 PSP patients and 8 age and gender matched subjects.

Methods

Subjects

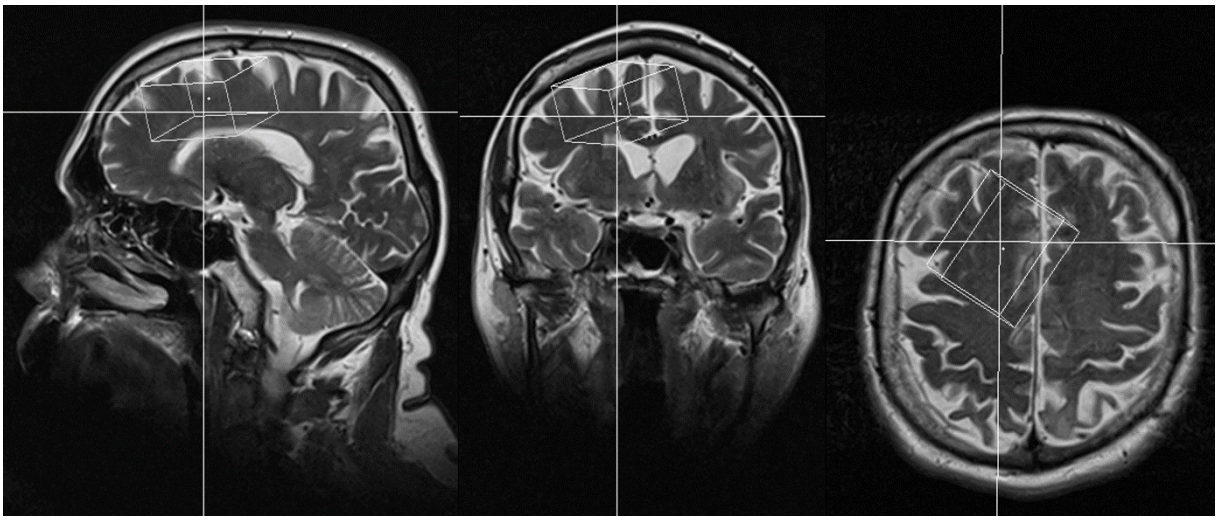
All participants signed the informed consent. The study was approved by the Ethics Committee of the 1st Faculty of Medicine and General University Hospital, Prague, Czech Republic and was in compliance with the Declaration of Helsinki. We included seven right handed patients with probable PSP according to the NINDS-SPSP clinical criteria [60] (3 males and 4 females), age ranged 59 - 76 years (median 66), disease duration 2 -10 years (median 5). Clinical evaluation was done with the Natural History and Neuroprotection in Parkinson Plus Syndromes–Parkinson plus scale (NNIPPS).[61] Neuropsychological testing consisted of the Montreal Assessment Battery (MOCA) and Frontal Assessment Battery (FAB). Two patients were treated with levodopa (daily dose 300 and 500 mg, respectively) and one with amantadine (daily dose 200 mg), while five patients were not taking any drugs.

In addition, eight right handed, healthy subjects (5 males and 3 females), age ranged from 56 to 74 years (median 67), were examined as a control group. A questionnaire was used to determine that all controls were free of any neurological or psychiatric illness and not taking any pharmacotherapy possibly interfering with neural transmission.

Spectra acquisition

GABA spectra were acquired always in the morning time, using whole-body 3T MR scanner (Trio, Siemens, Germany) using MEGA-PRESS (TE=68 ms, TR=1500 ms, 1024 time points, number of accumulations 256, excitation frequency 3 ppm) single-voxel sequence using head bird-cage transmit-receive head coil. The volume of interest (VOI) of cca 45 ml was placed in the right frontal brain region as depicted in Fig. 13.

Figure 13. The position of the volume of interest (VOI cca 45 ml) for the 1H MR spectra measurement in all three projections.



Automated shimming followed by the manual shimming were employed to achieve optimal spectra quality. 3 CHESS pulses (bandwidth = 50 Hz) were used for water suppression. Each signal accumulation was saved separately and referenced to the selected (first) signal accumulation based on the position of the maximum of the remaining water peak. All accumulations were summed up subsequently and processed by LCModel program.[149] Optimized LCModel basis set has been used for spectra fitting in the range 2.6 - 4.6 ppm. We calculated concentration [mM, laboratory units] of GABA.

