Correlates and Determinants of Eye Movements Across the Adult Lifespan

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List of Abbreviations

AD	Alzheimer's disease
APOE	apolipoprotein E
AVLT	Auditory Verbal Learning Test
b	unstandardised regression coefficient
β	standardised regression coefficient
CI	confidence interval
Corsi	Corsi block-tapping test
DZNE	Deutsches Zentrum für Neurodegenerative Erkrankungen/ German Center for Neurodegenerative Diseases, Bonn, Germany
EM	eye movement
Exp. Coef	exponentiated coefficient
f^2	Cohen's f^2
FDR	false discovery rate
FMRP	fragile X mental retardation protein
GWAS	genome-wide association study
ICH-GCP	International Council for Harmonisation Good Clinical Practice
IMBIE	Institute for Medical Biometry, Informatics and Epidemiology
IQR	interquartile range
ISCED	International Standard Classification of Education
LD	linkage disequilibrium

M	mean
MCI	mild cognitive impairment
N	number
OR	odds ratio
PRS	polygenic risk score
p	p-value
p_T	p-value threshold
Q-Q-plot	quantile-quantile-plot
RMSE	root mean square error
SD	standard deviation
SNP	single nucleotide polymorphism
SPEM	smooth pursuit eye movement
TMT	trail-making test
MWT-B	Mehrfachwahl-Wortschatz-Intelligenztest
WMC	working memory capacity

1. Abstract

Eye movements reflect a multitude of perceptual, cognitive and motor processes. However, it is unknown what added value an oculomotor test battery has to the study protocol of cohort studies on aging and age-related diseases. To answer this question, a better understanding of the determinants and correlates of eye movements across the adult life-span is required. I based my research on data from the Rhineland Study, which is a population-based cohort study in Bonn, Germany. The oculomotor battery in the Rhineland Study includes fixation, smooth pursuit, prosaccade and antisaccade tasks. To learn the extent to which eye movement measures can serve as markers for biological aging processes, I first examined age-related variability and sex differences in eye movement performance. The analyses revealed that age was associated with all but five antisaccade measures and blink rate during fixation. Sex differences in eye movement performance were present only in smooth pursuit velocity gain and blink rate during fixation, but they were of small size. Eye movements are also known to be altered in several diseases, including schizophrenia and Alzheimer's disease (AD). Since both aforementioned diseases represent polygenic disorders, genetic liability can be quantified using genetic risk scores. First, I investigated whether I could provide genetic evidence for antisaccades and smooth pursuit eye movements as endophenotypes for schizophrenia by associating genetic risk scores for schizophrenia with performance in the smooth pursuit and antisaccade tasks. I found that the genetic risk scores for schizophrenia were associated with antisaccade performance, supporting the endophenotype status of antisaccades for schizophrenia. I then tested which, if any, classical cognitive tasks or eye movement outcomes are sensitive to genetic susceptibility for AD. I found that Corsi forward performance and the probability of correcting antisaccade errors may be the best candidates to capture genetic liability for AD across the adult lifespan. Lastly, I investigated whether interindividual differences in cognitive performance are reflected in the mean pupil size during a fixation task. Pupil size was correlated with interindividual differences in speed of processing and response generation, but not with working memory or global cognition. On the basis of the overall work, I conclude that eye movement tasks add value to population-based studies on aging and age-related diseases as oculomotor data are language-independent and culture-free assessments of normal age-related and pathophysiological changes in brain activity.

2. Introduction and aims

Eye movement tasks were included in the study protocol of the Rhineland Study, a community-based cohort study on healthy aging (Breteler & Wolf, 2014), because they may add value to the cognitive test battery. However, as this is a unique feature of the Rhineland Study, there is no evidence of their utility coming from other cohort studies on aging and age-related diseases. The aim of my PhD thesis, therefore, was to obtain a better understanding of the correlates and determinants of eye movement performance in the general population.

2.1 Eye Movements and Brain Activity

The eyes are often referred to as the window to the soul. Even if the soul is a rather philosophical term (Menn, 2002) that is difficult to grasp, this saying suggests that the visual system is a rich source of information. This is supported by previous research showing that eye movements (the output of this system) reflect the operation of a multitude of perceptual, cognitive and motor processes, including attention, processing speed, decision making, working memory, learning, motion processing and inhibition (Hutton, 2008; Massen, 2004).

However, to get a better understanding of eye movements as the output of the visual system, we first have to understand the processing of visual input, which is illustrated in Figure 1. Visual processing starts when somebody looks at an object of interest, the target. The image of the target is first projected on the retina of the eyes, where it stimulates photoreceptors (Joukal, 2017). Then, the neural signals travel via the optic nerve through the optic chiasm and afterwards via the optic tract to the lateral geniculate nucleus of the thalamus (Joukal, 2017). From there, the visual input is sent through the optic radiation to the primary visual cortex in the occipital lobe and processed in the brain areas contralateral to the target location in the visual field (Joukal, 2017). Roughly speaking, visual processing in the dorsal “how/vision for action” stream in the parietal cortex serves to program movements and control their execution, whereas visual processing in the ventral “what/vision for perception” stream in the temporal lobe serves to visually process target features such as colour or shape to identify possible targets for action (Goodale et al., 1991; Milner & Goodale, 2008). However, this clear segregation of functions has been

challenged as it seems to be an oversimplification of visual processing in the brain areas (Freud et al., 2016). Still, it highlights that visual processing is not limited to the occipital cortex but involves several brain areas.

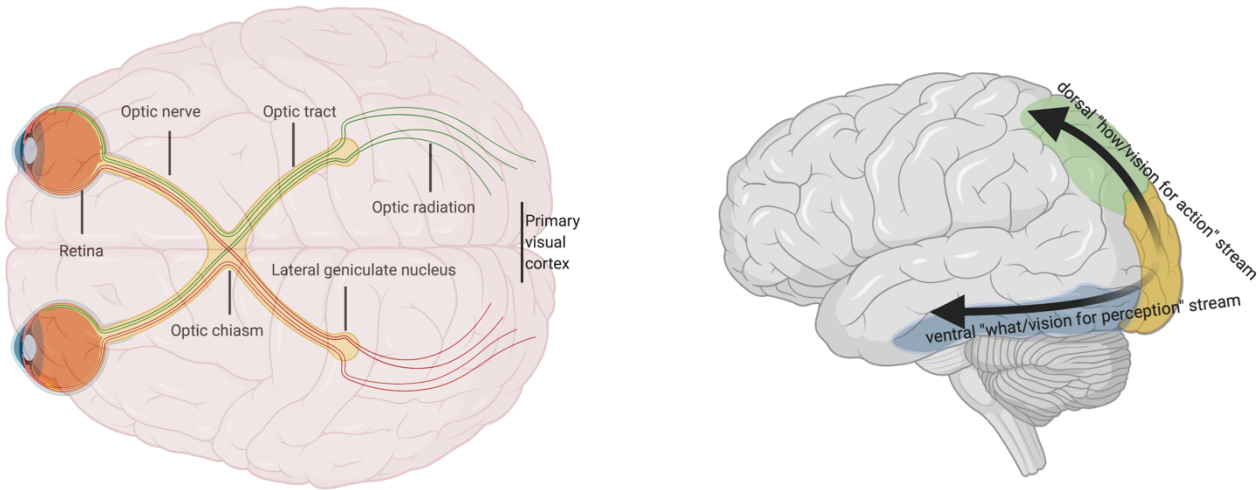

Figure 1. The visual pathway (created with BioRender.com)

