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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

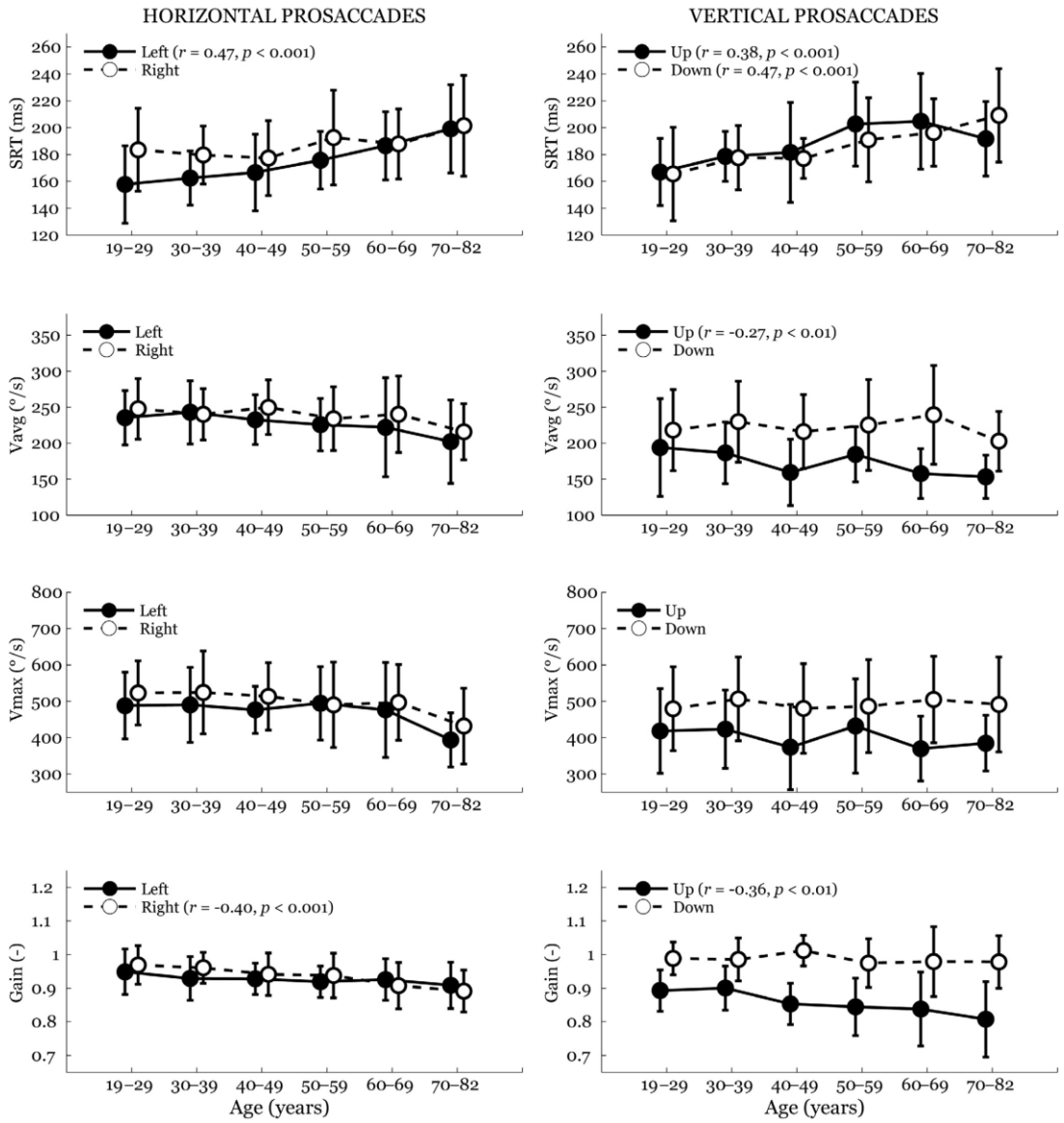

Fig. 1. Age-related changes of horizontal and vertical prosaccades. SRT: saccade reaction time; V_{avg}: average velocity; V_{max}: maximal velocity; left: target presented at the left side; right: target presented at the right side; up: target presented up; down: target presented down.

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

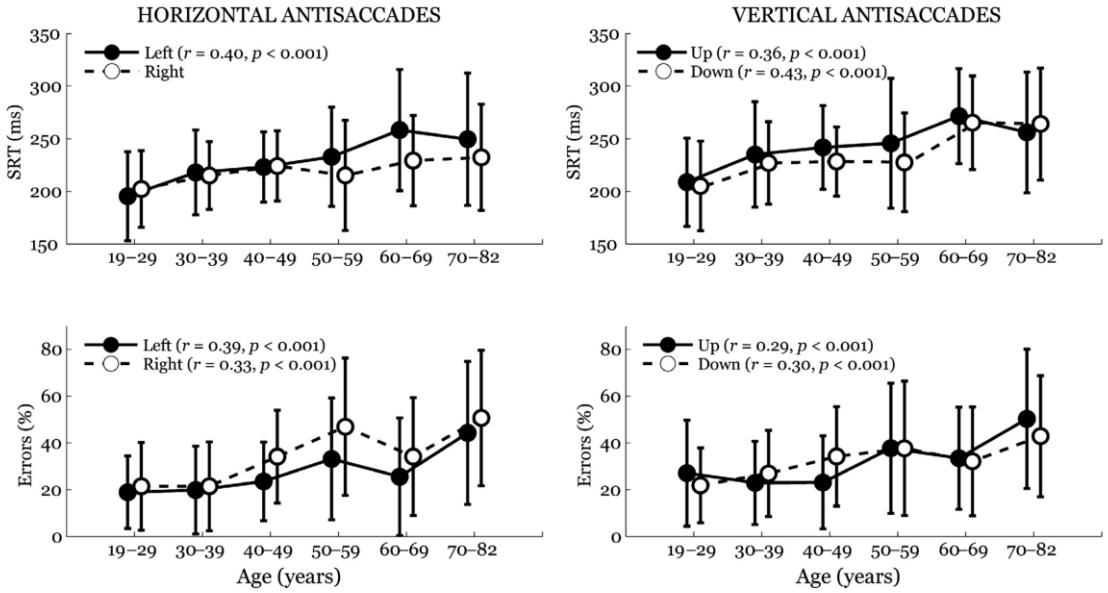

Fig. 2. Age-related changes of horizontal and vertical antisaccades. SRT: saccade reaction time; left: target presented at the left side, correct movement to the right; right: target presented at the right side, correct movement to the left; up: target presented up, correct movement downward; down: target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

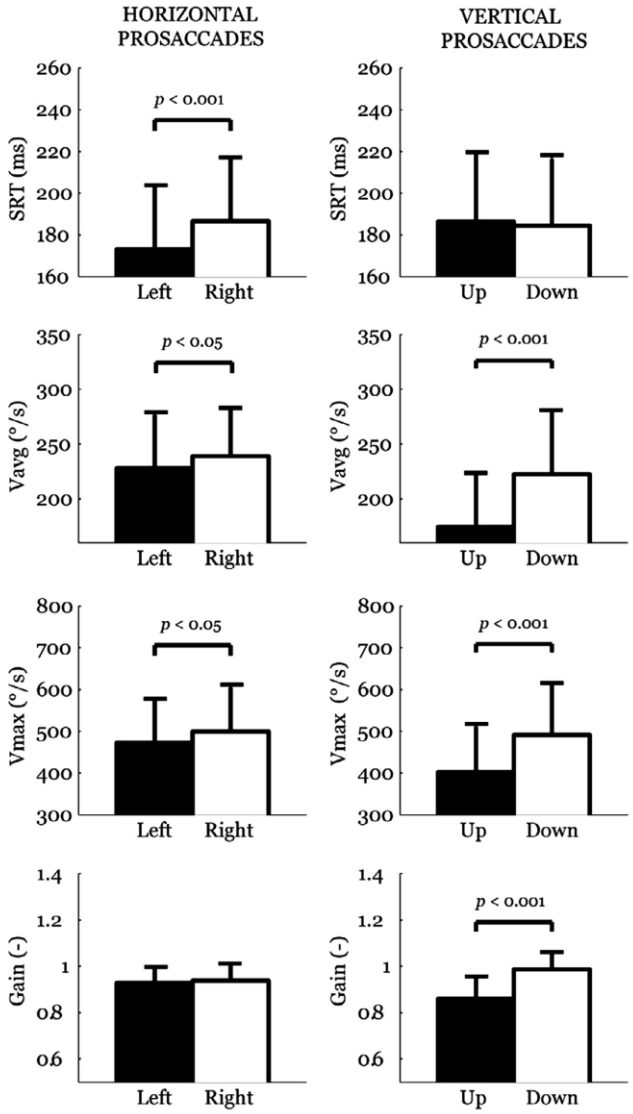

Fig. 3. Side/direction differences in prosaccades. SRT: saccade reaction time; V_{avg}: average velocity; V_{max}: maximal velocity; left: target presented at the left side; right: target presented at the right side; up: target presented up; down: target presented down.

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.

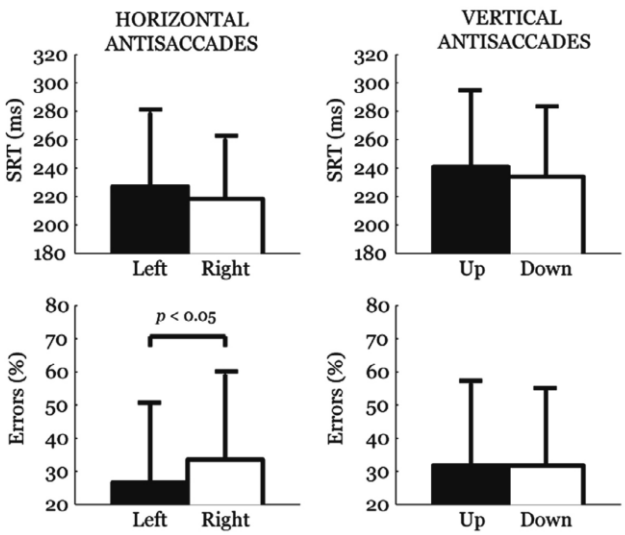

Fig. 4. Side/direction differences in antisaccades. SRT: saccade reaction time; left: target presented at the left side, correct movement to the right; right: target presented at the right side, correct movement to the left; up: target presented up, correct movement downward; down: target presented down,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

None reported. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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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,

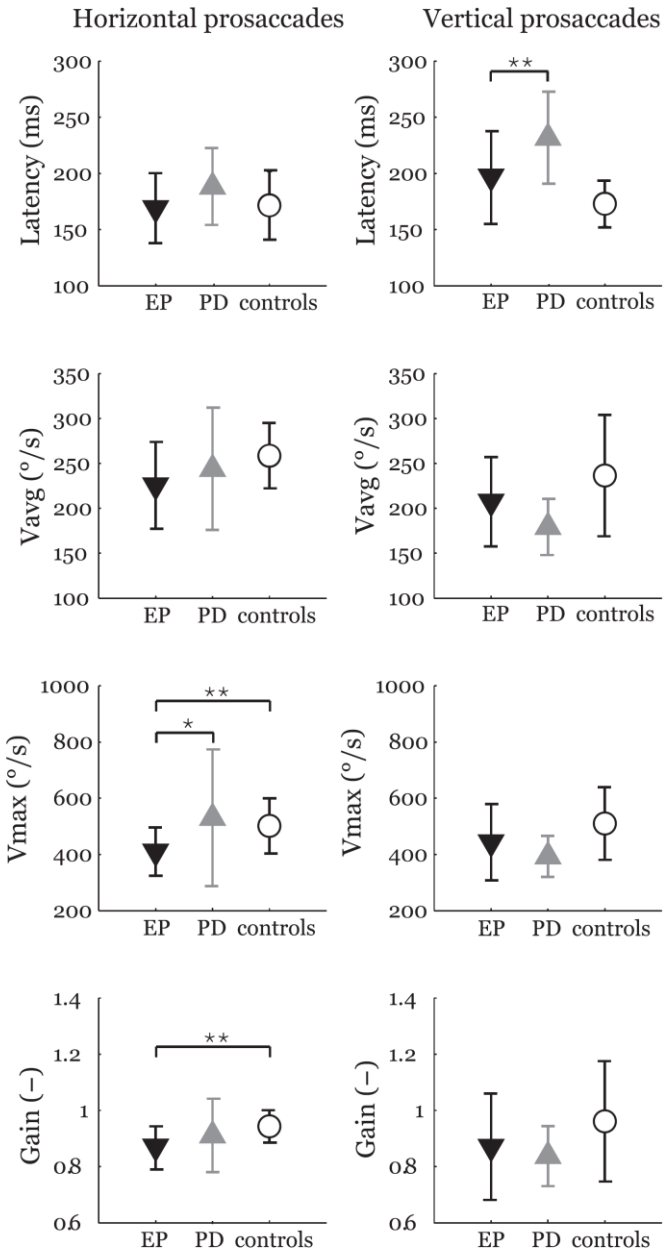

Figure 1. Latencies, average velocities (Vavg), maximal velocities (Vmax), and gains for horizontal (left) and vertical (right) prosaccades. Comparison of EP patients with PD and healthy control groups after Bonferroni adjustment: *$p<0.05$; **$p<0.01$; ***$p<0.001$. The symbols represent mean values 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-

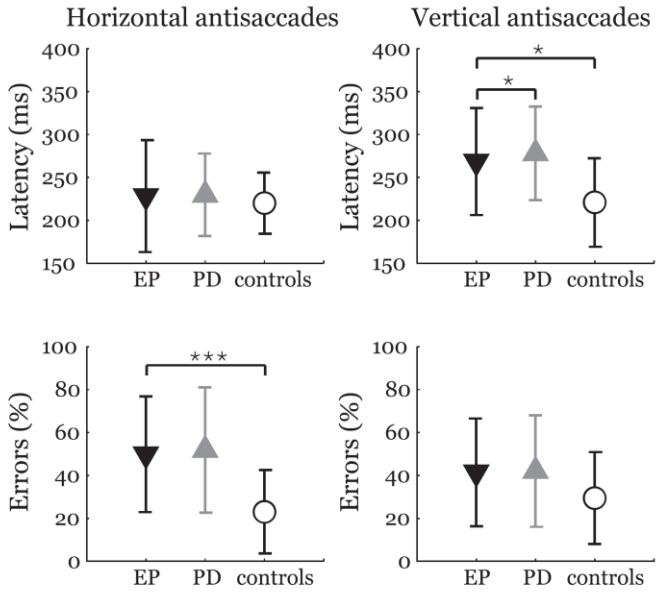

Figure 2. Latencies and error rates for horizontal (left) and vertical (right) antisaccades. Comparison of EP patients with PD and healthy control groups after Bonferroni adjustment: *$p<0.05$; **$p<0.01$; ***$p<0.001$. The symbols represent mean values and error bars standard deviations. EP = ephedrone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

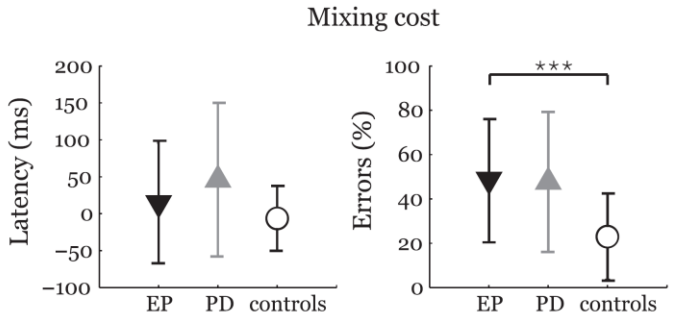

Figure 3. Latency and error rate for mixing cost. Comparison of EP patients with PD and healthy control groups after Bonferroni adjustment: *$p<0.05$; **$p<0.01$; ***$p<0.001$. The symbols represent mean values and error bars standard deviations. EP = ephedrone parkinsonism; PD = Parkinson's disease.
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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.