Oculomotor examination

Eye movements were examined using a binocular video-based eye tracker (mobile eBT Eye brain, Ivry-sur-Seine, France, www.eye-brain.com, 300 Hz sampling rate and 0.5° spatial resolution). Saccades were automatically detected according to a velocity threshold (Eye brain software) but were individually inspected and manually corrected by the experimenter if necessary. The left eye trace was analysed by default, however the right eye was used if the left eye signal was contaminated by artefacts. Saccades perturbed by blinks or other artefacts were discarded (less than 10% of the trials in all subjects). Saccades with a latency below 80 ms were considered anticipatory saccades and rejected, and SRT between 81 and 130 ms were considered "express saccades".[150]

Subjects were seated in a calm, dark room with their chin supported by a chin strap and their forehead in contact with a frontal support. They faced a flat, 26 inch LCD screen

(ProLite, Iiyama model PL 2600, size 550 mm x 344 mm) located 60 cm in front of them at eye level.

The RDE was assessed in one session of 30 minutes duration according to Bompas.[147] Each trial started with the appearance of the central fixation point. When the fixation point switched off, a small green target appeared at the right or at the left side of the screen. In some trials a bright irrelevant stimulus (the distractor) appeared in the opposite location at various delays relative to target onset. Participants were instructed to move their eyes as fast as they can to the target, ignoring any other stimuli. The fixation point was a small red square localized at the center of the screen (15 x 15 pixels; luminance: 120 cd/m2). The target stimulus: a small green square (15 x 15 pixels; luminance: 120 cd/m2), presented at 13deg eccentricity either on the left or on the right of fixation a black background. Distractor stimuli: larger light grey squares (20 x 20 pixels, luminance: 120 cd/m2) centred at 13deg eccentricity. Each trial began with the fixation point for 500 ms. The fixation point disappeared with the target onset which appeared randomly on the right (R) or left (L), for 300 ms. On some trials, the target appeared alone without distractor. Distractors were presented for 50 ms with 6 different stimulus onset asynchronies, ranging from 80 ms before (-20, -50, -80) to 80 ms after the target (20, 50, 80). We performed the trial in a fixed order: first paradigm without distractor, and then with distractor -80, 80, -50, 50, -20, 20. The target stimulus was randomly presented four times to the right and four times to the left side. The remote distractor effect (RDE) was considered as the percentage increase of latency of a correctly performed saccade in trials with distractor compared to the no-distractor condition. We also measured the frequency of occurrence of erroneous saccades towards the distractor.

Statistics

A Mann-Whitney U test for two independent samples was used for comparison between variables of PSP and healthy control group. Bonferroni adjustment was used to correct for the number of comparisons (30). The corrected level of significance was set as $p = 0.0017$.

Results

The NNIPPS score of PSP patients ranged from 64 to 116 (median 100) and the NNIPPS oculomotor subscore ranged from 9 to 15 (median 13). MOCA score of patients ranged from 9-24 (median 15.9) and FAB ranged from 8-16 (median 12.5). The GABA concentrations ranged from 0.18 to 2.10 mM (median 0.82) in the PSP group and from 0.55 to 2.40 mM (median 1.02) in the control group. (Table 6).

Table 6: Clinical characteristics of patients: NNIPPS Natural History and Neuroprotection in Parkinson Plus Syndromes–Parkinson plus scale; SD: standard deviation; MOCA: Montreal Assessment Battery; FAB: Frontal Assessment Battery.

	PSP			**Controls**		
	mean	SD	Range	mean	SD	Range
NNIPPS score	90,6	20,3	64–116	n/a	n/a	n/a
NNIPPS ocular subscore	12,1	2,2	9–15	n/a	n/a	n/a
MOCA	15,9	5,1	9–24	25,5	3,9	21–29
FAB	12,5	3,2	8–16	16,3	2,8	10–18
GABA levels	1,03	0,62	0.18–2.10	1,16	0,58	0.55–2.40

Considering spectra acquisition, there were no significant differences on GABA concentrations between PSP and control group ($p = 0.61$).

The RDE did not demonstrate any significant differences between patients and controls (Table 7). However when pooling the results of early and late distractor, we found significant increased number of errors in PSP compared to controls, and even if not significant, shorter saccade latencies in PSP than in controls.

Table 7: Results and statistics of the remote distractor effect (RDE)Time DP: time of distractor presentation; R: right; L: left; Lat: latency; 0: same time of target; (20)-(50)-(80): distractor presented 20, 50 or 80 ms after target; (m.20)-(m.50)-(m.80): distractor was presented 20, 50 or 80 ms before target.