Knowledge of the anatomy of the eye and visual pathway is also needed to understand why humans perform eye movements and do not solely rely on head and body movements to bring a target into their visual field. Eye movements serve to align a target with the fovea, the retinal area with the highest visual resolution (Lencer & Trillenberg, 2008). There are two classes of photoreceptors present in the retina: cones and rods (Joukal, 2017). Rods are extremely sensitive to light and exist in all retinal areas except the fovea (Hirsch & Curcio, 1989). Cones require stronger light and have diverse responses to light of different wavelengths (Joukal, 2017). The high visual resolution within the foveal area results from the high density of cones with small receptive fields (Hirsch & Curcio, 1989). To bring a target on the fovea, a rapid eye movement called saccade is executed (Delgado-García, 2000). In order to maintain a stationary target in the foveal area, the target is fixated (Adler & Fliegelman, 1934). Slowly moving targets elicit smooth pursuit eye movements (SPEM), which aim to keep the target within the foveal area by adjusting the eye velocity to the target velocity (Lencer & Trillenberg, 2008). It is estimated that humans perform about 200,000 saccades each day (Pratt et al., 2006). The choice which

target should be aligned with the fovea depends on attentional processes that can roughly be divided into bottom-up and top-down processes (Itti & Koch, 2001). Bottom-up attentional processes are elicited in the presence of salient targets (Itti & Koch, 2001) and can lead to the execution of reflex-like saccades towards the target (Massen, 2004; Mosimann et al., 2004). The salience of a target is determined by its visual characteristics such as its luminance, colour or motion (Itti & Koch, 2001). In contrast to bottom-up processes, top-down processes are characterised by a goal-directed decision to direct the attention towards a target (Itti & Koch, 2001). Top-down processes are associated with volitional saccades (Massen, 2004; Mosimann et al., 2004).

The brain areas involved in eye movement control are quite well characterised (Leigh & Zee, 2015). Fixation and saccade neurons are found in the superior colliculus (Munoz & Fecteau, 2002) and frontal eye fields (Hanes et al., 1998). Their activity levels are interdependent with higher activations in fixation neurons going along with lower activations in saccade neurons. Several brain areas are involved in the fine-tuning of saccades such as the cerebellum for spatially accurate saccades (Optican, 2005) or saccadic burst cells in the brainstem reticular formation that determine saccade velocity (Sparks, 2002). In general, volitional saccades require more complex sensorimotor transformations than reflex-like saccades (Munoz & Everling, 2004). For control of SPEMs, there are also numerous cortical and subcortical brain areas involved (Krauzlis, 2004; Lencer & Trillenberg, 2008). The adjustment of the eye velocity to the velocity of the smoothly moving target mainly depends on the medial superior temporal area and the frontal pursuit area in the frontal eye fields (Krauzlis, 2004; Tanaka & Lisberger, 2001).

Knowledge about visual processing and oculomotor control highlights that the saying from the beginning could be rephrased to “The eyes are a window to brain activity”.

2.2 Eye Movements as Biomarkers

One of the main objectives of the Rhineland Study is to identify biomarkers for neurodegenerative and other age-related diseases (Breteler & Wolf, 2014). Since the eyes can be regarded as a window to brain activity and since the neuroanatomy of the visual system is well understood (Joukal, 2017), eye movement performance may provide potential biomarkers for age-related and neuropathological brain changes. According to

the FDA-NIH Biomarker working group (2016), a biomarker is “a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or biological responses to an exposure or intervention, including therapeutical interventions. Molecular, histologic, radiographic, or physiologic characteristics are types of biomarkers.”

Eye movements could serve as physiologic characteristic for biological aging processes as previous studies have reported that performance in most eye movement measures changes with age (e.g. Munoz et al., 1998; Noiret et al., 2017; Peltsch et al., 2011; Shafiq-Antonacci et al., 1999; Sweeney et al., 2001). However, those studies have revealed inconsistent results for some eye movement measures such as the peak prosaccade velocity (Shafiq-Antonacci et al., 1999; Sweeney et al., 2001). The inconsistent results across studies are partly due to small sample sizes and the inclusion of participants of a limited age range. Further, except for one study (Bargary et al., 2017), previous studies did not take into account that eye movements may differ between men and women. However, sex is known to be a key cause of variation between humans (Brooks & Clayton, 2017) and sex differences have been reported for the brain metabolism in two brain regions that are critically involved in oculomotor control (Gur et al., 1995; Hu et al., 2013). Therefore, sex differences may also exist in eye movement performance and should be taken into account when investigating aging effects on eye movements. To evaluate the potential of eye movements as aging biomarkers, it is thus important to quantify the associations between age, sex and eye movement performance in different oculomotor tasks in a large, population-based sample. Characterising these normal age-related changes in eye movement performance is also important to distinguish them from pathophysiological changes.

Pathophysiological changes in eye movement performance have been reported in schizophrenia (Calkins et al., 2008) and in Alzheimer’s disease (Anderson & MacAskill, 2013). Schizophrenia (Pardiñas et al., 2018) and Alzheimer’s disease (Jansen et al., 2019) are both polygenic disorders, which implies that disease risk is partly determined by genetic risk and can be quantified using polygenic risk scores (PRS) (International Schizophrenia Consortium, 2009).

In the case of schizophrenia, it has long been known that patients with schizophrenia have impaired eye movement performance, as SPEM impairments were described as early as 1908 (Diefendorf & Dodge, 1908) and have since been extensively studied (Calkins et al., 2008; Holzman, 2000). Interestingly, first-degree healthy relatives of patients with schizophrenia show eye movement impairments in the same eye movement measures, but to a lesser degree (Calkins et al., 2008). This suggests a shared genetic basis between schizophrenia and eye movements, and impairments on those measures from the antisaccade and SPEM tasks have even been suggested as endophenotypes of schizophrenia (Braff et al., 2007). An endophenotype is a quantitative biological trait which is considered to be closer to the actions of the disease genes than disease symptoms (Gottesman & Gould, 2003). However, molecular genetic evidence for a shared genetic basis is largely missing as candidate gene studies have revealed inconsistent findings (Gatt et al., 2015; Greenwood et al., 2012; Haraldsson et al., 2009, 2010; Kattoulas et al., 2012; Rybakowski et al., 2002; Thaker et al., 2004). The recent development of PRS for schizophrenia offers, therefore, a new opportunity to provide molecular genetic evidence for a link between genetic risk for schizophrenia and eye movement performance.