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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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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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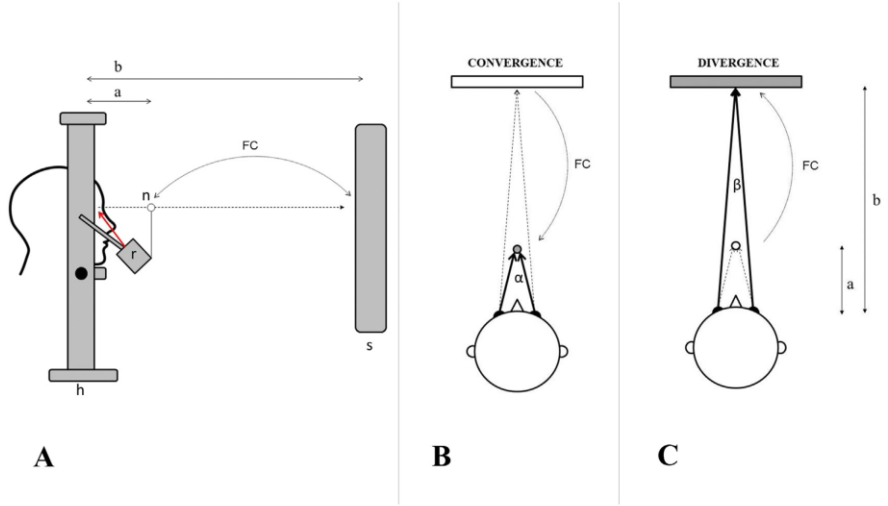

Fig. 1. Eye movement task employed. A: patient position; B: examination of convergence; C: examination of divergence; h: head support construction; r: eye movement recorder; n: near fixation point; s: screen; a = 10 cm; b = 60 cm; $\alpha = 43.6^{\circ}$; $\beta = 7.6^{\circ}$; FC: focus change.

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

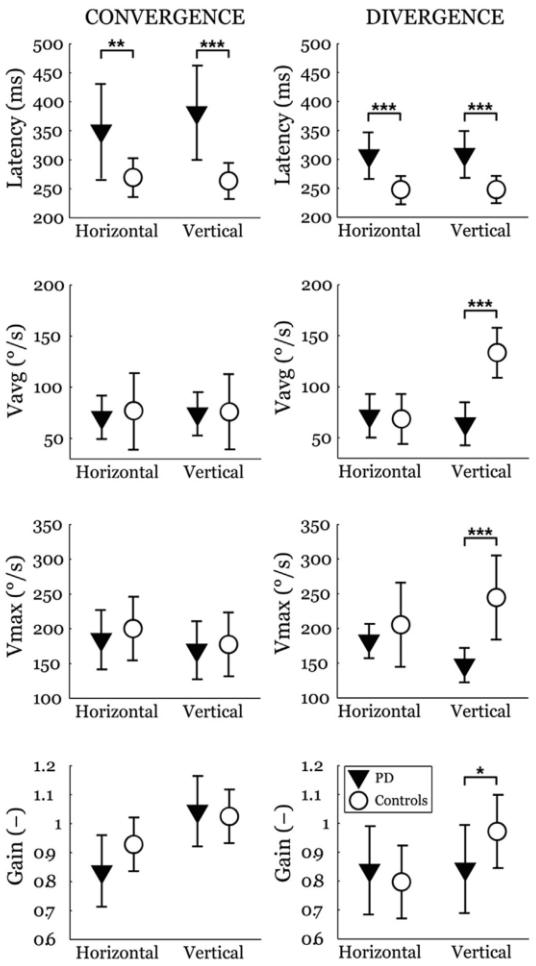

Fig. 2. Latencies, average velocities (Vavg), maximal velocities (Vmax), and gains for convergence and divergence. Comparison between PD and healthy control groups after Bonferroni adjustment: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

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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Disease-Specific Regions Outperform Whole-Brain Approaches in Identifying Progressive Supranuclear Palsy: A Multicentric MRI Study

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To identify progressive supranuclear palsy (PSP), we combined voxel-based morphometry (VBM) and support vector machine (SVM) classification using disease-specific features in multicentric magnetic resonance imaging (MRI) data. Structural brain differences were investigated at four centers between 20 patients with PSP and 20 age-matched healthy controls with T1-weighted MRI at 3T. To pave the way for future application in personalized medicine, we applied SVM classification to identify PSP on an individual level besides group analyses based on VBM. We found a major decline in gray matter density in the brainstem, insula, and striatum, and also in frontomedian regions, which is in line with current literature. Moreover, SVM classification yielded high accuracy rates above 80% for disease identification in imaging data. Focusing analyses on disease-specific regions-of-interest (ROI) led to higher accuracy rates compared to a whole-brain approach. Using a polynomial kernel (instead of a linear kernel) led to an increased sensitivity and a higher specificity of disease detection. Our study supports the application of MRI for individual diagnosis of PSP, if combined with SVM approaches. We demonstrate that SVM classification provides high accuracy rates in multicentric data—a prerequisite for potential application in diagnostic routine.

Keywords: magnetic resonance imaging, progressive supranuclear palsy, atypical parkinsonism, support vector machine classification, voxel-based morphometry

INTRODUCTION

Progressive supranuclear palsy (PSP) is a neurodegenerative disease with a clinical syndrome including atypical parkinsonism, supranuclear palsy, postural instability, and dementia. Neuropathologically, PSP is characterized by the accumulation of tau protein (tauopathy), resulting in neurofibrillary tangles and affecting both neurons and glial cells (Williams and Lees, 2009). It is associated with structural changes in the midbrain, also called "hummingbird" or "penguin sign".

Recently, several studies investigated the pattern of PSP-related structural brain changes using magnetic resonance imaging (MRI) in combination with an analysis technique called voxel-based morphometry (VBM). These studies present, at least partly, conflicting results. Many studies found a diminished gray matter density (GMD) in the left and right insulae (Brenneis et al., 2004; Padovani et al., 2006; Ghosh et al., 2012). Several papers reported a reduced GMD in the thalamus (Cordato et al., 2005; Boxer et al., 2006; Padovani et al., 2006; Shi et al., 2013), but other papers did not find GMD changes here (Brenneis et al., 2004; Ghosh et al., 2012). Volumetric analysis of structural MRI showed a significantly smaller putamen in PSP patients in comparison to healthy controls (Messina et al., 2011). Recently, quantitative and systematic meta-analyses have been introduced to imaging data to identify the prototypical neural correlates of neurodegenerative diseases (Schroeter et al., 2007, 2008, 2009). Meanwhile three comprehensive meta-analyses have applied methods like anatomical likelihood estimates or effect-size signed differential mapping to PSP studies (Shi et al., 2013; Shao et al., 2014; Yu et al., 2015). By investigating gray matter changes, these meta-analyses consistently identified the thalamus, basal ganglia, insula, and midbrain as the disease-specific core network of PSP.

Our study aimed at further investigating structural brain changes associated with PSP by using MRI and VBM using a multicentric approach. We included a cohort of 20 patients and 20 healthy control subjects. Note that 20 patients is a relatively large cohort because of the low prevalence of PSP. We included 11 patients from Germany (Ulm, Homburg, Leipzig) and 9 patients from the Czech Republic (Prague). In order to assess the effect of the different locations and scanning conditions, we analyzed the Czech participants separately, because all Czech participants were scanned under identical conditions. In addition, we also performed a conjunction analysis using the Czech and the German cohort. A high degree of similarity between the VBM results of both cohorts would demonstrate that the effects of PSP in terms of brain degeneration are large enough to be shown across different centers using VBM.

Another aim of the study was the individual classification of PSP patients and healthy controls by machine learning pattern recognition algorithms applied to imaging data with our multi-centric approach. Here, we performed support vector machine (SVM) classification (Chang and Lin, 2011) on the basis of GMD images obtained with VBM. In two recent studies, this approach was already used to classify PSP patients from healthy controls within a unicentric setting (Focke et al., 2011; Salvatore et al., 2014). We demonstrate that high accuracy rates can also be obtained across different centers. Furthermore, the availability of multicentric data for the training of a classifier is a major advantage in the reliable detection of a rare condition. Recently, multicentric SVM classification was shown to achieve high accuracy rates for the differentiation between Alzheimer's disease and frontotemporal lobar degeneration (Dukart et al., 2013). Dukart and colleagues used disease-specific regions of interest (ROI) for SVM feature selection instead of using all brain regions. The ROIs were selected according to comprehensive meta-analyses. Similar to their approach (Dukart et al., 2013) and in contrast to other recent work (Focke et al., 2011; Salvatore et al., 2014), we used disease-specific ROIs based on meta-analytically extracted prototypical neural networks for PSP (Shi et al., 2013; Shao et al., 2014; Yu et al., 2015) and performed SVM classification using the voxels within these ROIs for feature selection in addition to whole-brain analyses. Note that the ROIs are defined here (Shi et al., 2013; Shao et al., 2014; Yu et al., 2015) in an independent cohort in a data-driven manner. We compared the resulting accuracy rates with the results from a whole-brain approach, expecting higher accuracy rates within the ROI-based technique. In addition, we validated the influence of different kernel functions and different approaches of feature selection on results.

METHODS

Subjects

A cohort of 20 PSP patients (7 female, age 67.3 ± 7.8 years, mean ± standard deviation) was compared to a group of 20 age- and gender-matched healthy control subjects (8 female, age 66.3 ± 7.8 years). Multicentric data were obtained in Germany and the Czech Republic (see **Table 1** for demographic details). The German cohort of 11 patients (4 female, age 69.0 ± 9.3 years) and 11 controls (4 female, 68.1 ± 7.8 years) was chosen from the data of the Consortium for Frontotemporal Lobar Degeneration at the centers of Ulm, Homburg, and Leipzig. The Czech subcohort of 9 patients (3 female, 65.2 ± 5.4 years) and 9 controls (4 female, 64.2 ± 7.6 years) was selected in Prague under identical conditions and was, therefore, additionally used to perform a unicentric analysis. Mean age and age variability did not differ between cohorts as investigated by two-tailed two-sample *t*-tests and *F*-tests for equal variances (all comparisons $p > 0.2$). The study was approved by the local ethics committees (Ethics Committee of the General University Hospital in Prague, Czech Republic; Ethics Committee of the University of Ulm, Germany; Ethics Committee of the University of Leipzig, Germany; Ethics Committee of the Saarland Medical Board, Homburg, Germany). All participants were carefully informed about the study and gave signed written consent in accordance with the Declaration of Helsinki.

Data Acquisition

T1-weighted structural brain images were acquired at all four centers using the magnetization-prepared rapid gradient-echo (MP-RAGE) sequence implemented on 3T MAGNETOM scanners (Siemens, Erlangen, Germany; Prague: MAGNETOM Trio; Ulm: MAGNETOM Allegra; Homburg: MAGNETOM Skyra; Leipzig: MAGNETOM Verio). All images were acquired with a nominal resolution of $1 \times 1 \times 1\,\mathrm{mm}^3$. Further imaging parameters are listed in **Table 2**. Note that the same acquisition parameters were used in Homburg and Leipzig, whereas a slightly different set of parameters was used at the other two sites, Prague and Ulm (longer echo time with a smaller imaging bandwidth per pixel).

VBM Analysis

Image processing was performed using the VBM 8 toolbox rev. 435 (Structural Brain Mapping Group, University of Jena,

TABLE 1 | Demographical and scanner data for patients and control subjects.