Time DP	**Side**	**Parameter**	**PSP**		**Controls**		**Mann-Whitney U test**
sec	**R/L**	**Lat (ms)/Error (% x 100)**	**Median**	**IQR**	**Median**	**IQR**	***p***
no distractor	R	Lat	185	87,75	287,5	43,19	0,014
no distractor	L	Lat	198,63	68,75	297,25	82,75	0,1807
0	R	Lat	328,08	161,42	287,5	43,19	0,7308
0	R	Error	0,25	0,56	0,29	0,50	0,8842
0	L	Lat	187,5	90	336,5	205,08	0,0426
0	L	Error	0,5	0,35	0,25	0,63	0,143
20	R	Lat	294	166,81	316,38	101,88	0,3357
20	R	Error	0	0,5	0	0,13	0,3963
20	L	Lat	202,67	219,58	361	64,71	0,2141
20	L	Error	0,5	0,73	0	0,25	0,0118
50	R	Lat	223,33	188	331,88	113,83	0,3357
50	R	Error	0	0,25	0	0,13	0,5921
50	L	Lat	283,67	259	333,17	57,25	0,5974
50	L	Error	0,25	0,19	0	0,13	0,0351
80	R	Lat	178,25	210,44	322,5	70,75	0,152
80	R	Error	0,25	0,25	0	0	0,0513
80	L	Lat	247,75	99,23	332,79	47,25	0,0401
80	L	Error	0	0,25	0	0	0,359
m.20	R	Lat	255	116,13	343,5	105,29	0,0205
m.20	R	Error	0,5	0,38	0,25	0,38	0,519
m.20	L	Lat	265	181,38	344,92	50,83	0,1709
m.20	L	Error	0,75	0,38	0,29	0,38	0,0044
m.50	R	Lat	160,5	116,42	338,75	55,5	0,0932
m.50	R	Error	0,5	0,63	0,25	0,29	0,2918
m.50	L	Lat	333	264,25	352,5	56,38	0,9333
m.50	L	Error	0,75	0,46	0,38	0,5	0,0345
m.80	R	Lat	335,33	53,63	325	143,69	0,5167
m.80	R	Error	1	0,75	0,5	0,54	0,3465
m.80	L	Lat	437	144,5	326,75	41	0,1061
m.80	L	Error	0,75	0,19	0,13	0,75	0,0662
Summed data, all distractors							
no.distractor		Lat	190,63	75,88	306,75	45,71	0,0734
early distractors		Lat	263,50	165,69	353,93	44,18	0,152
		Error	0,65	0,24	0,28	0,41	0,027
late distractors		Lat	251,20	168,39	332,39	64,89	0,1893
		Error	0,35	0,17	0,10	0,15	0,0059

Discussion

Contrary to our expectations, we did not find any statistically significant differences in GABA concentrations between PSP patients and controls. Our negative results may be explained by the low number of investigated subjects or by the VOI where we measured GABA, namely the right frontal brain region. Indeed, measuring GABA transmission in PSP seems to be a difficult task, taking into account previous conflicting results. GABA/A receptors or glutamic acid decarboxylase (GAD) activity have been found to be diminished at the anterior cingulate cortex,[139] globus pallidus,[151] putamen, external pallidum, and hippocampus.[152, 153] Other authors found normal [154] or even increased GABA in autopsied brains of PSP patients.[155]

We failed also to demonstrate any statistically significant differences in the detailed RDE performance between PSP patients and controls. However when pooling all results between no distractor, early and late distractor, we obtain higher error rate and shorter latencies in PSP. Some authors argue that saccadic inhibition and the RDE reflect the same mechanism [156, 157] others that saccadic inhibition produces the major component of the RDE.[158] Our results are in line with the known loss of saccade inhibition reflecting prefrontal dysfunction in PSP.[104, 159]

We conclude that no clear relationship between increased RDE and abnormal GABA concentrations was revealed in the present small scale trial.

Larger studies would be needed to measure GABA transmission in the brain and its relation to distraction susceptibility and other behavioral features of PSP patients.

PART III

DISCUSSION

This thesis builds the first milestone of the constitution of a Videooculography laboratory in our faculty.

We provided a simple algorithm with video explanation, for students and clinicians on how to examine EM in a few minutes in the clinical practice.[41] This paper gave a general overview of the classification of eye movements, anatomy and neurophysiological description of the function of the EM system. We highlighted on the interest of the patient history, the accurate examination about position and movement during saccades and pursuit EM. At last, we described in this paper how to define principal patient symptoms as diplopia, oscillopsia, vertigo and the principal signs as strabismus, nystagmus, skew deviation, ocular tilt, square wave jerks, flutter, opsoclonus, eye muscles palsies, and disorders of saccades and pursuit. Finally our paper broadly describes the most commonly used technique, videooculography.

The normative study [42] aiming to review all normative studies allows new labs to facilitate the creation of their norms. We concluded that there is no difference between females and males, neither between the different educational levels of the subjects. Age is relevant for the analysis of EM. In fact, the EM system ages with the rest of all cognitive functions of the brain. We also concluded that EM should be investigated in the horizontal and vertical plane, due to the anatomical segregation of both kinds of EM. The eye movement paradigm should be simple and feasible to perform in a short time. This was probably the reason of the negative results of the PSP distraction study (too complicated and long). We conclude that with aging the reaction time of pro and antisaccades becomes longer. The velocity decreases, saccades became less accurate and the error rate for antisaccades increased, probably due to hemisphere aging and biomechanical changes of the eye. Metrics as velocity and accuracy did not change with aging, indicating probable preservation of brainstem and cerebellum in the lifespan of the patient.