For individuals with Alzheimer's disease, it has also been known that they have impaired eye movement performance (Crawford et al., 2013; Garbutt et al., 2008; Noiret et al., 2017). However, the sensitivity of eye movements in capturing genetic risk for Alzheimer's disease compared to traditional cognitive tasks has not yet been assessed. PRS for Alzheimer's disease provide an opportunity to examine the sensitivity of eye movement tasks and traditional cognitive tasks in capturing genetic risk for Alzheimer's disease.

2.3 Pupillometry

Pupillometry data derived from video-based eye-tracking are a rich source of information. Pupil dilations have been linked to activity in the superior colliculus and the locus coeruleus, the main source of norepinephrine in the central nervous system (Joshi & Gold, 2020). Structural integrity of the locus coeruleus and regulation of norepinephrine levels are key components for cognitive function and can, therefore, serve as a basis for understanding higher-order cognitive abilities (Mather & Harley, 2016; Tsukahara & Engle, 2021). Thus, the pupils can also be regarded as a window to brain activity in the locus coeruleus-norepinephrine system.

Interestingly for cognitive research, pupil dilations have been found to be related to mental effort (van der Wel & van Steenbergen, 2018). However, it remains controversial whether or not interindividual differences in pupil diameter during a fixation task are related to interindividual differences in fluid intelligence or working memory performance (Bornemann et al., 2010; Heitz et al., 2008; Tsukahara & Engle, 2021; Tsukahara et al., 2016; Unsworth et al., 2020). Since more than half of the studies on this topic originate from two laboratories and were mainly conducted in small samples with limited age range, this question requires investigation in a large, independent sample. Further, it should be extended to additional cognitive test and domain scores and investigated whether these associations are age-dependent.

2.4 Thesis Outline

The aim of this thesis was to investigate correlates and determinants of eye movement performance in the general population. The potential of eye movement measures as biomarkers for normal biological aging processes are reported in Chapter 3. In this chapter, the eye movement test battery of the Rhineland Study is also described in detail. In addition, Supplement 1 provides an overview and explanation of all eye movement measures of the Rhineland Study. Whether there is evidence for a genetic link between schizophrenia and antisaccade and SPEM performance is presented in Chapter 4. Chapter 5 deals with the question of how sensitive eye movements are in capturing genetic risk for Alzheimer's disease in comparison to traditional cognitive tasks. In Chapter 6, I examine the extent to which interindividual differences in cognitive test and domain scores are reflected in the mean pupil size during a fixation task. Finally, in Chapter 7, I briefly discuss whether the results of my research projects support the inclusion of oculomotor tasks in population-based studies on aging and age-related diseases, and give directions for future research.

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3. Strong Age but Weak Sex Effects in Eye Movement Performance in the General Adult Population: Evidence from the Rhineland Study

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Strong age but weak sex effects in eye movement performance in the general adult population: Evidence from the Rhineland Study

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ABSTRACT

Assessing physiological changes that occur with healthy ageing is prerequisite for understanding pathophysiological age-related changes. Eye movements are studied as biomarkers for pathological changes because they are altered in patients with neurodegenerative disorders. However, there is a lack of data from large samples assessing age-related physiological changes and sex differences in oculomotor performance. Thus, we assessed and quantified cross-sectional relations of age and sex with oculomotor performance in the general population. We report results from the first 4,000 participants (aged 30–95 years) of the Rhineland Study, a community-based prospective cohort study in Bonn, Germany. Participants completed fixation, smooth pursuit, pro-saccade and antisaccade tasks. We quantified associations of age and sex with oculomotor outcomes using multivariable linear regression models. Performance in 12 out of 18 oculomotor measures declined with increasing age. No differences between age groups were observed in five antisaccade outcomes (amplitude-adjusted and unadjusted peak velocity, amplitude gain, spatial error and percentage of corrected errors) and for blink rate during fixation. Small sex differences occurred in smooth pursuit velocity gain (men have higher gain) and blink rate during fixation (men blink less). We conclude that performance declines with age in two thirds of oculomotor outcomes but that there was no evidence of sex differences in eye movement performance except for two outcomes. Since the percentage of corrected antisaccade errors was not associated with age but is known to be affected by pathological cognitive decline, it represents a promising candidate preclinical biomarker of neurodegeneration.

1. Introduction

As life expectancies increase, the prevalence of age-related neurological disorders rises (Jaul & Barron, 2017). A thorough understanding of brain changes in healthy aging is a prerequisite to understanding pathophysiological changes underlying neurodegenerative disorders. One functional domain that is impaired in many neurodegenerative disorders is the control of eye movements (EMs) (Anderson & MacAskill, 2013). EMs are controlled by distributed brain system at the interface of perception, cognition and motor control. The neuroanatomy of EMs is well understood (Luna et al., 2008) and examinations are brief and well-tolerated by people of all ages (Noiret et al., 2017). Multiple cognitive processes are involved in EMs, including attention, working memory and learning (Hutton, 2008). Consequently, EMs provide a suitable model for investigating both pathological and normal cognitive changes that occur with age.

The most commonly used oculomotor tasks are the fixation, smooth pursuit eye movement (SPEM), prosaccade and antisaccade tasks. Fixations serve to maintain the alignment of a stationary target with the fovea (Lencer & Trillenberg, 2008). SPEMs are elicited in an attempt to keep the retinal image of a moving target on the fovea (Lencer & Trillenberg, 2008). A saccade is a rapid EM executed to bring an object of interest onto the fovea (Hallett, 1978); prosaccades are saccades towards a sudden-onset peripheral target, whereas antisaccades are

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saccades in the opposite direction (Hallett, 1978).

Good fixation performance reflects high spatial accuracy and low saccade frequency. In SPEM, closely pursuing the target means that eye and target velocity correspond (indicated by the velocity gain) and that saccade frequency is low. For prosaccades and antisaccades, fast saccade initiation (low latency), high peak velocities and high spatial accuracy are indicators of optimal performance. Spatial accuracy of saccades can be indicated by different measures, including amplitude gain and spatial error. Amplitude gain reflects the average landing position relative to the target with values below 100% indicating that the saccade amplitude was too low (saccade undershot the target) and values above 100% indicating that the saccade amplitude was too high (saccade overshot the target). A value of 100% indicates that the saccade perfectly landed on the target. Spatial error reflects the mean deviation from the target position. In the antisaccade task, the antisaccade error rate (percentage of trials where the first saccade is erroneously made towards the target) and the percentage of corrected antisaccade errors are additionally measured, with lower error rates and higher correction rates indicating better performance.

Neurodegenerative conditions are characterised by selective oculomotor deficits (Anderson & MacAskill, 2013). For example, individuals with Alzheimer's disease (Crawford et al., 2013; Garbutt et al., 2008), Parkinson's disease (Antoniades et al., 2015), Huntington's disease (Blekher et al., 2006) and mild cognitive impairment (Levy, Lavidor, & Vakil, 2018) make more antisaccade errors than age-matched controls. Moreover, individuals with Alzheimer's disease correct a substantially lower proportion of antisaccade errors compared to controls (Crawford et al., 2013; Garbutt et al., 2008; Noiret et al., 2018). Further, SPEMs have lower velocity gain in Alzheimer's disease (Garbutt et al., 2008) and Parkinson's disease (Pinkhardt et al., 2012).