Patient ID	Age (years)	Sex	City	Scanner	Control ID	Age (years)	Sex	City	Scanner
P08DE	55	m	Ulm	Allegra	C39DE	73	m	Ulm	Allegra
P42DE	74	f	Ulm	Allegra	C54DE	78	m	Ulm	Allegra
P40DE	61	f	Ulm	Allegra	C72DE	75	f	Ulm	Allegra
P48DE	65	m	Ulm	Allegra	C82DE	73	m	Ulm	Allegra
P50DE	64	m	Ulm	Allegra	C85DE	71	f	Ulm	Allegra
P72DE	60	m	Ulm	Allegra	C91DE	71	m	Ulm	Allegra
P55DE	79	f	Leipzig	Verio	C64DE	54	f	Leipzig	Verio
P79DE	70	m	Leipzig	Verio	C80DE	64	m	Leipzig	Verio
P15DE	85	m	Homburg	Skyra	C22DE	72	m	Leipzig	Verio
P32DE	67	f	Homburg	Skyra	C49DE	61	f	Leipzig	Verio
P92DE	79	m	Homburg	Skyra	C46DE	57	m	Leipzig	Verio
P01CZ	68	m	Prague	Trio	C04CZ	65	f	Prague	Trio
P08CZ	69	f	Prague	Trio	C06CZ	66	f	Prague	Trio
P12CZ	64	m	Prague	Trio	C07CZ	72	f	Prague	Trio
P15CZ	61	f	Prague	Trio	C09CZ	58	m	Prague	Trio
P18CZ	61	m	Prague	Trio	C19CZ	54	m	Prague	Trio
P21CZ	75	f	Prague	Trio	C25CZ	61	m	Prague	Trio
P26CZ	69	m	Prague	Trio	C29CZ	56	m	Prague	Trio
P32CZ	58	m	Prague	Trio	C31CZ	70	f	Prague	Trio
P33CZ	62	m	Prague	Trio	C34CZ	76	m	Prague	Trio

CZ, Czech Republic; DE, Germany; f, female; m, male.

TABLE 2 | Acquisition parameters of the MP-RAGE sequence at all four imaging centers.

Imaging center	Prague	Ulm	Leipzig	Homburg
Scanner	Trio	Allegra	Verio	Skyra
Software	syngo MR B17	syngo MR A30	syngo MR B17	syngo MR D11
Flip angle	10°	8°	9°	9°
Repetition time(ms)	2,300	2,200	2,300	2,300
Echo time(ms)	4.43	4.38	2.98	2.96
Inversion time(ms)	900	1,200	900	900
Bandwidth(Hz/Px)	150	130	238	240
FoV	240 × 256	256 × 256	240 × 256	240 × 256
Image matrix	240 × 256 × 160	256 × 256 × 208	240 × 256 × 176	240 × 256 × 176
Nominal resolution(mm^3)	1 × 1 × 1	1 × 1 × 1	1 × 1 × 1	1 × 1 × 1

MP-RAGE, magnetization-prepared rapid gradient-echo; FoV, field of view; Px, pixel.

Department of Psychiatry, Germany) with Statistical Parametric Mapping 12 rev. 6,470 (The Wellcome Trust Centre for Neuroimaging, UCL, London, UK) and MATLAB 8.6 (R2015b, MathWorks, Inc, Natick, MA). GMD images were generated using the unified segmentation approach that presents a probabilistic framework combining image registration, tissue classification, and bias correction (Ashburner and Friston, 2005). Each voxel within the GMD images contains a measure of gray matter probability obtained by the unified segmentation approach. In order to account for volume changes during normalization, GMD was scaled by the amount of non-linear deformation that is also called modulation. To meet the assumptions of random field theory, GMD images were finally smoothed with a Gaussian kernel of 8-mm full-width at half-maximum (FWHM). Voxel-wise statistical analysis was performed with the general linear model implementing a two-sample *t*-test to compare PSP patients with healthy participants, controlling for age, sex, and total intracranial volume. Clusters were detected using a voxel-threshold of $p < 0.001$. To correct for multiple comparisons, a minimum cluster size of $k > 1{,}000$ was chosen to detect significant clusters with $p < 0.05$, family-wise error (FWE) corrected threshold on the cluster level (Nichols and Hayasaka, 2003).

To study effects induced by a single center, and to assess between-center variability arising from different location and hardware, statistical analyses were performed separately with patients and controls from Prague (unicenter approach) and with patients and controls from the German centers (multicenter

approach). Due to the smaller numbers of patients in both subcohorts, a voxel-threshold of $p < 0.005$ was used. However, a minimum cluster size of $k > 1{,}000$ was again used to report significant clusters at $p < 0.05$, FWE-corrected. A conjunction analysis was performed including the Czech and the German cohort to investigate the overlap of the results between both groups of participants.

To test the variability between the German centers, a second conjunction analysis was performed using two cohorts generated by merging the participants from Prague and Ulm, and by merging the participants from Prague, Homburg, and Leipzig (Homburg and Leipzig used identical scanning parameters, see **Table 2**). In both cohorts, two-sample *t*-tests were performed to detect significant GMD differences between patients and controls using the same threshold as in the conjunction analysis described above (voxel-threshold of $p < 0.005$ in combination with a minimum cluster size of $k > 1{,}000$). Results of both analyses were combined using a conjunction analysis to investigate the overlap.

SVM Analysis

In order to differentiate PSP patients from healthy controls, SVM classification was performed with GMD images using the libSVM software package rev. 3.18 (Chang and Lin, 2011). The libSVM package offers open source software using the sequential minimization optimization algorithm (Platt, 1998) supporting SVM classification and regression. Classification accuracy was obtained by cross-validation using the "leave one out" approach by generating a set of 400 models, leaving a patient and a control subject out when building the classifier. Thereafter, it was checked if both remaining data sets were classified correctly. Sensitivity and specificity were computed from the number of correctly classified patients and controls. To assess the stability of classification results depending on kernel type and feature selection, the analysis was performed with two different kernels (linear, polynomial) and two different approaches of feature selection. First, voxels were used with SPM's gray matter tissue probability map using different minimum gray matter probabilities between 0 and 80% (all thresholds are listed in **Table 3**). Before thresholding, the gray matter tissue probability map was interpolated to meet the resolution of the GMD images obtained by using the VBM toolbox as described above. The gray matter tissue probability map was also smoothed with a Gaussian kernel of 8-mm FWHM, which is the same filter that was applied to the GMD images.

The second approach of feature selection was based on disease-specific ROIs. ROIs were extracted from comprehensive meta-analyses on structural MRI changes in the gray matter in PSP from the literature (systematic literature search in PubMed on June 14, 2016, search terms: supranuclear palsy, meta-analysis, VBM). Based on three relevant meta-analyses (Shi et al., 2013; Shao et al., 2014; Yu et al., 2015), the disease-specific ROIs included the thalamus, basal ganglia, midbrain, and insula, because these brain regions were consistently impaired across all meta-analyses. Note that the definition of disease-specific ROIs in our study was based on comprehensive meta-analyses independent from our data and conducted across whole-brain studies only. ROIs were defined with the WFU-Pickatlas across the aforementioned brain regions. Finally, a model was generated from all patients and controls, and weights of voxels most relevant for SVM classification were detected using the libSVM package (Chang and Lin, 2011).

RESULTS

The VBM analyses revealed a major decline in GMD in PSP patients compared to healthy controls. In particular, a

TABLE 3 | Accuracy, sensitivity, and specificity of support vector machine (SVM) classification with cross-validation generating 400 models excluding a patient and a healthy control when building the classifier.

	Linear kernel						Polynomial kernel					
	PSP	**Ac (%)**	**HC**	**Ac (%)**	**Sens (%)**	**Spec (%)**	**PSP**	**Ac (%)**	**HC**	**Ac (%)**	**Sens (%)**	**Spec (%)**
0.8	**200**	50	**320**	80	71	62	**240**	60	**308**	77	72	66
0.7	**259**	65	**360**	90	87	72	**313**	78	**335**	83	83	79
0.6	**305**	76	**343**	86	84	78	**320**	80	**336**	84	83	81
0.5	**295**	74	**341**	85	83	76	**320**	80	**329**	82	82	80
0.4	**281**	70	**340**	85	82	74	**317**	79	**337**	84	83	80
0.3	**281**	70	**339**	85	82	74	**317**	79	**337**	84	83	80
0.2	**282**	70	**339**	85	82	74	**318**	80	**338**	84	84	80
0.1	**282**	70	**340**	85	82	74	**318**	80	**338**	84	84	80
0.001	**281**	70	**340**	85	82	74	**318**	80	**338**	84	84	80
M1	**299**	75	**360**	90	88	78	**337**	84	**345**	86	86	85
M2	**339**	85	**330**	82	83	84	**340**	85	**323**	81	82	84
M3	**337**	84	**342**	86	85	84	**340**	85	**294**	74	76	83
M4	**299**	75	**357**	89	87	78	**340**	85	**316**	79	80	84

Columns with progressive supranuclear palsy (PSP) patients and healthy controls (HC) show the number of correctly classified participants for two different ways of generating models using a linear and a polynomial kernel. Feature selection was performed with SPM's gray matter tissue probability map using different thresholds, and with three different masks generated by the WFU Pickatlas (Masks: M1 = putamen+pallidum+midbrain; M2 = M1+caudate; M3 = M2+thalamus; M4 = M3+insulae).: Ac, Accuracy; Sens, Sensitivity; Spec, Specificity.

diminished GMD was observed in the brainstem, thalamus, left and right anterior insulae, and also in wide regions in the putamen extending to the pallidum (**Figure 1**, color-coded in yellow/orange). Less prominent differences were detected in lateral orbitofrontal and frontomedian regions. The same regions were obtained when investigating GMD differences in the multicentric German cohort with patients and controls from three different centers (Ulm, Homburg, and Leipzig, **Figure 2**, color-coded in yellow/orange). The German PSP patients showed a diminished GMD in the brainstem, insula, and putamen/pallidum in both hemispheres. Smaller effects were also obtained in frontomedian regions.

The conjunction analysis between the results obtained with the Prague cohort and German cohort revealed a consistent picture of GMD decline in PSP patients compared to controls. We obtained a remarkably large overlap of brain regions affected by PSP in both analyses with the German and Czech participants (**Figure 3**, colored in yellow). The overlap revealed a reduced GMD in patients not only in the putamen and pallidum but also in the insular cortex (colored in yellow). The German cohort showed prominent GMD reductions in the brainstem (colored in red), while the Prague cohort also showed diminished GMD in frontomedian regions (colored in blue).

Figure 4 shows the conjunction between PSP-related GMD decrease obtained from two different cohorts obtained by

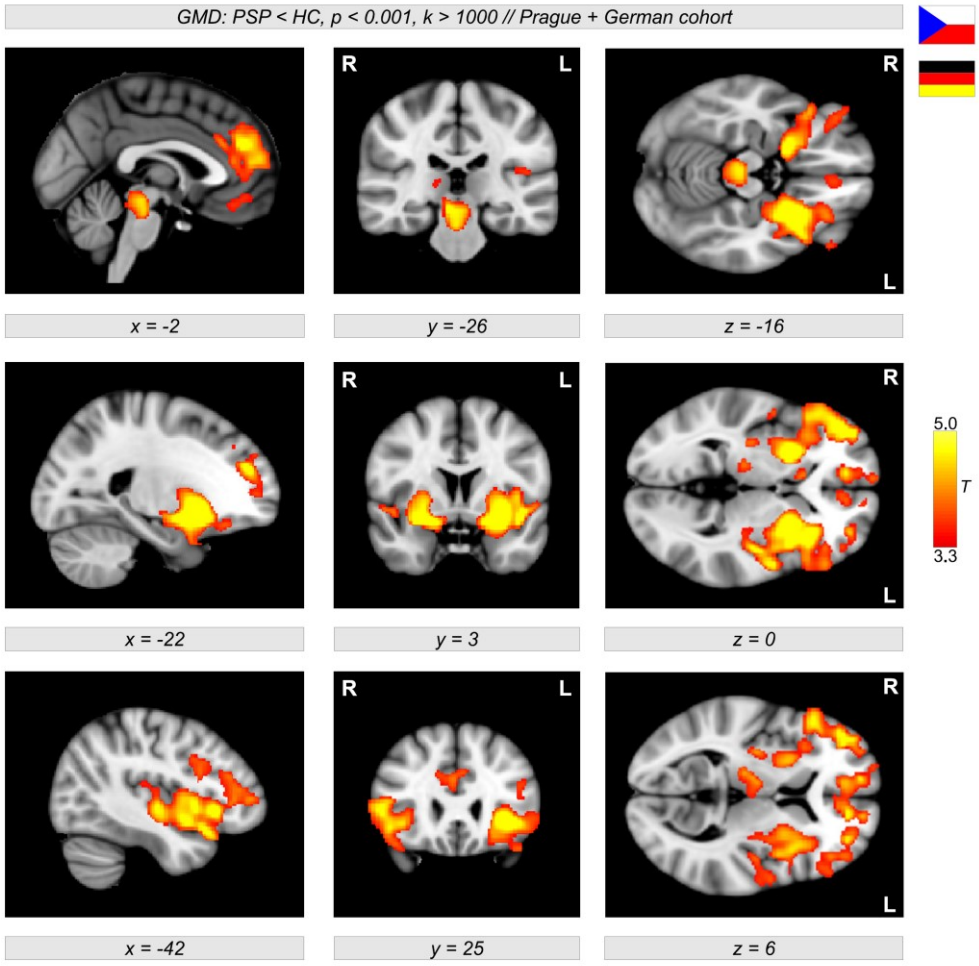

FIGURE 1 | Sagittal, coronal, and axial brain slices showing significant gray matter density (GMD) differences between 20 patients with progressive supranuclear palsy (PSP) and 20 age- and sex-matched healthy controls (HC) (color-coded in red/yellow, $p < 0.001$, $k > 1{,}000$, controlled for multiple comparisons using family-wise error-correction with $p < 0.05$ on the cluster level). In PSP patients, a diminished GMD was observed in the brain stem, insula, frontal cortex, and also in wide regions of the putamen extending to the pallidum.