Another interesting finding of our study was the high error rate of elderly healthy subjects, with preserved capacity to monitor their mistakes. This should be taken in account in studies with elderly patients, for example, investigating frontal involvement in PSP or temporal dementias. We finally propose an index to be used in the clinical practice, comparing the SRT of horizontal pro- and antisaccades. This index should be >1, and it may be useful to analyse diseases with prolongation of the SRT as the Corticobasal syndrome.

Convergence insufficiency and blurred vision are common non-motor symptoms of patients with PD and atypical Parkinsonism. We examined VEM in a group of PD patients compared to controls,[43] but also in a small group of patients with PSP, MSA and EP. Because of the burden of statistical power the results of the last three groups of patients weren't published. We found that the SRT increases in all patients groups. Parkinson disease patients have milder disturbances compared to EP, and the atypical parkinsonian syndromes that show additional decreased velocity and accuracy for convergence and divergence. We found that velocity and accuracy are more effected for divergence than for convergence, pointing to a separate control of both EM. We have demonstrated objectively for the first time that diplopia, blurred vision or near vision in parkinsonian syndromes, is secondary to disturbances in the VEM pathways.

We have investigated EM in patients with iRBD. We found two groups of patients, one with pure iRBD with similar EM to controls, and one with few (asymptomatic) signs for Parkinsonism with similar EM disturbances as patients with PD. This second group of patients had indeed long SRT for vertical saccades and a high error rate in the antisaccade task. We observed that these abnormalities were correlated with bad performance on the MOCA test but did not correlate with disease duration nor the results of the UPDRS III part.

In the electrophysiological paper we investigated with simultaneous intraoperative microelectrode recordings basal ganglia neuronal activity during scanning eye movements and visually guided saccades in PD.[118] We detected 130 neurons located in the STN, 30 in the SNr and 23 in the GPi, and 20% of these neurons were related to EM activity. Neurons related to scanning eye movements were unrelated to saccades. The number of eye movements made while watching the photographs was higher than while watching the black screens. The results agree with the role of the basal ganglia in the EM control. Our results

suggested that each of the explored structures – STN, SNr and GPi contains a relatively high percentage of neurons involved in the execution and/or control of eye movements.

The ephedrone study was for me one of the most challenging of all the studies realized during my thesis. I was impressed by the severity of neurological symptoms of these very young men, who worsened from year to year with no hope for treatment. Due to the bad economic situation of Georgia, all patients are obliged to live with their families, with immense economic and social consequences. We tried with this study to advance the knowledge of pathophysiology Ephedrone damage, hoping that new ways for treatment could be open. We found slow and hypometric horizontal EM as we can see in Gaucher disease, long SRT and high error rate of antisaccades reflecting cortical involvement.[131] We failed to demonstrate the previously described autonomic symptoms in these patients, by lacking of urinary symptoms, orthostatic hypotension or changes in pupil reactions.

The last submitted study, probably the more ambitious, had unfortunately negative results. PSP patients were videotaped for clinical assessment, they underwent fMRI, Spectroscopy and videooculography. The fMRI study was done in collaboration with the Max Plank institute and Prof. Karsten Müller submitted results in 2016. We were interested in the positive response of few PSP patients to the GABA analog Zolpidem. We hypothesized that patients had less GABA, more distraction and tried to objectively demonstrate it by measuring GABA spectra with spectroscopy and distraction with VOG. Unfortunately our results were inconclusive, probably due to the low number of patients.

CONCLUSION

The basal ganglia and its projections to cerebral cortex, thalamus and brainstem are involved in numerous bran functions such as motor control, sensorimotor integration and cognition. Eye movement's physiology is strongly related to the function of the basal ganglia and findings in PD and other parkinsonian syndromes could demonstrate it.

This thesis provides new insights into pathophysiology and differential diagnosis of PD and other parkinsonian syndromes through EM. It demonstrates the role of the basal ganglia in scanning EM. We suggest that aging influences EM, and that the cerebral control of convergence and divergence VEM are segregated. We offer the first description about disrupted EM control in asymptomatic iRBD patients, suggesting dysfunction of cortical processing in prodromal PD.

Several of our hypotheses were confirmed: i) blurred vision in PD, PSP, MSA and EP patients is due to dysfunction of VEM and this was objectively demonstrated; ii) occulomotricity could be a marker for pre-clinical PD; iii) basal ganglia are involved in scanning eye movements; iv) Eye movements in EP are different from PD. We failed to confirm that PSP patients are more distractible, and have a bigger remote distractor effect (RDE) due to lower GABA levels.

We leave a VOG laboratory in a great team. Our results hopefully will help clinicians, medical students and researchers to approach EM understanding.

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