Aging in the absence of neurodegenerative disease is also associated with changes in EM performance. Increased prosaccade and antisaccade latency with advancing age (e.g. Munoz et al., 1998; Noiret et al., 2017; Peltsch et al., 2011; Shafiq-Antonacci et al., 1999; Sweeney et al., 2001) and stable antisaccade peak velocity (e.g. Shafiq-Antonacci et al., 1999; Sweeney et al., 2001) are relatively consistent findings. However, as studies on aging effects have mostly used small sample sizes, have included participants of limited age range and have explored only a few EM parameters, aging effects on other EM outcomes are less clear. For example, antisaccade error rate was found to increase with age in most (e.g. Fernandez-Ruiz et al., 2018; Klein et al., 2000; Shafiq-Antonacci et al., 1999; Sweeney et al., 2001) but not all studies (e.g. Noiret et al., 2017) and peak prosaccade velocity was found to either decline with age (e.g. Sweeney et al., 2001) or to remain stable (e.g. Shafiq-Antonacci et al., 1999).

Biological sex is a key cause of variation between humans (Brooks & Clayton, 2017) and a candidate to affect EMs because sex differences are known to exist in brain metabolism in bilateral visual cortex and cerebellum (Gur et al., 1995; Hu et al., 2013), two regions critically involved in EM control. Sex differences in EMs are, however, almost entirely unexplored. One recent study of 1,058 participants reported sex differences in half of the assessed EM outcomes, with men outperforming women in most of them (Bargary et al., 2017). However, that sample was young (mean age = 22 years, range = 16–40 years) and consisted mostly of university students, limiting the capacity for wide-ranging conclusions about sex differences in the general population and across the adult lifespan. This is critical, given evidence of interactions between age and sex in brain metabolism (Kakimoto et al., 2016).

There is thus a strong need to thoroughly characterise the effects of age, sex and their possible interactions on EMs. In this study, we report a comprehensive assessment of age and sex effects on EM performance across the adult life span using data from fixation, SPEM, prosaccade and antisaccade tasks in the Rhineland Study. This study provides the largest and most representative sample for the investigation of the associations of age and sex with EM performance to date.

2. Materials and methods

2.1. Participants

Data analysis is based on the first 4,000 participants of the Rhineland Study (age range = 30–95 years), who underwent baseline assessments between March 2016 and July 2019. The study sample comprised all participants with data in at least one of the four tasks (N = 3,682). The Rhineland Study is an on-going community-based cohort study in Bonn, Germany. Study inclusion criteria are living in one of the two geographically defined areas in Bonn, being 30 years or older and having sufficient command of the German language to provide written informed consent. The study procedures were approved by the ethics committee of the Medical Faculty of the University of Bonn and carried out in accordance with the recommendations of the International Council for Harmonisation Good Clinical Practice standards (ICH-GCP).

2.2. Eye movement recording

Testing took place in a quiet, darkened room in one of two identical recruitment centres. Participants sat in a height-adjustable chair in front of a 22-inch monitor (1680x1050 pixels) whilst resting their chin on a chinrest and their arms on the desk in front of them. Viewing distance between eyes and monitor was 70 cm. EMs were recorded using video-based infrared oculography (EyeLink 1000 and EyeLink 1000 Plus; SR Research Ltd.) at 1,000 Hz.

2.3. Procedure and oculomotor tasks

EM tasks were programmed using ExperimentBuilder (SR Research Ltd.). The target was a white (RGB 255,255,255) circle 0.35° in diameter presented on black (0,0,0) background. After a horizontal-vertical five-point calibration, participants performed fixation, SPEM, prosaccade and antisaccade tasks in fixed order. There was no break between the fixation, SPEM and prosaccade tasks, and participants were instructed to follow the target with their eyes as closely as possible whilst keeping their head still. The antisaccade task was first explained and then practiced with six trials.

In the fixation task, participants had to fixate the target at the centre ($x = 0°$, $y = 0°$), the left ($x = –9.63°$, $y = 0°$), the right ($x = 9.63°$, $y = 0°$), the top ($x = 0°$, $y = 9.63°$) and the bottom ($x = 0°$, $y = –9.63°$). The order within which the target was presented in these positions was randomised across participants but eccentric locations were always followed by the central location. The central position thus had to be fixated four times in total. The target appeared at each eccentric location for 10 s and at the central location for 5 s each time.

In the smooth pursuit task, the target moved between ±9.63° in a sinusoidal waveform in the horizontal plane ($y = 0°$) at a frequency of 0.5 Hz. The target began in the centre, moved left and then completed ten full cycles with a total duration of 21 s.

The prosaccade task was a horizontal 'step' task, comprising 30 trials. In each trial the target appeared first in the centre ($x = 0°$, $y = 0°$) for a random duration of 1–2 s (average 1.5 s). Then it stepped to a peripheral position ($x=±9.63°$, $y = 0°$), where it remained for 1 s before returning to the centre for the next trial. An equal number of steps to the left and right were presented in a random order for each participant.

The antisaccade task began with six practice trials followed by 30 trials. The trial procedure was the same as in the prosaccade task. The only difference was the instruction, as participants were instructed to look at the target whilst in the centre but to immediately look to the mirror image position of the target when it stepped to the periphery.

2.4. Outcome variables

Fixations were defined as periods of at least 100 ms duration without blinks or saccades. We calculated the spatial error of gaze position

during fixation (root mean square error, RMSE, in degree of visual angle), as well as saccade frequency (saccades/second) and blink rate (blinks/second).

All eye movements with velocity $< 30°/s$ and duration ≥ 50 ms were classified as SPEM. SPEM outcomes were mean velocity gain and saccade frequency (saccades/second). SPEM velocity gain was calculated by dividing eye velocity by target velocity and multiplying by 100. A value of 100 indicates perfect eye-target velocity match, whilst values below or above 100 indicate that eye movements are slower or faster than the target, respectively.

In the saccade tasks, saccades were automatically detected on the basis of a minimum velocity criterion (velocity $\geq 60°/s$) or on the basis of minimum velocity and minimum acceleration criteria (velocity $\geq 22°/s$ and acceleration $\geq 3800°/s^2$). Trials were considered valid when there was a fixation on the central fixation point that started at least 100 ms before peripheral target onset and that was no more than 3° off the central fixation point. No saccade or blink was allowed to occur during this interval. Additionally, saccades had to end before the peripheral target timed out for a trial to be considered valid. Saccades with amplitude $< 1°$ or latency < 80 ms were excluded.