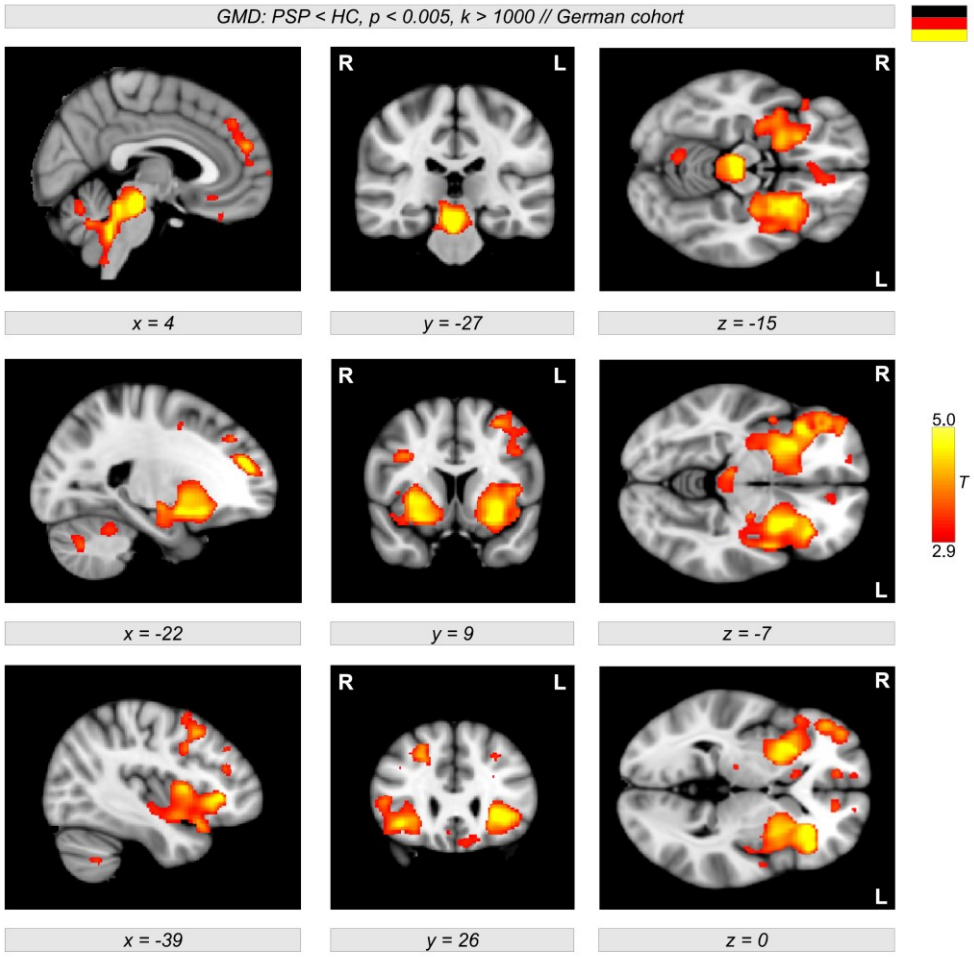

FIGURE 2 | Orthogonal brain sections showing significant gray matter density (GMD) differences between patients with progressive supranuclear palsy (PSP) and healthy controls (HC) (color-coded in red/yellow, $p < 0.005$, $k > 1{,}000$) in the German cohort. Significant clusters are shown using family-wise error-correction with $p < 0.05$ on the cluster level.

merging the participants from Prague and Ulm, and by merging the participants from Prague, Leipzig, and Homburg. An overlap of GMD decrease was observed in the thalamus, putamen, insula, and frontomedian and frontolateral regions (**Figure 4**, color-coded in yellow).

Using SVM classification with a polynomial kernel and a feature selection of voxels within SPM's gray matter mask with different minimum gray matter probabilities, PSP patients were identified with classification accuracy up to 80%. Healthy controls were detected with classification accuracy up to 84% (**Table 3**). The resulting sensitivity was up to 84% and the specificity was up to 81%. Interestingly, we received similar results using different gray matter probabilities ≤60% when choosing voxels within SPM's gray matter mask (**Table 3**). This demonstrates the robustness of SVM classification with respect to feature selection dependent on the minimum gray matter tissue probability. Using a linear kernel, accuracy values were generally lower for the PSP patients at around 70%.

The disease-specific ROI-based SVM approach focusing on the pallidum, putamen, caudate nucleus, thalamus, midbrain, and insula as PSP's core network generally outperformed the classification with all gray matter voxels of the brain with respect to disease detection and specificity of classification (**Table 3**, **Figure 5**). Using different ROIs and a polynomial kernel, we obtained accuracy rates above 84% for detecting PSP patients, which outperformed all other whole-brain approaches using minimum gray matter probabilities (see rows with masks M1–M4 in **Table 3**). Accuracies for detecting healthy control subjects

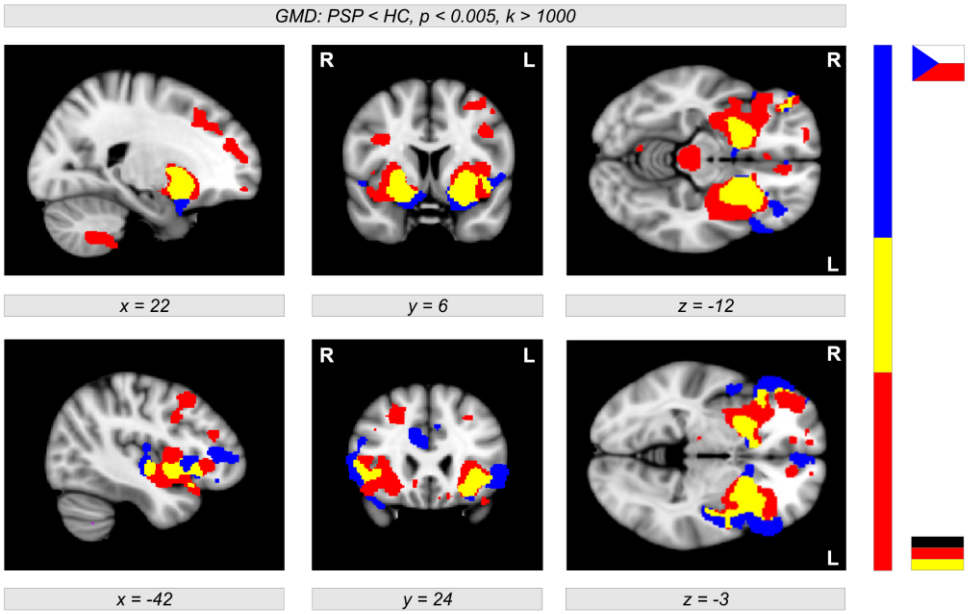

FIGURE 3 | Conjunction analysis showing both the unicentric Prague cohort (color-coded in blue) and the multicentric German sample (Ulm, Homburg, and Leipzig, color-coded in red, see also Figure 2) showing significant gray matter density (GMD) differences between patients with progressive supranuclear palsy (PSP) and healthy controls (HC) ($p < 0.005$, $k > 1{,}000$). The overlap (color-coded in yellow) shows a reduced GMD in patients in the putamen extending to the pallidum, and also in the insular cortex. The German cohort showed prominent GMD reductions in the brainstem, while the Prague cohort also showed diminished GMD in frontomedian regions.

varied between 74 and 86%, depending on the ROI. However, the specificity was always above 83%.

Finally, when using a model for all patients and healthy controls, relevant voxels for classification were observed in the brainstem, putamen, pallidum, and caudate nuclei, but also in cerebellar regions (**Figure 5**). Thus, using the whole-brain approach (first row in **Figure 5**), we observed the same regions that are discussed in the context of PSP in recent VBM studies (Shi et al., 2013; Shao et al., 2014; Yu et al., 2015).

DISCUSSION

Several recent studies aimed at investigating structural gray matter differences between PSP patients and controls using VBM (Price et al., 2004; Cordato et al., 2005; Ghosh et al., 2012). In line with these findings, we show a disease-related GMD decrease using a multicentric approach based on a relatively large sample of patients. A recent meta-analysis (Shi et al., 2013) investigating nine VBM studies with a total of 143 PSP patients suggested a crucial role of the insula and basal ganglia in PSP, which is corroborated by our findings. However, the most prominent finding (Shi et al., 2013) was a GMD decline in the thalamus, which we obtained when restricting the analysis to patients from Prague, Leipzig, and Homburg (see **Figure 4**, brain regions colored in red). Note that the thalamus is not included in the list of principal areas affected by the disease (Keith-Rokosh and Ang, 2008). Finally, we also found a major GMD reduction in gray matter regions in the vicinity of the brainstem, which suggests atrophy and seems to reflect the so-called hummingbird sign proposed as a pathognomonic entity in PSP.

In a more recent meta-analysis, Yu et al. (2015) studied 39 VBM publications investigating brain atrophy in PSP, corticobasal degeneration, and multisystem atrophy (MSA). In total, 176 PSP patients were included in this analysis. In line with our findings and the literature (Shi et al., 2013; Shao et al., 2014), they reported brain atrophy in insular brain regions and the thalamus. Our major result of GMD decrease in the putamen and pallidum appears divergent from Yu et al. (2015): While we excluded MSA patients from our cohort associating striatal GMD decline with PSP, Yu and colleagues (Yu et al., 2015) showed striatal GMD reduction solely for MSA, but not PSP. The authors claim that striatal atrophy might distinguish MSA from PSP (see Figure 2 in Yu et al., 2015). This is not in line with our findings, which show a more complex and widespread involvement of brain regions with PSP.

To distinguish different patterns of neurodegenerative diseases, SVM classification across GMD maps can be a useful tool beyond group comparisons. However, the question remains as to whether it is possible to achieve a robust dissociation between atypical Parkinson syndromes due to different patterns of brain degeneration. In a recent study, Focke et al. (2011) used SVM classification with a relatively small number of 10

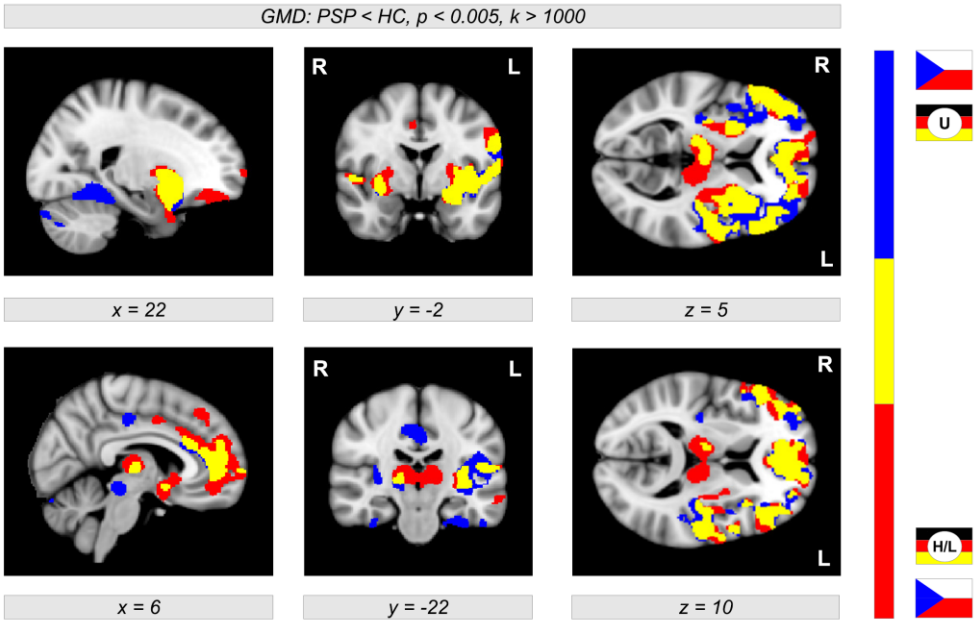

FIGURE 4 | Conjunction analysis showing significant gray matter density (GMD) differences ($p < 0.005$, $k > 1{,}000$) in two groups of participants merging the participants from Prague and Ulm (U), and the participants from Prague, Homburg, and Leipzig (H/L). The overlap (color-coded in yellow) shows major reductions of GMD in patients with progressive supranuclear palsy (PSP) in the thalamus, putamen, insula, and also in frontomedian and frontolateral brain regions in comparison with healthy controls (HC).

PSP patients compared to 21 idiopathic Parkinson's disease (IPD) patients and 22 healthy controls. They did not observe a significant distinction between PSP patients and healthy controls based on GMD images (sensitivity 20%; accuracy 65.6%; see Table 4 in Focke et al., 2011). This is quite surprising considering the major GMD differences between PSP patients and controls that we observed. We achieved sensitivity, specificity, and accuracies above 80%. Relevant voxels for the SVM classification were located in exactly the same regions as detected with the VBM statistics investigating atrophy in PSP patients vs. controls. This major difference between our findings and the results of Focke et al. (2011) might be due to different disease stages or different sample sizes of the PSP patients. Probably, a sample of 10 PSP patients is a limitation for achieving sufficient sensitivity for classification. On the other hand, we also analyzed our German (11 patients) and Czech (9 patients) cohorts separately and received very similar GMD differences in both analyses (shown by the conjunction analysis in **Figure 3**). Unfortunately, Focke et al. (2011) did not show VBM comparisons between PSP patients and healthy controls, but only GMD differences between PSP and IPD; hence, whether the obtained differences are predominantly based on PSP or IPD cannot be disentangled. This within-disease comparison might be a reason why they (Focke et al., 2011) did not detect GMD changes in the striatum and insula, because of neurodegeneration in PSP and IPD in the same brain regions. Their cerebellar findings might be related to IPD and not to PSP. Note that both meta-analysis studies (Shi et al., 2013; Yu et al., 2015) do not report involvement of the cerebellum in PSP. It is even more interesting that Focke et al. (2011) obtained a significant SVM classification between PSP and IPD. However, they did not report a significant classification between patients and healthy controls— neither for PSP nor for IPD patients.

A more recent study used SVM classification with quite a large number of 28 PSP patients by performing a principal components analysis (PCA) on T1-weighted structural images (Salvatore et al., 2014). In contrast to the study of Focke et al. (2011), they also obtained high accuracy rates in comparisons of PSP or IPD patients with healthy controls with very similar patterns of relevant voxels for both conditions (Salvatore et al., 2014). Furthermore, they were able to dissociate PSP and IPD directly with relevant voxels mainly detected in the thalamus (Shi et al., 2013) and also in the cerebellum, which is in line with previous findings (Focke et al., 2011). In agreement with our results, Salvatore et al. (2014) detected relevant voxels in the medial part of the midbrain, whereas the striatum and insula did not contribute to their classification. This seems surprising because of the involvement of these regions in PSP as consistently shown in our study and previous meta-analyses (Shi et al., 2013; Yu et al., 2015). Whereas Salvatore et al. (2014) performed the SVM

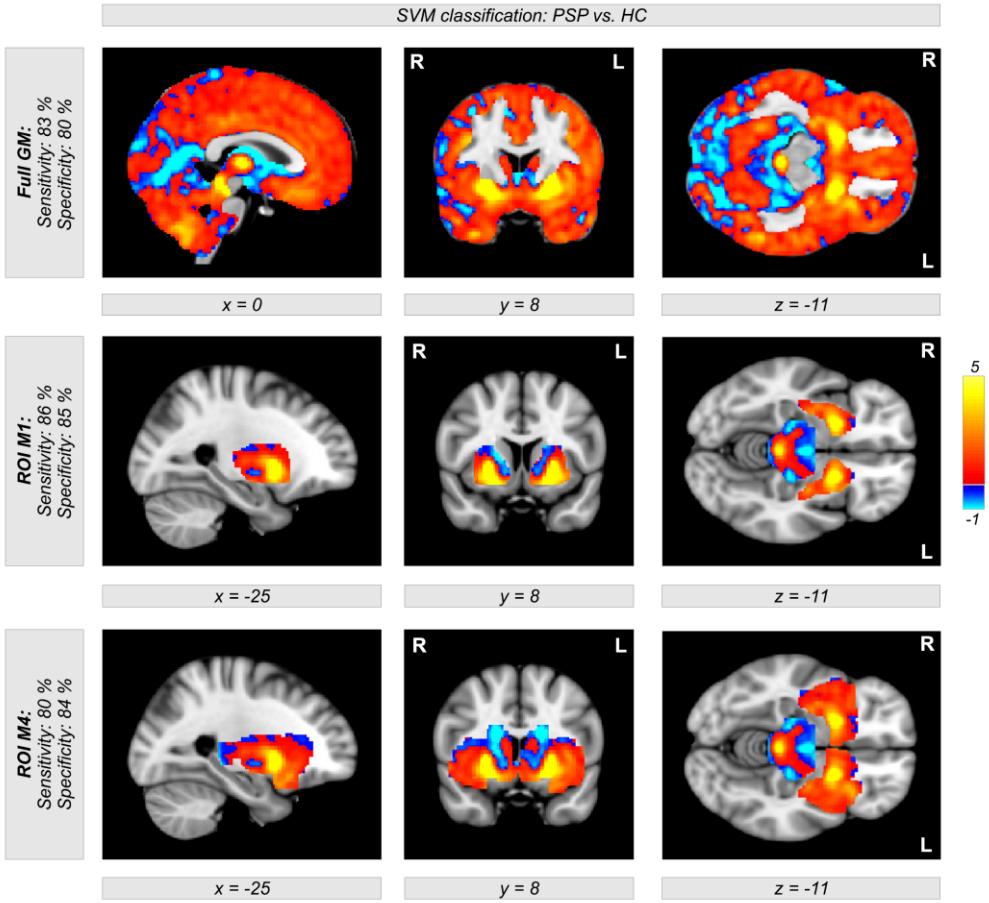

FIGURE 5 | Weights of voxels most relevant for support vector machine (SVM) classification between both groups of patients with progressive supranuclear palsy (PSP) and healthy controls (HC) after SVM training. Note that these weights are relative and have no applicable units. Relevant voxels are located in the brain stem, putamen, and pallidum. Classification accuracy was obtained with a polynomial kernel using cross-validation generating a set of 400 models leaving a patient and a control subject out when building the classifier. SVM classification was performed on all voxels within the SPM's gray matter mask (tissue probability >0.4, top row). The middle and the bottom row show the same analysis using regions of interest instead of all gray matter voxels (middle row: M1 = putamen+pallidum+midbrain; bottom row: M4 = M1+caudate+thalamus+insulae).

analysis using a PCA on T1-weighted images, we applied SVM to the GMD images. The combination of the SVM technique with the VBM approach might be more sensitive for relating gray matter changes in the striatum and insula to PSP.

Both previous SVM studies (Focke et al., 2011; Salvatore et al., 2014) applied a linear kernel for SVM classification, which reflects the default setting in the libSVM software package. However, other kernels might be more suitable, leading to higher accuracy rates and an improved sensitivity and specificity. In a recent study, Huppertz et al. (2016) performed SVM classification using a radial basis function (RBF) kernel. However, the extraction of SVM weighting factors is mathematically only defined for the linear kernel and not for an RBF kernel. Therefore, the SVM approach was repeated with a linear kernel to identify the most relevant regions for classification (Huppertz et al., 2016). Motivated by a recent paper demonstrating the advantage of using a polynomial kernel showing an improved accuracy when dissociating mild cognitive impairment from Alzheimer's disease (Belmokhtar and Benamrane, 2012), we also used a polynomial kernel for PSP disease classification. We clearly obtained an improved accuracy when comparing the accuracy rates with the use of a polynomial and a linear kernel. Note that the polynomial kernel allows kernel parameters that enable a change in the balance between specificity and sensitivity. In the future, this might be helpful when using sensitive approaches for disease detection.

In contrast to previous studies that used whole-brain approaches (Focke et al., 2011; Salvatore et al., 2014), we also

showed that classification can be improved when using a disease-specific ROI-based approach for SVM feature selection. ROIs were defined in independent and comprehensive cohorts in a data-driven manner with meta-analyses across whole-brain studies (Shi et al., 2013; Shao et al., 2014; Yu et al., 2015) avoiding circular approaches. This is in line with previous work comparing the meta-analytically inspired ROI-based approach with the whole-brain approach for feature selection. Combining disease-specific ROI approaches with several imaging modalities can improve the classification accuracy that was shown for identification of and differentiation between Alzheimer's disease and frontotemporal lobar degeneration (Dukart et al., 2011, 2013). Therefore, future studies might combine ROI-based feature selection with additional imaging modalities, such as diffusion tensor imaging sensitive to changes in white matter or positron-emission tomography sensitive to metabolism, or clinical features and biomarkers from serum/cerebrospinal fluid for PSP detection that can help us to understand the specific pattern of disease-related brain atrophy in PSP. Further development of SVM-based classification might complement the radiologist's MRI-based diagnostics for PSP disease detection and characterization.

In sum, our study investigated structural brain differences between PSP patients and healthy controls. To pave the way for future application in personalized medicine, we applied SVM classification to identify PSP on an individual level. Using VBM, we found a major decline in GMD in the brainstem, insula, putamen, pallidum, and also frontomedian regions. SVM classification yielded high accuracy rates in multicentric data, a prerequisite for application in diagnostic routine. Focusing analyses on disease-specific ROIs and using an advantageous kernel led to higher accuracy rates. Our study supports the application of MRI for individual diagnosis of PSP, if combined with SVM approaches. Classification results might also be improved by advantageous kernel and feature selection.

AUTHOR CONTRIBUTIONS

General conception: KM, RJ, CB, JT, JH, HM, ER, MS; Study design: KM, RJ, MS; Data analysis: KM; Figures: KM; Drafting the manuscript: KM; Final preparation of the article: KM, RJ, HM, MS; Members of the FTLD consortium: KF, AL, JK, MO, MS.

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Short communication

GABA spectra and remote distractor effect in progressive supranuclear palsy: A pilot study

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ABSTRACT

Disturbances of the gamma-aminobutyric-acid (GABA) system have been suspected of contributing to the pathophysiology of progressive supranuclear palsy (PSP). The ability to rapidly resolve competitive action decisions, such as shifting the gaze to one particular stimulus rather than another, can be predicted by the concentration of GABA in the region of the frontal cortex relevant to eye movements. For this reason, our study measured GABA levels in seven PSP patients and eight healthy controls, using proton magnetic resonance spectroscopy, and assessed the relationship of these measurements to the remote distractor effect (RDE), an eye-movement paradigm investigating competitive action decisions. No significant differences were found in either frontal-eye-field GABA levels or RDE between PSP patients and controls.

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1. Introduction

Progressive supranuclear palsy (PSP) is an atypical parkinsonian syndrome characterized by supranuclear ophthalmoplegia, axial dystonia, pseudobulbar palsy, early falls and subcortical dementia [1]. Cerebral cortical hypometabolism due to the combined loss of interneurons containing benzodiazepine receptors and differentiation of the cerebral cortex from distant brain regions has proved to be related to the pathophysiology of PSP [2]. Previous studies have shown that PSP patients may improve their fine motor skills, dexterity and voluntary saccadic eye movements with zolpidem (a benzodiazepine receptor [subtype BZ1] agonist) treatment [3–7]. To test the hypothesis that alterations in gamma-aminobutyric acid-ergic (GABAergic) transmission underlie the motor symptoms of PSP, the present study aimed to analyze 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 [8]. GABA levels have been correlated with a susceptibility to distraction when examined by an eye-movement paradigm, the remote distractor effect (RDE) [9]. The RDE consists of a delay of saccades with simple visual targets when an irrelevant stimulus appears elsewhere in the visual field [10]. The RDE involves cell populations coding for visually guided saccades and distractor inhibition at either the level of the superior colliculus or within the cortical eye fields [11]. Sumner et al. [12] showed that healthy subjects with higher GABA levels have more efficient suppression of distractors, resulting in lower RDEs.

Based on these observations, it was assumed that PSP patients would probably show higher RDE and lower GABA spectra in the frontal cortex, including the frontal eye field. To assess this theory, GABA levels, measured by 3 T MRS, and the RDE were investigated in seven PSP patients and eight age- and gender-matched subjects (controls).

2. Methods

2.1. Subjects

All participants gave their informed consent. The study was approved by the Ethics Committee of the First faculty of medicine and General University Hospital, Prague, Czech Republic, and was in compliance with the Declaration of Helsinki. The study included seven right-handed patients with probable PSP, according to National institute of neurological disorders and stroke (NINDS)/Society for PSP (SPSP) clinical criteria [13], comprising three men and four women (age range: 59–76 [median: 66] years; disease duration: 2–10 [median: 5] years). Clinical evaluation was based on functional measures of the Neuroprotection and natural history in Parkinson plus syndromes (NNIPPS)–Parkinson plus scale (PPS) [14]. Neuropsychological testing consisted of the Montreal cognitive assessment (MOCA) battery of tests and the Frontal assessment battery (FAB). Two patients were being treated with levodopa (300 mg/day and 500 mg/day, respectively) and one with amantadine (200 mg/day), whereas five patients were not taking any drugs.

In addition, eight healthy, right-handed subjects (five men and three women), ages 56–74 (median: 67) years, were included as the control group. A questionnaire was used to determine that all controls were free of any neurological or psychiatric illness and were not taking any pharmacotherapy that could possibly interfere with neural transmission.

2.2. Spectral acquisition

GABA spectra were acquired always in the morning, with a whole-body 3 T MR scanner (MAGNETOM Trio, Siemens, Munich, Germany) and MEGA-PRESS spectroscopy (echo time [TE]: 68 ms; repetition time [TR]: 1500 ms; time points: 1024; number of accumulations: 256; excitation frequency: 3 ppm) in a single-voxel sequence, using a birdcage transmit–receive head coil. The volume of interest (VOI; cca 45 mL) was placed in the right frontal brain region (Fig. 1). Automated shimming was followed by manual shimming to achieve optimal spectral quality. Three chemical-shift selective saturation (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 remaining water peak. All accumulations were subsequently summed up and processed using an LCModel software program [15]. The optimized LCModel basis set has been used for fitting spectra in the 2.6–4.6 ppm range. The calculated concentration (mM, laboratory units) was used to express GABA measurements.

2.3. Oculomotor examination

Eye movements were examined using a binocular video-based eye-tracking device (Mobile EBT, e(ye)BRAIN, Ivry-sur-Seine, France), with a 300-Hz sampling rate and 0.5° spatial resolution. Saccades were automatically detected according to a velocity threshold (e[ye]BRAIN software), but were individually inspected and manually corrected by the experimenter if necessary. The left eye trace was analyzed by default, although the right eye was used if the left eye's signal was contaminated by artifacts. Saccades perturbed by blinks or other artifacts were discarded (< 10% of trials of all subjects). Saccades with a latency < 80 ms were considered anticipatory saccades and rejected, and sacchadic reaction times (SRTs) of 81–130 ms were considered "express saccades" [16].

Each participant was seated in a quiet dark room, chin supported by a chinstrap and forehead in contact with a frontal support, facing a flat 26-inch liquid-crystal display (LCD) screen (Iiyama ProLite, model PL 2600, size 550 mm × 344 mm) located 60 cm in front of the subject at eye level.

The RDE was assessed in one 30-min session, as described by Bompas and Sumner [10]. Each trial started with the appearance of the central fixation point. When the fixation point was switched off, a small green target appeared on either the right or left side of the screen. In some trials, a bright irrelevant stimulus (the distractor) appeared on the opposite side after a variable delay relative to the target appearance. Participants were instructed to move their eyes as fast as possible to the target, ignoring any other stimuli. The fixation

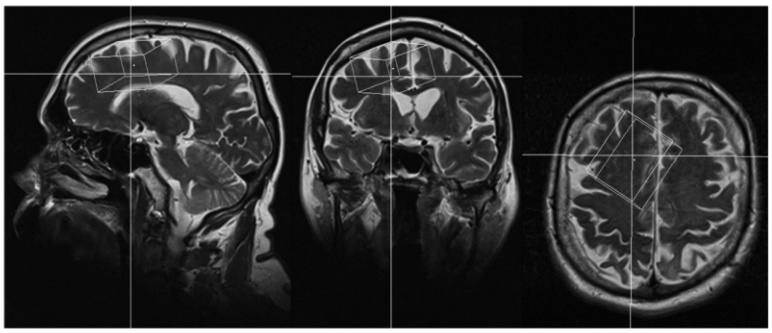

Fig. 1 – Placement of volume of interest (VOI; cca 45 mL) for ^{1}H nuclear magnetic resonance (NMR) spectral measurement in all three projections.

point was a small red square in the center of the screen (15 × 15 pixels; luminance: 120 cd/m^2); the target stimulus was a small green square (15 × 15 pixels; luminance: 120 cd/m^2) appearing at a 13° eccentricity on either the left or right of the fixation point on a black background. Distractor stimuli were larger light-gray squares (20 × 20 pixels, luminance: 120 cd/m^2) centred at a 13° eccentricity.