For both saccade tasks, we calculated mean latencies, mean peak saccadic velocities, mean amplitude gain and mean spatial error for directionally correct saccades on valid trials. A directionally correct prosaccade was counted when the initial saccade was in the direction of the peripheral target. A correct antisaccade was counted when the initial saccade was performed in the opposite direction of the peripheral target. The saccade latency was defined as the time (in ms) from target appearance to saccade initiation. For the calculation of the mean peak saccadic velocities, the average of the peak saccade velocities from all trials was calculated. The mean amplitude gain was calculated by dividing eye position by target position and multiplying this value by 100. A value of 100 indicates a saccade with perfect spatial accuracy, whilst values below or above 100 indicate that saccades undershot or overshot the target position, respectively. To calculate mean spatial error, target step amplitude was first subtracted from the saccade amplitude of the initial saccade, with the difference then divided by the target step amplitude. Following this, the value was multiplied by 100 and the absolute value was taken. This measure indicates the deviation of landing position from (mirrored) target position. The units are percentages to indicate relative deviation from the target step amplitude.

Due to the main sequence relationship of saccades (i.e. the strong correlation between saccade amplitude and peak velocity) (Bahill et al., 1975; Dodge & Cline, 1901), we also calculated amplitude-adjusted peak velocities, dividing peak velocity by amplitude gain. For the antisaccade task, we additionally calculated the antisaccade error rate, antisaccade costs (antisaccade latency minus prosaccade latency) and the percentage of corrected antisaccade errors.

2.5. Missing and invalid data

Across tasks, 318 out of 4,000 participants had no EM data. Missing data were primarily due to technical issues during data acquisition or post-processing of the data (75.4%), with a lesser number of missing cases due to contraindications (8.5%), exclusion after visual inspection of data (8.2%), non-compliance (5.7%), or refusal (0.6%). A few cases (1.3%) had no data due to at least two of the aforementioned reasons, which results from independent evaluations of data quality for the different EM tasks.

At task level, we excluded participants from the entire prosaccade or antisaccade task if they had < 7 valid trials in the task (number of participants excluded from the antisaccade task: 91; prosaccade task: 18). Participants with > 4 antisaccade errors were required to have performed at least one corrective saccade to ensure that participants understood the task instructions (number of participants excluded: 1).

All saccade outcomes except antisaccade error rate and percentage of corrected antisaccade errors were calculated only if a participant had ≥ 7 trials that were correct and therefore could be included in the calculation. For the percentage of corrected antisaccade errors the criterion was set to ≥ 5 direction errors.

For blink rate during fixation we excluded participants who had a value that was more than three times the interquartile range above the third quartile of their age group (30–39, 40–49, 50–59, 60–69, 70–79, 80+) because such values could reflect signal loss that was falsely classified as blink.

2.6. Statistical analysis

Skewed EM outcomes (prosaccade and antisaccade latency and spatial error, as well as mean spatial error, saccade frequency and blink rate during fixation) were log transformed.

We generated one scatterplot for each EM outcome for a first visual inspection of the association of age with EM performance and possible interaction effects between age and sex (Supplement A).

We quantified change in EM performance per one-year increase in age and differences in EM performance between men and women by using a separate multivariable regression model for each EM outcome (except for the EM outcome correction rate of antisaccade errors, see explanation below). All initial models included age and sex as independent variables with further adjustment for best-corrected visual acuity and educational level. Next, we included an additional term of age^2 in each model to evaluate potential nonlinear relationships between age and EM performance. Age and age^2 were mean-centred to prevent collinearity (Iacobucci et al., 2016). Missing covariate data were imputed using predictive mean matching (Hmisc package, 10 bootstrap replicates). For a detailed description of the model assumptions of the multivariable regression models see Supplement B.

To compare the strength of age and sex effects on EM outcomes, we calculated Cohen's f^2, which measures the proportion of variance in the outcome that is uniquely accounted for by either age or sex (Cohen, 1988). We presented the effect sizes visually in a forest plot. As a rule of thumb, $f^2 = 0.02$ indicates a small effect, $f^2 = 0.15$ indicates a medium effect and $f^2 = 0.35$ indicates a strong effect (Cohen, 1988, pp. 410–414). Further information on the calculation of f^2 is in Supplement B. The effect sizes did not only allow us to make a ranking of the strength of association but also allowed us to evaluate whether those aging effects that were significant in the regression model were of relevant effect size. We wanted to rule out the possibility that associations just became significant due to high statistical power resulting from our large sample size. Thus, we considered the results of the regression models and the effect sizes together in the interpretation of the results.

To evaluate whether relations between age and EMs differed between men and women, we constructed an additional model for each outcome, which included age*sex and age^2*sex terms in addition to age, age^2, sex, best-corrected visual acuity and education. For each EM outcome we carried out a likelihood-ratio test that compared the interaction model to the model without interaction terms.

Since the percentage of corrected antisaccade errors was severely skewed (most participants corrected 100% of their errors), we fitted a one-inflated beta regression model instead of a multivariable linear regression model (gamlss package). The one-inflated beta regression model is a mixture model consisting of two parts. The first part models whether or not somebody corrected all direction errors by using a logistic regression model and the second part is a beta regression model that models the data of those participants who did not correct all direction errors. Since this model requires that all values range from 0 to 1, we first transformed the variable by dividing it by 100.

Additionally, we calculated for each age group the standard deviation as a measure of interindividual variability in performance and inspected whether the variability within each group increased from the youngest to the oldest age groups.

We further examined whether the stability of performance during a test differed across age. We assessed this intraindividual variability in

performance by calculating for each participant for latency, amplitude gain, spatial error and peak velocity in both saccade tasks as well as for SPEM velocity gain the standard deviation in performance across all valid trials (saccade tasks) or segments of pursuit (SPEM). We calculated for each outcome a multivariable regression model that included age and sex as predictors and best-corrected visual acuity and education as potential confounders. All outcomes except for intraindividual variability in smooth pursuit velocity gain were log-transformed due to a high skewness of the regression residuals. In a second step we added age^2 to the model to evaluate nonlinear associations.

Statistical analyses were carried out in R using an alpha level of 0.05. Point estimates of association are presented with 95% confidence intervals.

3. Results

3.1. Study sample

Table 1 gives descriptive characteristics of the study sample. The sample had a high level of education and high best-corrected visual acuity.

3.2. Age effects

The associations between age and EM performance are displayed in Table 2. The strength of association of each EM outcome with age is presented visually in Fig. 1 (forest plot). Taking the results of the regression models and the effect sizes together, we concluded that there were age-related performance declines in log of spatial error (RMSE) ($f^2 = 0.05$) and log of saccade frequency ($f^2 = 0.07$) during fixation, SPEM velocity gain ($f^2 = 0.21$), saccade frequency during SPEM ($f^2 = 0.04$), log of prosaccade ($f^2 = 0.28$) and antisaccade latency ($f^2 = 0.21$), prosaccade amplitude gain ($f^2 = 0.04$), log of prosaccade spatial error ($f^2 = 0.06$), amplitude-adjusted and unadjusted peak prosaccade velocity (both $f^2 = 0.02$), antisaccade error rate ($f^2 = 0.09$) and antisaccade costs ($f^2 = 0.04$). Nonlinear effects of age, indicating more rapid performance decline with advancing age, were statistically significant in all of these outcomes. Since f^2 represents the combined effect size of both linear and nonlinear associations of age with EM performance, aging effects were numerically strongest in saccade latencies and SPEM velocity gain. In contrast, amplitude-adjusted peak antisaccade velocity did not show any significant decline with advancing age ($p = 0.859$). In addition, antisaccade peak velocity, antisaccade amplitude gain, antisaccade spatial error and log of blink rate during fixations all had very low linear and nonlinear associations with age ($f^2 \leq 0.01$) and are, therefore, also considered to be relatively stable across age. Associations between best-corrected visual acuity and EM outcomes were – if present – of negligible size ($f^2 \leq 0.004$) and did therefore not account for differences in EM performance.