Each trial began with the fixation point displayed for 500 ms, before disappearing when the target appeared randomly on either the right (R) or left (L) for 300 ms. On some trials, the target appeared without the distractor. Distractors were presented for 50 ms with six different stimulus onset asynchronies, ranging from 80 ms before (−20, −50, −80) to 80 ms after (20, 50, 80) the target appeared. The trials were always performed in a fixed order: first, the paradigm without the distractor and then with the distractor (−80, 80, −50, 50, −20, 20). The target stimulus was randomly presented four times on the R and four times on the L. The RDE was calculated as the percentage increase in latency of a correctly performed saccade in trials with *vs* without the distractor. The frequency of erroneous saccades towards the distractor was also measured.

2.4. *Statistical analysis*

The Mann–Whitney U test for two independent samples was used for comparisons between variables in the PSP patients *vs* healthy controls. Bonferroni adjustment was performed to correct for the number of comparisons ($n = 30$). The corrected level of significance was set at $P = 0.0017$.

3. Results

The NNIPPS score for the PSP patients ranged from 64 to 116 (median 100), and the NNIPPS oculomotor subscore ranged from 9 to 15 (median 13). MOCA scores ranged from 9 to 24 (median 15.9), and the FAB ranged from 8 to 16 (median 12.5). GABA concentrations ranged from 0.18 to 2.10 (median 0.82) mM in the PSP group, and from 0.55 to 2.40 (median 1.02) mM in the controls (Table 1).

Considering spectral acquisitions, there were no significant differences in GABA concentrations between the PSP and control groups ($P = 0.61$). There were also no significant differences between patients and controls in RDE (Table 2). However, when the results of the distractor trials were pooled, a significantly greater number of errors were made by the PSP patients compared with the controls and, although not significant, saccadic latencies were shorter for PSP patients than for controls.

Table 1 – Clinical characteristics of progressive supranuclear palsy (PSP) patients and matching controls.

	PSP			Controls		
	Mean	SD	Range	Mean	SD	Range
NNIPPS–PPS score	90.6	20.3	64–116	NA	NA	NA
NNIPPS ocular subscore	12.1	2.2	9–15	NA	NA	NA
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

SD: standard deviation; NNIPPS–PPS: neuroprotection and natural history in Parkinson plus syndromes–Parkinson plus scale; n/a: not applicable; MOCA: Montreal cognitive assessment; FAB: frontal assessment battery; GABA: gamma-aminobutyric acid.

4. Discussion

Contrary to our expectations, no statistically significant differences were found in GABA concentrations between PSP patients and controls. These negative results might be explained by the small number of investigated subjects or by the VOI in which GABA was measured – namely, the right frontal brain region. In fact, measuring GABA transmission in PSP is evidently a difficult task, if the previous conflicting results are taken into account. Whereas GABA(A) receptors and glutamic acid decarboxylase (GAD) activity have been found to be diminished at the anterior cingulate cortex [2], globus pallidus [17], putamen, external pallidum and hippocampus [18,19], other authors have found normal [20] or even increased GABA levels in the autopsied brains of PSP patients [21].

The present study has also failed to demonstrate any statistically significant differences in RDE between PSP

Table 2 – Statistical results for remote distractor effect (RDE) trials in patients with progressive supranuclear palsy (PSP) *vs* controls.

Time of DP (sec)	Side (R/L)	Parameter (% × 100)	PSP median	IQR	Controls median	IQR	Mann–Whitney U test (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
−20	R	Lat	255	116.13	343.5	105.29	0.0205
−20	R	Error	0.5	0.38	0.25	0.38	0.519
−20	L	Lat	265	181.38	344.92	50.83	0.1709
−20	L	Error	0.75	0.38	0.29	0.38	0.0044
−50	R	Lat	160.5	116.42	338.75	55.5	0.0932
−50	R	Error	0.5	0.63	0.25	0.29	0.2918
−50	L	Lat	333	264.25	352.5	56.38	0.9333
−50	L	Error	0.75	0.46	0.38	0.5	0.0345
−80	R	Lat	335.33	53.63	325	143.69	0.5167
−80	R	Error	1	0.75	0.5	0.54	0.3465
−80	L	Lat	437	144.5	326.75	41	0.1061
−80	L	Error	0.75	0.19	0.13	0.75	0.0662
Pooled (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

DP: distractor presentation; R: right; L: left; Lat: latency; 0: same time as target; 20/50/80: 20/50/80 ms after target; −20/−50/−80: 20/50/80 ms before target.

patients and controls. However, when pooling all the results of trials with no distractor, and early and late distractors, a higher error rate and shorter latency times were obtained in PSP patients. Some authors argue that saccadic inhibition and the RDE reflect the same mechanism [22,23]; others contend that saccadic inhibition comprises the majority of the RDE [24]. Our present results are in line with the known loss of saccadic inhibition, reflecting prefrontal dysfunction in PSP [25,26].

In conclusion, no clear relationship between an increased RDE and abnormal GABA concentrations was revealed in our present small-scale pilot trial. Thus, larger studies are needed to measure GABA transmission in the brain, and its relationship to distraction susceptibility and other behavioral features of PSP patients.

Disclosure of interest

The authors declare that they have no competing interest.

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REGULAR RESEARCH PAPER

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Eye movements in idiopathic rapid eye movement sleep behaviour disorder: High antisaccade error rate reflects prefrontal cortex dysfunction

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Abstract

Abnormalities of eye movements have been reported in patients with Parkinson's disease (PD). However, it is unclear if they occur in the prodromal stage of synucleinopathy represented by idiopathic rapid eye movement sleep behaviour disorder (iRBD). We thus aimed to study eye movements in subjects with iRBD and in de novo PD, to assess if their abnormalities may serve as a clinical biomarker of neurodegeneration. Fifty subjects with polysomnography-confirmed iRBD (46 male, age 40–79 years), 18 newly diagnosed, untreated PD patients (13 male, age 43–75 years) and 25 healthy controls (20 male, age 42–79 years) were prospectively enrolled. Horizontal and vertical ocular prosaccades and antisaccades were investigated with video-oculography. All patients completed the MDS-UPDRS and the Montreal Cognitive Assessment. In addition, a neuropsychological battery was performed on iRBD subjects. When compared with healthy controls, both de novo PD patients and iRBD subjects showed increased error rates in the horizontal antisaccade task ($p < 0.01$, $p < 0.05$ respectively). In the iRBD group, the error rates in horizontal and vertical antisaccades correlated with performances in the Prague Stroop Test and the Grooved Pegboard Test, as well as with motor scores of the MDS-UPDRS. De novo PD patients showed a lower gain ($p < 0.01$) compared with controls. In conclusion, the increased error rate in the antisaccade task of iRBD and PD patients reflects a dysfunction of the dorsolateral prefrontal cortex and is related to the impairment of executive functions and attention.

KEYWORDS
eye movements, Parkinson's disease, rapid eye movement sleep behaviour disorder, saccades, video-oculography

1 | INTRODUCTION

Idiopathic rapid eye movement (REM) sleep behaviour disorder (iRBD) is characterized by a loss of muscle atonia, dream enactment and complex motor behaviours occurring during REM sleep. (Ferini-Strambi & Zucconi, 2000; Schenck, Bundlie, Ettinger, & Mahowald, 1986; St Louis, Boeve, & Boeve, 2017) iRBD converts into a manifest neurodegenerative synucleinopathy phenotype, such as Parkinson's disease (PD), multiple system atrophy (MSA) and dementia with Lewy bodies (DLB) in up to 92% of cases within 15 years (St Louis et al., 2017). Several brain structures at the cortical (frontal

cortex) and subcortical (hypothalamus, thalamus and pontine nuclei) levels are involved in the regulation of REM sleep, but their particular role in the pathophysiology of iRBD is still not completely understood (St Louis et al., 2017). Current research efforts have focused on finding sensitive clinical biomarkers for the early prediction of neurodegeneration in iRBD. To date, several predictive markers of PD and other synucleinopathies have been identified, such as hyposmia (Barber et al., 2017; Mahlknecht et al., 2015; Postuma, Berg, et al., 2015; Postuma, Gagbon, Vendette, & Montplaisir, 2009; Postuma, Iranzo, et al., 2015), impaired colour vision (Postuma et al., 2009; Postuma, Berg, et al., 2015; Postuma, Iranzo, et al., 2015) and neuroimaging markers (Iranzo et al., 2010; Meles et al., 2017; Pytigorskaya et al., 2017).

Eye movement analysis is a suitable tool to investigate brain function and to elucidate the pathophysiology of neurodegenerative diseases (Antoniades & Kennard, 2015). Studies of eye movement metrics in PD showed hypometric saccades, normal or mildly increased latency and an increased error rate in the antisaccadic paradigm (Antoniades, Demeyere, Kennard, Humphreys, & Hu, 2015; Leigh & Zee, 2006). These abnormalities have been attributed to dysfunction in circuits connecting the basal ganglia, brainstem, cerebellum and prefrontal cortex — circuits that have a pivotal role in eye movement control (Condy, Wattiez, Rivaud-Péchoux, Tremblay, & Gaymard, 2007; Ploner, Gaymard, Rivaud-Péchoux, & Pierrot-Deseilligny, 2005; Rascol et al., 1989; White, Saint-Cyr, Tomlinson, & Sharpe, 1983). Results from the antisaccade task demonstrated a higher error rate in patients with MSA and DLB (Mosimann et al., 2005) than it did for healthy subjects (Brooks et al.,). An increased error rate in the antisaccade task can be attributed to the patient´s dysfunction of the prefrontal cortex (Condy et al., 2007; Ploner et al., 2005). This is supported by previous studies which showed that poor antisaccade error rate performance correlates with neuropsychological test measures of prefrontal cortical function (Levy, Mendell, & Holzman, 2004; Postuma, Berg, et al., 2015; Postuma, Iranzo, et al., 2015). Higher antisaccadic error rates were also found in early-stage drug-naive PD patients (Antoniades & Kennard, 2015; Antoniades et al., 2015). Eye movement abnormalities have been presumed to occur even in the pre-symptomatic phase of neurodegenerative disorders; however, there have been no studies characterizing eye movement metrics in iRBD performed so far (Antoniades & Kennard, 2015).

The main goal of the present study was to verify whether eye movement abnormalities are detectable in iRBD subjects and whether these abnormalities are similar to those found in early PD patients. Additionally, we analysed eye movement parameters in conjunction with clinical and neuropsychological measures to confirm their relations.

2 | METHODS

2.1 | A. Subjects

Three groups of study subjects were enrolled at the Department of Neurology and Center of Clinical Neuroscience, Charles University and General University Hospital in Prague. Each participant provided written, informed consent. The study was approved by the Ethics Committee of the General University Hospital in Prague, Czech Republic, and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Fifty iRBD subjects (46 men, 4 women), with a mean age of 65.6 (standard deviation [*SD*], 7.6) years, were diagnosed with iRBD according to the International Classification of Sleep disorders diagnostic criteria, third edition (American Academy of Sleep Medicine, 2014). Eighteen de novo, untreated PD patients (13 men, 5 women; mean age of 62.6 [*SD* 9.4] years) were diagnosed based on the established clinical criteria (Postuma, Berg, et al., 2015; Postuma, Iranzo, et al., 2015); mean disease duration was 1.6 (*SD*, 1.3; range, 1.0–6.0) years. In addition, 25 normal control (NC) healthy subjects were enrolled (20 men, five women), with a mean age of 66.4 (*SD* 9.0) years and without any history or signs of neurological or psychiatric disorders. No subject complained of visual discomfort.

None of these iRBD patients had overt Parkinsonism or dementia. Dementia was defined according to the DSM-V criteria for major neurocognitive disorders. Parkinsonism was defined according to the MDS clinical diagnostic criteria as clearly present bradykinesia, in combination with either rest tremor, rigidity, or both (Postuma, Berg, et al., 2015; Postuma, Iranzo, et al., 2015).

Clinical examination in all subjects was performed by a movement disorders specialist (P.D., V.I. or E.R.), using the Movement Disorders Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part III—motor scale. Eye movements were examined by an eye movement specialist.

Cognitive function in iRBD subjects and in patients with untreated PD was assessed by a certified neuropsychologist (O.B. or, T.N.) using the Montreal Cognitive Assessment (MoCA) (Kopecek et al., 2017). Additionally, iRBD subjects underwent an extended neuropsychological battery, including tests of: (a) attention (Trail Making Test, Part A [TMT-A] and Prague Stroop Test [PST]), (b) working memory (Digit Span Backwards [DSB] and Letter-Number Sequencing from the Wechsler Adult Intelligence Scale, Third Revision [LNS]), (c) executive function (Trail Making Test, Part B [TMB-B] and verbal fluency [VF]), (d) explicit memory (Rey Auditory Verbal Learning Test delayed recall [RAVLT] and Memory Binding Test delayed recall [MBT]), and (e) psychomotor and motor speed of upper limbs (Symbol Digit Modalities Test [SDMT] and Grooved Pegboard Test [GPT]) (Kane, 1991).

2.2 | B. Video-oculography recording, apparatus and experimental paradigm

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 (Bonnet et al., 2013). Two different tasks were performed in the same order in a single visit lasting 20 min without interruption of the examination. We examined: (a) prosaccades (a saccade towards a target) in horizontal and vertical planes, and (b)

antisaccades (a voluntary eye movement made in the direction opposite to the side where a stimulus is presented) in horizontal and vertical planes (Figure 1). Subjects were seated in a quiet, 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 Liquid Crystal Display screen (ProLite, Iiyama model PL 2,600, size 550 × 344 mm) located 60 cm in front of them at eye level.

(1) Simple prosaccades, horizontal and vertical: a green central fixation point (15 × 15 pixels; luminance, 120 cd/m^2) was presented for a pseudorandom duration. Following a 200-ms (millisecond) gap after the fixation point was turned off, a green peripheral target (15 × 15 pixels, luminance, 120 cd/m^2) appeared for 1,000 ms, in a random order right or left, up or down at 11,86° from the central fixation point. Twenty-eight saccades were recorded in each plane (horizontal and vertical). Latency (the time between the target onset and the beginning of eye movement [ms]), average velocity (Vavg, degree per second [°/s]), maximal velocity (Vmax, [°/s]) and gain (ratio of subject's saccadic amplitude to desired saccadic amplitude) were analysed. Lower gain (<1) reflects saccadic hypometria and higher gain (>1) hypermetric saccades. Mean values were obtained for each subject for each side and direction.

(2) Simple antisaccades, horizontal and vertical: in this task, the colour of the central fixation point and of the peripheral stimulus was red, whereas all other parameters remained the same. However, unlike performing simple prosaccade task, subjects were instructed to look as quickly as possible at the opposite direction of the peripheral stimulus. Thirty-two saccades were recorded in each plane. Only latency of antisaccades and number of errors were measured. Because there was no target in the correct gaze direction, neither velocity nor gain could be measured in this task.

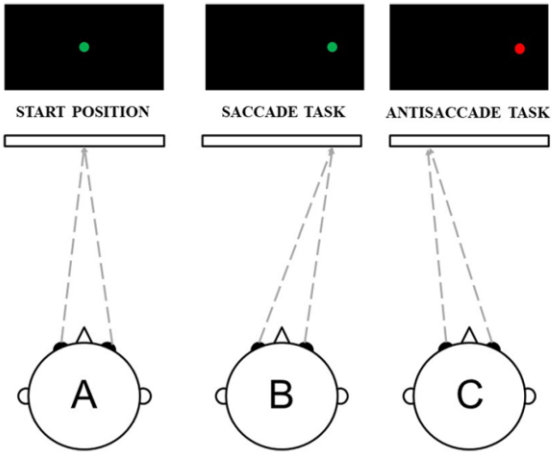

FIGURE 1 Eye movement examination trial for prosaccade and antisaccade tasks. (a) Start position, subject looks at the target (green) in the middle of the screen; (b) prosaccade task, subject performs a saccade towards the target (green); (c) antisaccade task, subject makes a voluntary eye movement in the direction opposite to the side where a stimulus (red) is presented

Saccades with a latency below 80 ms or above 1000 ms, and/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 hemifield away from the target following a prosaccade instruction, or towards the target following an antisaccade instruction.

2.3 | C. Statistical analysis

The average eye movement metric was calculated across all saccades for each subject. Group differences for each eye movement metric were evaluated across all three groups (PD, Rapid Eye Movement Sleep Behaviour Disorder and NC) using an analysis of variance with the post hoc Tukey–Kramer test. Pearson correlations were also applied to test for significant relationships. Because of the exploratory character of the study, corrections for multiple comparisons were not applied and the level of significance was set at $p < 0.05$. The classification performance (sensitivity/specificity) of the relevant features was calculated using binary logistic regression with leave-one-out cross-validation. The overall indication of diagnostic accuracy was reported as area under the curve (AUC) obtained from the operating characteristic curve.

3 | RESULTS

Table 1 provides clinical characteristics of the PD and iRBD subjects.

The measures of simple prosaccades can be seen in Figure 2. In horizontal prosaccades, significant intergroup differences were found

TABLE 1 Clinical characteristics of the PD and iRBD patients

	iRBD (*n* = 50)	PD (*n* = 18)
Mean age (years)	65.64 (*SD* 7.6, range, 40–79)	62.57 (*SD* 9.4, range, 43–75)
Men	92% (*n* = 46)	72% (*n* = 13)
Positive history of Parkinson's disease in family	4% (*n* = 2)	22% (*n* = 4)
RBD presence (%)	100% (*n* = 50)	0%
Antidepressant therapy	14% (*n* = 7)	11% (*n* = 2)
Anti-Parkinsonian therapy	0%	0%
Clonazepam therapy	2% (*n* = 1)	5% (*n* = 1)
Mean age of disease onset (years)	60.4 (*SD* 9.0, range 35–77)	60.5 (*SD* 10.3, range 37–74)
Mean symptoms duration (years)	5.3 (*SD* 4.7, range 1.0–26.0)	1.6 (*SD* 1.3, range 1.0–6.0)
Mean MDS-UPDRS III score	5.3 (*SD* 4.9, range 0.0–24.0)	32.7 (*SD* 14.3, range 8.0–63.0)
MoCA	24.3 (*SD* 4.2, range 19–30)	23.8 (*SD* 8.2, range 18–28)

PD: Parkinson's disease; iRBD: Rapid Eye Movement Sleep Behaviour Disorder; *SD*: standard deviation.

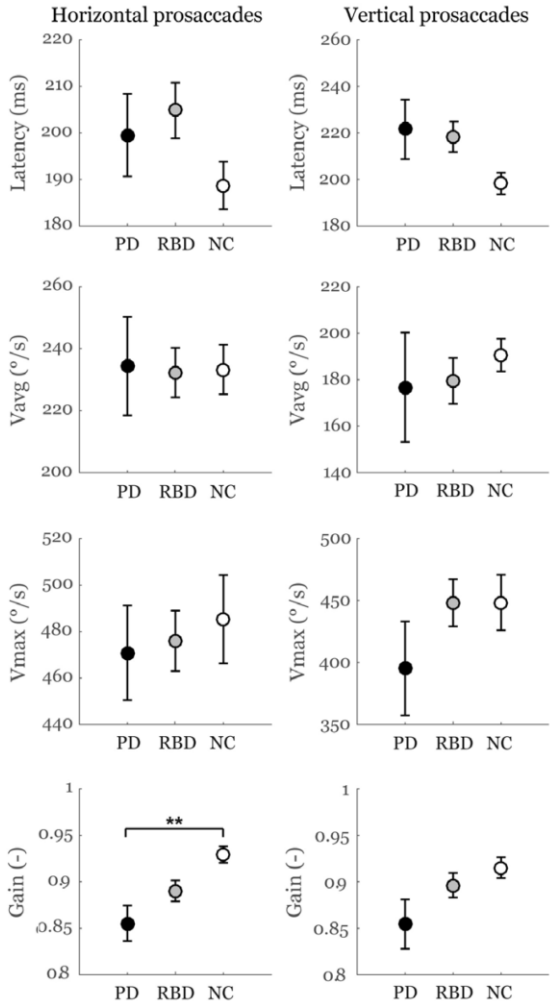

FIGURE 2 Results of horizontal and vertical prosaccades. The circles represent mean values and error bars represent standard error mean values. Analysis of variance was used to test for group differences with $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$. PD: Parkinson's disease; iRBD: rapid eye movement sleep behaviour disorder; NC: normal control; Vavg: average velocity; Vmax: maximal velocity.

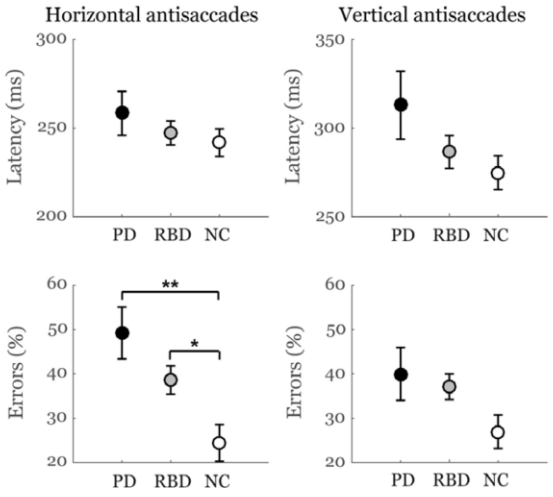

FIGURE 3 Results of horizontal and vertical antisaccades. The circles represent mean values and error bars represent standard error mean values. Analysis of variance was used to test for group differences with $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$. PD: Parkinson's disease; iRBD: rapid eye movement sleep behaviour disorder; NC: normal control.

in the gain ($F_{2,91} = 5.7$, $p = 0.005$, $\eta^2 = 0.13$), mainly reflecting the differences between PD and NC ($p < 0.01$).

The results of antisaccades can be seen in Figure 3. The only significant differences between groups concerned errors in horizontal antisaccades ($F_{2,98} = 6.8$, $p = 0.002$, $\eta^2 = 0.15$), which mainly reflected differences between PD and NC ($p < 0.01$) and between iRBD and NC ($p < 0.05$). A trend towards differences between RBD and NC was also observed for errors in vertical antisaccades (*t* test: $t(73) = 2.1$, $p = 0.04$). A combination of two measures related to errors in horizontal and vertical antisaccades was able to separate the PD and NC groups with AUC 0.82 (sensitivity, 71.4%; specificity, 77.8%) and RBD and NC groups with AUC 0.70 (sensitivity, 71.9%; specificity, 63.4%).

When looking for trends between errors in antisaccades and clinical data in the iRBD group, positive correlations were found between the MDS-UPDRS III score and errors in horizontal antisaccades ($r = 0.52$, $p = 0.0001$) as well as with errors in vertical antisaccades ($r = 0.49$, $p = 0.0003$). A weak correlation was also found between the MoCA score and errors in vertical antisaccades ($r = -0.31$, $p = 0.03$). In addition, several moderate correlations were detected between performance in antisaccades and neuropsychological test results. The errors in horizontal antisaccades correlated with PST-D (naming colours) ($r = 0.41$, $p = 0.004$) as well as with the GPT ULL (upper left limb) ($r = 0.44$, $p = 0.002$). Errors in vertical antisaccades correlated with the scores of PST-D ($r = 0.57$, $p < 0.0001$), PST-W (weak interference condition) ($r = 0.46$, $p = 0.001$), GPT URL (upper right limb) ($r = 0.47$, $p = 0.0009$) and GPT ULL ($r = 0.57$, $p < 0.0001$).

4 | DISCUSSION

The current study revealed eye movement abnormalities in individuals with idiopathic RBD. Because iRBD is considered as the prodromal stage of synucleinopathies, we can assume that observed oculomotor abnormalities represent markers of prodromal neurodegeneration. This hypothesis is further supported by the observed correlations between MDS-UPDRS part III and errors in antisaccades.

We found an increased error rate in the antisaccade task in both PD and iRBD patients, compared with controls. This suggests an

TABLE 2 Results of neuropsychological tests in iRBD subjects

Test	iRBD
TMT-A	42.3 (*SD* 19.6, range 23.0–116.0)
TMT-B	108.6 (*SD* 64.5, range 10.0–384.0)
TMT-B (errors)	1.2 (*SD* 1.5, range 0.0–6.0)
TMT-B/A	2.7 (*SD* 1.5, range 0.3–10.4)
Stroop D	14.5 (*SD* 3.6, range 10.0–31.0)
Stroop W	16.9 (*SD* 3.1, range 11.0–24.0)
Stroop C	32.1 (*SD* 8.4, range 15.0–54.0)
Stroop I	38.4 (*SD* 12.5, range 25.0–100.0)
Stroop C/D	2.2 (*SD* 0.5, range 1.5–3.4)
Stroop I/D	2.7 (*SD* 0.7, range 1.7–6.3)
VF vegetables	13.0 (*SD* 3.2, range 5.0–22.0)
VF animals/clothes	18.3 (*SD* 3.1, range 12.0–24.0)
VF action	18.2 (*SD* 5.2, range 6.0–29.0)
GPT URL	80.7 (*SD* 20.9, range 11.0–153.0)
GPT ULL	88.8 (*SD* 17.4, range 63.0–135.0)

iRBD: rapid eye movement sleep behaviour disorder; *SD*: standard deviation; TMT-A: Trail Making Test, Part A; TMT-B: Trail Making Test, Part B; Stroop: Stroop naming colour test; VF: verbal fluence; GPT URL: Grooved Pegboard Test for upper right limb; GPT ULL: Grooved Pegboard Test for upper left limb.

involvement of the inhibitory control of reflexive saccades, ensured by the dorsolateral prefrontal cortex (Condy et al., 2007; Ploner et al., 2005). As cognitive and executive functions of the prefrontal cortex are facilitated by dopamine via actions on D1/D2 receptors on pyramidal neurons in the prefrontal cortex (Floresco, ; Jenni, Larkin, & Floresco, 2017), it can be assumed that the observed changes may be related to the reduction in dopamine transport demonstrated in iRBD, which is consistent with a wide range of functional neuroimaging examinations (Iranzo et al., 2010, 2011). Accordingly, in previous studies, an increased error rate in the antisaccadic task has been described in our PD patients even in an early stage of the disease (Antoniades et al., 2015) and improvement of the antisaccades (marked by a decreased error rate) after a dose of levodopa has been documented (Hood et al., 2007).

Saccadic hypometria, which is considered one of the most consistent ocular motor abnormalities in PD (Antoniades & Kennard, 2015; Rottach, Riley, DiScenna, Zivotofsky, & Leigh, 1996) and was discovered in our patients with de novo PD, was not clearly pronounced in iRBD patients. This suggests that hypometria appears later in the disease course, in line with the progression of PD motor signs.

Interestingly, several relationships were found between neuropsychological test performance and VOG results in iRBD. Namely, horizontal antisaccades showed correlations with measures of visual scanning and sustained attention (Stroop naming colour; PST-D). This correlation may reflect a specific contribution of eye movement abnormalities to impaired sustained visual attention in iRBD, whereas preserved interference (PST-C) possibly recruits different mechanisms that are independent of saccadic eye movements (Ploner et al., 2005).

These findings were further corroborated by moderate associations found between tasks involving eye-hand coordination, such as GPT, and vertical antisaccades. GPT is considered a biomarker for nigrostriatal denervation in PD (Bohnen, Kuwabara, Constantine, Mathis, & Moore, 2007), but it appears to contain a cognitive component (Bezdicek et al., 2014). Thereafter, the association observed may be indicative of a cognitive impairment in iRBD that is reflected by impaired antisaccades as well.