Table 1
Descriptive Characteristics of the Total Sample and Stratified by Sex.

	Total sample	Women	Men
Number of participants, N (%)	3682 (100)	2107 (57.2)	1575 (42.8)
30–39 years	647 (17.6)	345 (16.4)	302 (19.2)
40–49 years	721 (19.6)	433 (20.6)	288 (18.3)
50–59 years	976 (26.5)	580 (27.5)	396 (25.1)
60–69 years	712 (19.3)	415 (19.7)	297 (18.9)
70–79 years	481 (13.1)	259 (12.3)	222 (14.1)
80+ years	145 (3.9)	75 (3.5)	70 (4.4)
Age, M (SD) in years	54.7 (14.1)	54.6 (13.7)	54.8 (14.5)
Education level, N (%)	3646 (99.0)	2083 (98.9)	1563 (99.2)
High	1915 (52.5)	988 (47.4)	927 (59.3)
Middle	1659 (45.5)	1040 (49.9)	619 (39.6)
Low	72 (2.0)	55 (2.6)	17 (1.1)
Best-corrected visual acuity, N (%)	3661 (99.4)	2099 (99.6)	1562 (99.2)
High (≥0.8)	3168 (86.5)	1801 (85.8)	1367 (87.5)
Middle (0.32–0.63)	465 (12.7)	284 (13.5)	181 (11.6)
Low (<0.32)	28 (0.8)	14 (0.7)	14 (0.9)

Note. N = number of participants, M = mean, SD = standard deviation. Education level was determined using the International Standard Classification of Education 2011 (ISCED) and was coded as low (lower secondary education or below), middle (upper secondary education to undergraduate university level) and high (postgraduate university study). Assessment of best-corrected visual acuity was based on visual scores from the right eye and was measured using an automated refractometer (Ark-1 s, NIDEK CO., Tokyo, Japan). Categorization of the visual acuity values was based on the guidelines of the International Council of Ophthalmology.

Table 3 depicts the results for the association between age, sex and percentage of corrected antisaccade errors. Age influenced whether or not all antisaccade errors were corrected but had no influence on the percentage of corrected antisaccade errors in those participants who corrected less than 100% of their errors. When age^2 was added to the model, it showed the same pattern of results as the linear age term.

The descriptive results of EM performance for each age group showed that interindividual differences in performance within each age band increased with age, particularly in saccade frequency during fixation, SPEM velocity gain, saccade latencies, antisaccade error rate and costs, but remained rather stable in all other outcomes (Supplement C).

Intraindividual variability in EM performance increased with age in all modelled outcomes except prosaccade and antisaccade peak velocity (Supplement D). The age effect was statistically significant for prosaccade peak velocity but Cohen's f^2 indicates that the effect is negligible ($f^2 < 0.01$). Age-related changes were strongest for prosaccade and antisaccade latency (prosaccades: $f^2 = 0.14$, antisaccades: $f^2 = 0.10$) and small for all other modelled outcomes ($0.01 \leq f^2 \leq 0.04$).

3.3. Sex differences

Sex was significantly associated with eight EM outcomes (Table 2). However, Cohen's f^2 indicated that these sex differences were small in SPEM velocity gain (higher gain in men) and blink rate during fixation (fewer blinks in men) (both $f^2 = 0.03$), with all other sex differences being negligible in size ($f^2 \leq 0.01$) (Fig. 2).

3.4. Interaction effects between age and sex

Testing for interactions between age and sex and between age^2 and sex yielded interactions for SPEM velocity gain, log of prosaccade latency, peak prosaccade velocity, amplitude-adjusted peak velocity in both saccade tasks, and antisaccade error rate (Supplement E). Inspection of the scatterplots (Supplement A) revealed that women had higher peak prosaccade velocity, amplitude-adjusted peak prosaccade and antisaccade velocity than men until approximately the age of 65 years, after which the direction of the effect reversed. Sex differences in log of prosaccade latency, antisaccade error rate and SPEM velocity gain increased across the measured age range (Supplement A).

4. Discussion

With nearly 4,000 participants and men and women almost equally represented from age group 30 to age group 80+, this is the largest and most representative study of associations of age and sex with EM performance to date. Our findings clarify the heterogeneous results from previous studies with smaller sample sizes, enable a ranking of age effects on EM performance and thereby contribute to a better understanding of EM changes that occur with age and in neurodegenerative disorders.

4.1. Age effects

We observed age-related decline in EM performance in 12 of 18 outcomes, with the largest declines in saccade latencies and SPEM velocity gain. However, EM performance was relatively stable across age

Table 2
Associations between Age, Sex and Eye Movement Outcomes.