The current study revealed eye movement abnormalities in individuals with idiopathic RBD. Because iRBD is considered as a prodromal stage of synucleinopathies, we assume that observed oculomotor abnormalities represent markers of prodromal neurodegeneration. This hypothesis is further supported by the observed correlations between MDS-UPDRS part III and eye movement abnormalities.

We acknowledge that we did not perform specific neuropsychological testing in PD and NC. In addition, our PD patients did not exhibit RBD symptoms, whereas PD with and without RBD may represent distinct disease phenotypes (Romenets et al., 2012). However, as numbers of errors in antisaccades in RBD subjects clearly intermediated between those of PD patients and healthy controls, we believe that errors of antisaccades represent a surrogate measure of prefrontal cortex involvement paralleling the severity of motor involvement independently of Parkinsonian phenotype.

In summary, we demonstrate that eye movement abnormalities correspond to early prefrontal cortex involvement in iRBD patients. This observation has been corroborated by correlations with the results of neuropsychological testing. The present findings broaden the range of markers reflecting subclinical neurodegeneration in iRBD and show the potential of eye movement examination as a tool for research of neurodegenerative diseases.

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CONFLICT OF INTERESTS

No conflicts of interest to declare.

AUTHORS CONTRIBUTION

J.H. conducted the eye movement examination (in cooperation with O.U. and C.B.), and collected and analysed data. J.R. made a statistical analysis and T.S. was our technical support. O.B. and T.N. took care of neuropsychological examination. K.Š., P.D., V.I. and P.D. carried out clinical testing of the subjects and made a diagnosis of RBD. E.R. acted as supervisor throughout the study. All authors contributed to the design of the study and manuscript preparation.

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ORIGINAL ARTICLE

Eye movement abnormalities are associated with brainstem atrophy in Wilson disease

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Abstract

Backgrounds This study aims to characterize eye movement abnormalities in Wilson disease and examine their association with the degree of brainstem atrophy.

Methods Twenty patients (10 males, mean age 46.8, SD 8.9 years) with genetically confirmed neurological WD on stable anti-copper treatment and 20 age- and sex-matched healthy subjects were examined. Eye movements, including prosaccade and antisaccade tasks, were evaluated using infrared videooculography. MRI was performed using 1.5 T system, and T_2-weighted images were used for the measurement of midbrain and pontine area on mid-sagittal slices. Clinical severity was assessed using the Unified Wilson's Disease Rating Scale (UWDRS).

Results Compared to healthy controls, WD patients showed prolonged latencies of horizontal prosaccades and hypometry of both horizontal ($p = 0.04$) and vertical ($p = 0.0046$) prosaccades. In the antisaccade task, WD patients showed prolonged latency of both horizontal ($p = 0.04$) and vertical antisaccades ($p = 0.047$) and increased error rate of vertical antisaccades ($p = 0.04$). There is a significant association between midbrain area and horizontal latencies ($r = -0.53$; $p = 0.02$) and vertical maximum speed in prosaccades ($r = 0.47$; $p = 0.04$). The pons area inversely correlated with horizontal prosaccade and antisaccade latencies ($p = 0.007$).

Conclusions We showed impairments of ocular saccades such as prolonged latencies, hypometry, and increased error rate in antisaccades. The strong association between prolonged latencies of prosaccades and the brainstem atrophy suggests that VOG might serve as a sensitive electrophysiological marker of brainstem dysfunction in WD.

Keywords Wilson disease · Eye movement · Brainstem

Introduction

Wilson disease (WD) is an autosomal recessive hereditary disease characterized by dysfunctional ATP7B copper transporting protein. This alteration causes the accumulation of copper in the brain and liver [1] that leads to progressive neurodegeneration. The structures most vulnerable to the toxic effect of copper in the brain are the basal ganglia (BG); other structures including the brainstem, cerebellum, and cortico-subcortical regions may also be affected [2, 3]. Dysfunction of the aforementioned structures is manifested typically by symptoms such as tremor, dysarthria, drooling, ataxia, parkinsonism, and/ or dystonia [4]. Additionally, other neurological abnormalities, such as REM sleep behavior disorder, polyneuropathy, or oculomotor dysfunction, were also described in WD [5–7]. In general, all neurological symptoms diminish during anti-copper therapy, but, due to irreversible central nervous system (CNS) damage, residual symptoms are often detectable even after treatment lasting several years [4, 5].

In WD patients with neurological manifestation (neuro-WD), several abnormalities of eye movement (EM) were described such as slow horizontal and vertical saccades [8, 9], abnormal vertical smooth pursuit [10], and increased antisaccadic latency and error rate [6]. Similar abnormalities of EM are often present in patients with neurodegenerative

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diseases. Notably, specific EM abnormalities related to atrophy of distinct brainstem regions were detected by videooculography (VOG) in patients with progressive supranuclear palsy (PSP) and multisystem atrophy (MSA) [11]. In neuro-WD patients, magnetic resonance imaging (MRI) shows brainstem atrophy frequently reaching severity comparable to that is seen in PSP [12]. Additionally, midbrain diameter in neuro-WD patients inversely corresponds to neurological severity [13]. However, the association between EM abnormalities and brainstem atrophy in WD was not studied so far. In this study, we thus aimed to analyze EM in WD using VOG and to assess their relation to global neurological severity and to the degree of regional brainstem atrophy.

Methods

Subjects

Twenty WD patients (10 males, mean age 46.8, SD 8.9 years) with genetically confirmed neurological WD on stable anti-copper treatment and 20 age- and sex-matched healthy controls (10 males, mean age of 46.4, SD 9.0) without any history or signs of neurological or psychiatric disorders were enrolled. No subject complained of visual discomfort. Clinical examination in all WD patients was performed by a movement disorders specialist using the Unified Wilson's Disease Rating Scale (UWDRS) including its activities of daily living (ADL) subscale (UWDRS II) and objective neurological examination subscale (UWDRS III) [14]. (Table 1).

Subjects were enrolled at the Department of Neurology and Center of Clinical Neuroscience, Charles University and General University Hospital in Prague. Each participant provided written informed consent. The study was approved by the Ethics Committee of the General University Hospital in Prague, Czech Republic, and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Videooculography recording, apparatus, and experimental paradigm

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. Two different tasks were performed in the same order one after the other in a single visit with duration of 20 min without a break. We examined (1) prosaccades (a saccade toward a target) in horizontal and vertical directions and (2) antisaccades (a voluntary eye movement made in the direction opposite to the side where a stimulus is presented) in horizontal and vertical directions. Subjects were seated in a quiet, 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. A green central fixation point (15 × 15 pixels; luminance: 120 cd/m2) was presented for a pseudorandom duration.

(1) Simple prosaccades were horizontal and vertical: the fixation point was turned off, and 200 ms (millisecond) later (gap), a green peripheral target (15 × 15 pixels, luminance 120 cd/m^2) appeared during 1000 ms at 11.86°, in a random order right or left, up or down. Twenty-eight saccades were recorded. Latency (a time between the target onset and the beginning of eye movement; [ms]), average velocity (Vavg, degree per second [°/s]), maximal velocity (Vmax, [°/s]), and gain (ratio of subject's saccadic amplitude to desired saccadic amplitude) were analyzed. Lower gain (< 1) reflects saccadic hypometria and higher gain (> 1) hypermetric saccades. Mean values were obtained for each subject for each side and direction.

(2) Simple antisaccades were horizontal and vertical: in this task, the color of the central fixation point was red (15 × 15 pixels, luminance 120 cd/m^2). Target locations were presented in a random order at 11.86° in the horizontal and vertical direction. Subjects were instructed to look as quickly as possible in the direction opposite to the peripheral target. Thirty-two saccades were recorded. Latency and error rate were extracted first for each direction and then for each subject.

Saccades with a latency below 80 ms or above 1000 ms and/or an amplitude below 1° were excluded from analysis, but these represented < 1% of all trials. Mean latency was determined only for correct antisaccades. Directional errors were defined as saccades initially directed toward the hemifield away from the target following a prosaccade instruction or toward the target following an antisaccade instruction.

MRI acquisition and analysis

MRI was performed using 1.5 T whole body Philips Achieva system. T_2-weighted images (axial slices covering entire brain area, resolution 0.5 × 0.5 × 1 mm^3, TE = 233 ms, TR = 2250 ms) were used for the measurement of midbrain and pontine area. MR images were first resliced to the sagittal plane, and areas of midbrain and pons were defined on mid-sagittal images according to the method described by Oba et al. [15]. In short, the caudal edge of midbrain was defined as line passing through the superior pontine notch and the inferior edge of the quadrigeminal plate; caudal edge of pons was defined as a line parallel to the first line passing through the inferior pontine notch. The area of the midbrain was traced manually above the first line (excluding the tectum). The area of the pons was outlined manually between the first and second line (Fig. 1).

Table 1 Clinical data of WD patients; *F* female; *M* male; *NA* not available, *K-F ring* Kayser-Fleischer ring; *UWDRS* Unified Wilson's Disease Rating Scale

				Type of the first neurological symptom											Genetics of ATP7B															
ID	Sex	Age at the time of examination	first neurological symptom (Age)	tremor	depression	dysartria	instability	anxiety	pain	cognitive deficit	Parkinsonian syndrom	dystonia	dysarthria	discoordination of limbs	p.[His1069Gln];[His1069Gln]	p.[His1069Gln];[Ala1135Glnfs*13]	p.[His1069Gln];[Ala1139Ile]	p.[His1069Gln];[Arg778Gly]	p.[His1069Gln];[Met769Hisfs*26];[Asp1267Val]	p.[Gly710Ser];[His1069Gln]	p.[His1069Gln];[Gly1176Arg]	p.[Lys1248Thrfs*83];[-]	p.[His1069Gln];[Val1262Phe]	Liver Cu (ug/g)	K-F ring	Medication onset (age)	Actual dose of D-PEN (mg)	Actual dose of Zn (mg)	UWDRS II	UWDRS III	UWDRS total score	Other neurological treatment
WD01	F	44	36		+									+	+									467	+	37	900	0	5	27	32	none
WD02	F	27	33	+											+									1736	+	39	600	100	6	11	17	propranolol
WD03	M	60	27	+	+	+	+				+				+	+								234	+	28	150	200	14	34	48	amantadinum, botulotoxinum
WD04	M	54	31									+			+									1247	+	17	1200	50	5	27	32	amantadinum, clonazepamum,levodopum, carbidopum
WD05	M	42	30												+									617	-	6	1200	0	0	8	8	none
WD06	F	48	29	+											+									1028	+	29	0	0	2	30	32	clonazepamum
WD07	F	51	29	+		+									+									487	+	30	900	0	3	6	9	none
WD08	M	42	20	+		+											+							1284	+	21	0	150	1	19	20	natrii valproas, procyclidinum
WD09	M	41	15			+	+								+									NA	+	15	0	100	7	39	46	clonazepamum, carbamazepinum, levetiracetamum, gabapentinum
WD10	F	37	36	+				+							+									704	+	37	600	0	0	1	1	alprazolamum, citalopramum
WD11	M	58	32		+										+									295	+	33	0	150	6	0	6	clonazepamum
WD12	F	40	40	+														+						1137	-	41	600	100	11	22	33	none
WD13	M	54	26	+		+									+									724	+	27	600	150	25	37	62	biperidenum, citalopramum, mirtazapinum, gabapentinum
WD14	M	46	18										+	+					+					NA	+	19	900	0	2	18	20	zuclopenthixolum, cyproteronum
WD15	M	26	57								+									+				NA	+	58	600	150	10	40	50	levodopum, carbidopum, levodopum, benserazidum
WD16	F	44	26	+																	+			NA	+	26	900	0	3	4	7	none
WD17	M	41	29	+																			+	887	+	30	0	200	10	50	60	biperidenum, milnacipranum
WD18	F	41	38	+											+									NA	+	39	0	150	2	4	6	clonazepamum
WD19	F	51	22											+	+									411	+	24	900	0	11	18	29	pregabalinum
WD20	F	50	30	+		+																	+	509	+	33	600	50	0	7	7	none

Statistical analysis

The average eye movement metric was calculated across all saccades for each subject. As the one sample Kolmogorov-Smirnov did not indicate non-normally distributed EM features, differences between groups (WD vs. controls) were calculated using independent t-test. Pearson correlations were then applied to test for significant relationships between EM and clinical and MRI scores. Due to the exploratory character of the study, corrections for multiple comparisons were not applied, and the level of significance was set at $p < 0.05$.

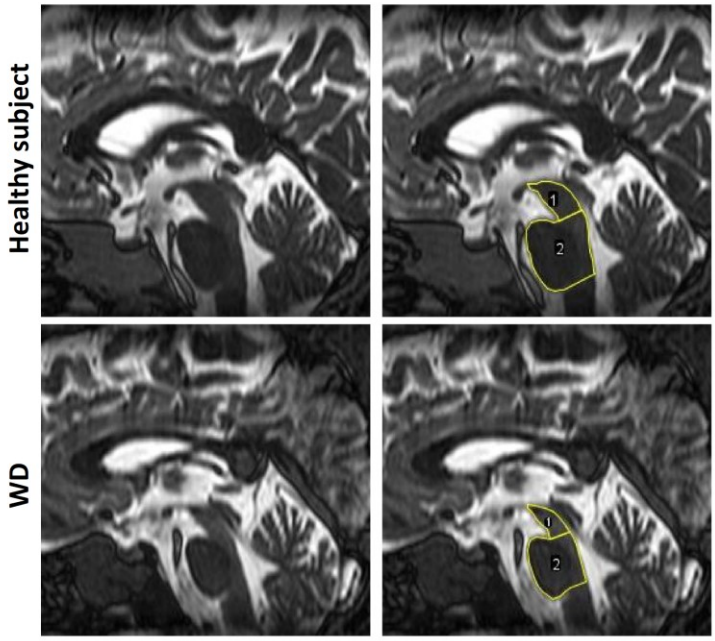

Fig. 1 Segmentation of midbrain and pons atrophy. Magnified midsagittal images of sample healthy subject (upper row) and WD patient (bottom row). Segmentations used for the calculation of midbrain (1) and pons (2) area are shown on the right. Please note the brainstem atrophy in WD patient

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