Fixation Task				**Smooth Pursuit Task** (N = 3665)			
Outcome	**Predictor**	**b (95%-CI)**	**p-value**	**Outcome**	**Predictor**	**b (95%-CI)**	**p-value**
Log of spatial error (RMSE) [log °]; N = 3662	Age	0.02 (0.02, 0.02)	**< 0.001**	Velocity gain [%]	Age	−4.90 (−5.20, −4.50)	**< 0.001**
	Sex	0.00 (−0.01, 0.01)	0.518		Sex	4.69 (3.72, 5.67)	**< 0.001**
Log of saccade frequency [log(N/s)]; N = 3662	Age	0.04 (0.04, 0.04)	**< 0.001**	Saccade frequency [N/s]	Age	0.10 (0.10, 0.10)	**< 0.001**
	Sex	0.02 (0.01, 0.03)	**0.002**		Sex	−0.10 (−0.14, −0.07)	**< 0.001**
Log of blink rate [log(N/s)]; N = 3613	Age	0.00 (0.00, 0.00)	**< 0.001**				
	Sex	−0.02 (−0.02, −0.02)	**< 0.001**				
Prosaccade Task (N = 3651)				**Antisaccade Task**			
Outcome	**Predictor**	**b (95%−CI)**	**p-value**	**Outcome**	**Predictor**	**b (95%-CI)**	**p-value**
Log of latency [log ms]	Age	0.02 (0.02, 0.02)	**< 0.001**	Log of latency [log ms];	Age	0.02 (0.02, 0.02)	**< 0.001**
	Sex	0.01 (0.00, 0.01)	**0.001**	N = 3184	Sex	−0.00 (−0.01, 0.00)	0.126
Amplitude gain [%]	Age	−1.00 (−1.10, −0.80)	**< 0.001**	Amplitude gain [%];	Age	−0.35 (−1.10, 0.41)	0.368
	Sex	−0.44 (−0.89, 0.02)	0.057	N = 3184	Sex	−0.88 (−2.84, 1.08)	0.380
Log of spatial error (RMSE) [log %]	Age	0.04 (0.03, 0.04)	**< 0.001**	Log of spatial error (RMSE) [log %];	Age	0.01 (0.01, 0.02)	**< 0.001**
	Sex	0.03 (0.02, 0.04)	**< 0.001**	N = 3184	Sex	0.01 (0.00, 0.03)	0.116
Peak Velocity [°/s]	Age	−5.00 (−6.44, −3.56)	**< 0.001**	Peak Velocity [°/s];	Age	−2.06 (−3.90, −0.22)	**0.028**
	Sex	−3.08 (−6.93, 0.77)	0.117	N = 3184	Sex	−3.26 (−8.03, 1.51)	0.180
Amplitude-adjusted peak velocity	Age	−0.01 (−0.03, 0.00)	**0.047**	Amplitude-adjusted peak velocity;	Age	0.00 (−0.02, 0.02)	0.859
	Sex	−0.01 (−0.05, 0.03)	0.610	N = 3184	Sex	−0.01 (−0.06, 0.05)	0.841
				Error rate [%]; N = 3555	Age	5.02 (4.45, 5.58)	**< 0.001**
					Sex	−3.85 (−5.38, −2.34)	**< 0.001**
				Costs [ms]; N = 3172	Age	5.83 (4.67, 7.00)	**< 0.001**
					Sex	−4.39 (−7.41, −1.37)	**0.004**

Note. The table displays the change per 10-years of age and the mean sex difference in performance for different eye movement outcomes. N = number, b = unstandardized regression coefficient, 95%-CI = 95%-confidence interval. Unstandardized regression coefficients were obtained from the following multivariable linear regression model: EM outcome ~ b_0 + age*b_1 + sex*b_2 + educational level + best-corrected visual acuity + residual error. Unstandardized regression coefficients for age indicate the change in outcome variable per 10-years of age. Each unstandardized regression coefficient for sex expresses the difference in eye movement outcome between men and women with women as reference group.

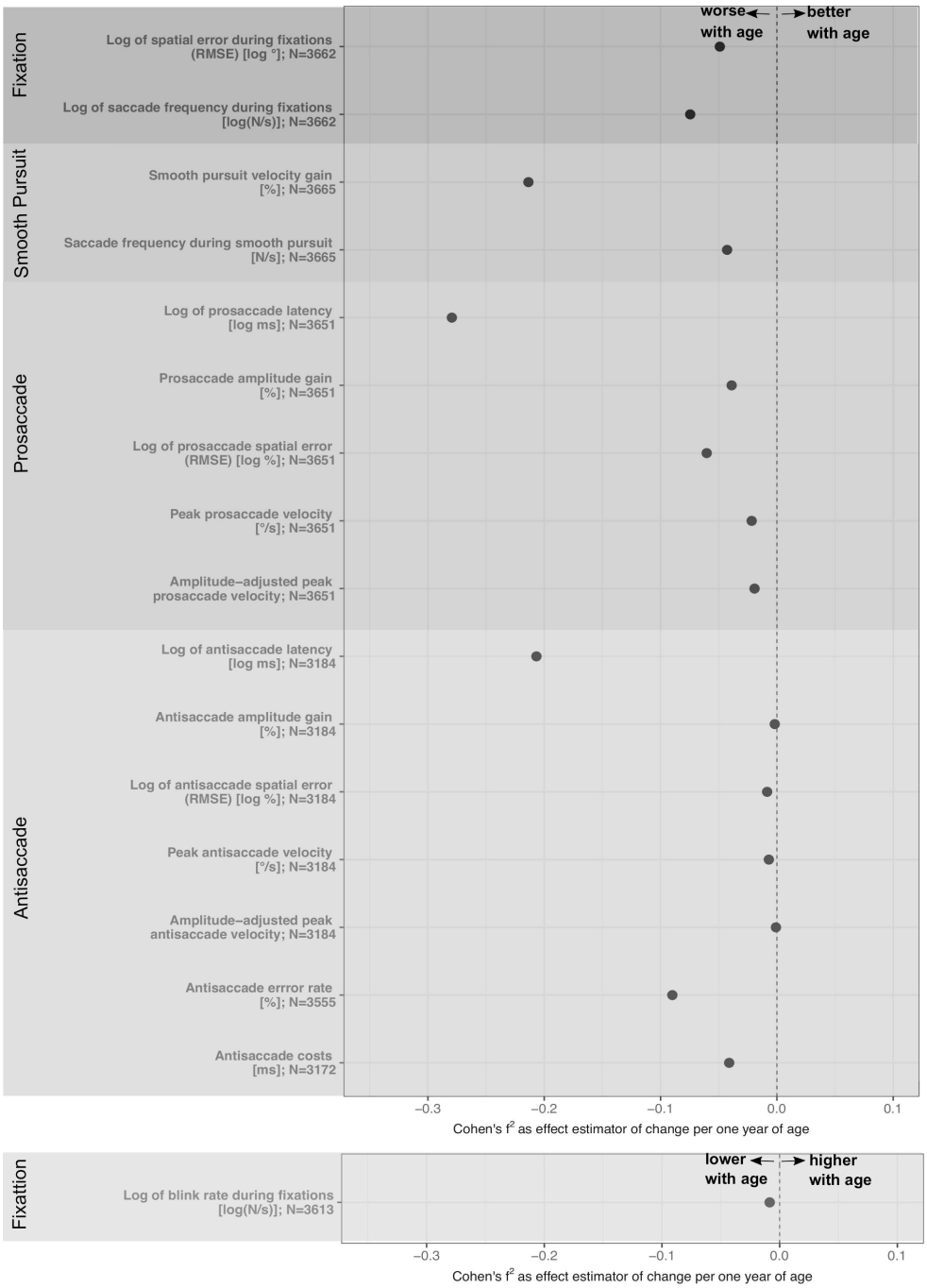

Fig. 1. Effect Sizes (Cohen's f^2) for Change in Eye Movement Performance per Year of Age. Cohen's f^2 indicates the effect size of change per one year of age for different eye movement outcomes (see y-axis). Some outcomes have been reversed so that all outcomes that show a decrease in performance across the lifespan have the effect size depicted on the left side of the vertical line and all outcomes that cross the vertical line indicate lifetime stability. For blink rate during fixation, performance cannot be classified as good or bad and therefore the trend (higher or lower with age) is indicated.

Table 3
Associations between Age, Sex and Percentage of Corrected Antisaccade Errors.

	Coefficient	Beta regression model - Exp. Coef. (95%- CI)	p-value	Logistic regression model - Exp. Coef. (95%-CI)	p-value
Percentage of corrected antisaccade errors; N = 2369	Intercept	3.32 (2.64, 4.18)	**< 0.001**	2.51 (1.76, 3.62)	**< 0.001**
	Age	1.00 (0.99, 1.00)	0.108	0.97 (0.96, 0.97)	**< 0.001**
	Sex	1.02 (0.88, 1.19)	0.768	1.40 (1.13, 1.72)	**0.002**

Note. For the EM outcome "percentage of corrected antisaccade errors" we calculated a one-inflated beta regression model, which is a mixture model consisting of a logistic regression and a beta regression model. The table displays the exponentiated coefficients (Exp. Coef) and their 95%-confidence intervals. The logistic regression part models whether or not somebody corrects all mistakes is associated with age and sex. The beta regression part models the associations between age, sex and the proportion of corrected errors in those participants who did not correct all of their errors. The exponentiated coefficients represent odds ratios.

in blink rate during fixation and in five antisaccade outcomes, namely amplitude-adjusted and unadjusted peak velocity, as well as amplitude gain, spatial error and percentage of corrected antisaccade errors (in those who corrected not all of their errors). In all outcomes that declined with age, we observed an accelerated decline with advancing age as indicated by significant age^2 terms. Generally, interindividual variability in performance increased with age, particularly in saccade frequency during fixation, SPEM velocity gain, saccade latencies, antisaccade error rate and costs across age groups, suggesting that age reinforces existing interindividual differences and that some individuals age more successfully than others. Intraindividual variability in performance also increased with age, except for antisaccade and prosaccade peak velocity, which indicates less stable task performance with increasing age for most EM outcomes.

The age-related increases in RMSE ($f^2 = 0.05$) and saccade frequency ($f^2 = 0.07$) during fixation demonstrated decreased fixational stability with advancing age. Fixations and saccades are interdependent because higher activations in fixation neurons go along with lower activations in saccade neurons. Fixation and saccade neurons are found in the superior colliculus (Munoz & Fecteau, 2002) and frontal eye fields (Hanes et al., 1998). Therefore, these results suggest age-related changes in activity patterns in saccade and fixation neurons.

In the smooth pursuit task, we observed lower velocity gain ($f^2 = 0.21$) and higher saccade frequency ($f^2 = 0.04$) with advancing age. Reduced velocity gain is typically associated with increased saccade frequency, as many saccades are used to compensate for slow SPEMs (Lencer & Trillenberg, 2008). Whilst numerous cortical and subcortical areas are involved in SPEMs (Krauzlis, 2004; Lencer & Trillenberg, 2008), the medial superior temporal area (Krauzlis, 2004) and the frontal pursuit area in the frontal eye fields (Tanaka & Lisberger, 2001) have been associated with on-line gain control in terms of direction and speed of SPEMs. Thus, the moderate decrease in velocity gain with advancing age suggests age-related changes that affect these areas. However, it is also plausible that age has small effects on many involved brain areas, which lead in sum to age-related decreases in SPEM velocity gain.

Aging had the strongest effects on saccade latencies (prosaccades: $f^2 = 0.28$; antisaccades: $f^2 = 0.21$), which supports the findings of previous studies (Munoz et al., 1998; Noiret et al., 2017; Peltsch et al., 2011; Shafiq-Antonacci et al., 1999; Sweeney et al., 2001) and extends this to the general adult population. Saccade latency depends on processes such as attention, target expectation, speed of target detection, response-related decision-making and response execution (Hutton, 2008). Thus, increases in saccade latencies indicate age-related slowing in saccade execution but it remains unclear which of the aforementioned components cause age-related decline.

Antisaccade costs also increased with age ($f^2 = 0.04$), suggesting that antisaccade latencies showed a disproportionally higher increase than prosaccade latencies. The cognitive processes required for saccade execution are more complex for antisaccades than prosaccades (Munoz & Everling, 2004); therefore, age may affect execution speed of complex cognitive processes. According to parallel programming models, a reflex-like prosaccade and a voluntary antisaccade are programmed in parallel; a successful antisaccade is executed if it reaches the activation threshold earlier than the prosaccade (Massen, 2004). Thus, the higher increase in antisaccade latencies corresponds to the finding of higher antisaccade error rate ($f^2 = 0.09$) with age, given that prolonged antisaccade programming is expected to increase the likelihood of antisaccade errors (Massen, 2004).

Performance in prosaccade amplitude gain ($f^2 = 0.04$) and spatial error ($f^2 = 0.06$) decreased with age, whereas antisaccade amplitude gain and spatial error remained relatively stable. Spatial accuracy of prosaccades is mainly influenced by cerebellar integrity (Optican, 2005), whereas programming of antisaccade amplitudes relies heavily on non-standard sensorimotor transformations in posterior parietal cortex (Herweg et al., 2014) and frontal eye fields (Moon et al., 2007). Since cerebellar brain volume declines with age but the (inferior) parietal lobe appears to be spared (Raz et al., 2001), prosaccade but not antisaccade spatial accuracy may be expected to decline with age. Additionally, previous research shows that Parkinson's disease patients differ from controls in prosaccade but not antisaccade gain (Mosimann et al., 2005), making hypometric prosaccades (Mosimann et al., 2005). This pattern of EMs supports the need to further investigate cerebellar involvement in Parkinson's disease (Wu & Hallett, 2013) and stresses the importance of understanding age-related brain changes as a prerequisite for understanding pathological brain changes. However, it should be noted that measures of saccade performance are highly sensitive to task design. Specifically, the amplitude of the target step was fixed on either side in the saccade tasks, which might have reduced the difficulty to perform spatially accurate saccades. This issue is particularly pertinent for the antisaccade task, where multiple target eccentricities place greater demands on sensorimotor transformations (Herweg et al., 2014). Therefore, it remains unclear whether the antisaccade task would have been more sensitive in detecting (age-related) differences in spatial accuracy if the target eccentricity had been varied.

Amplitude-adjusted peak velocity was relatively stable across the investigated age range for antisaccades, but showed a small decline for prosaccades ($f^2 = 0.02$), corresponding to previous research (Sweeney et al., 2001). Intraindividual variability in peak velocity was constant across the investigated age range in both tasks. In terms of neurophysiology, peak saccadic velocities are determined by the duration, number of spikes generated and maximal firing rate of saccadic burst cells in the brainstem reticular formation (Sparks, 2002). Since humans perform about 200,000 saccades each day, these cells can be considered to be continuously trained (Pratt et al., 2006). Amplitude-adjusted peak velocity was significantly lower for antisaccades compared to prosaccades. This means that maximal firing rates of burst cells and firing durations are lower for antisaccades than prosaccades and might explain why age-related differences occurred only for prosaccades. Further, this finding indicates that maximal firing rates of burst cells decline at some point despite constant training.

4.2. Sex differences

Sex differences were mostly absent or negligible. We found small sex differences in blink rate during fixation (men blinked less, $f^2 = 0.03$) and SPEM velocity gain (men had higher gain, $f^2 = 0.03$). SPEM velocity gain

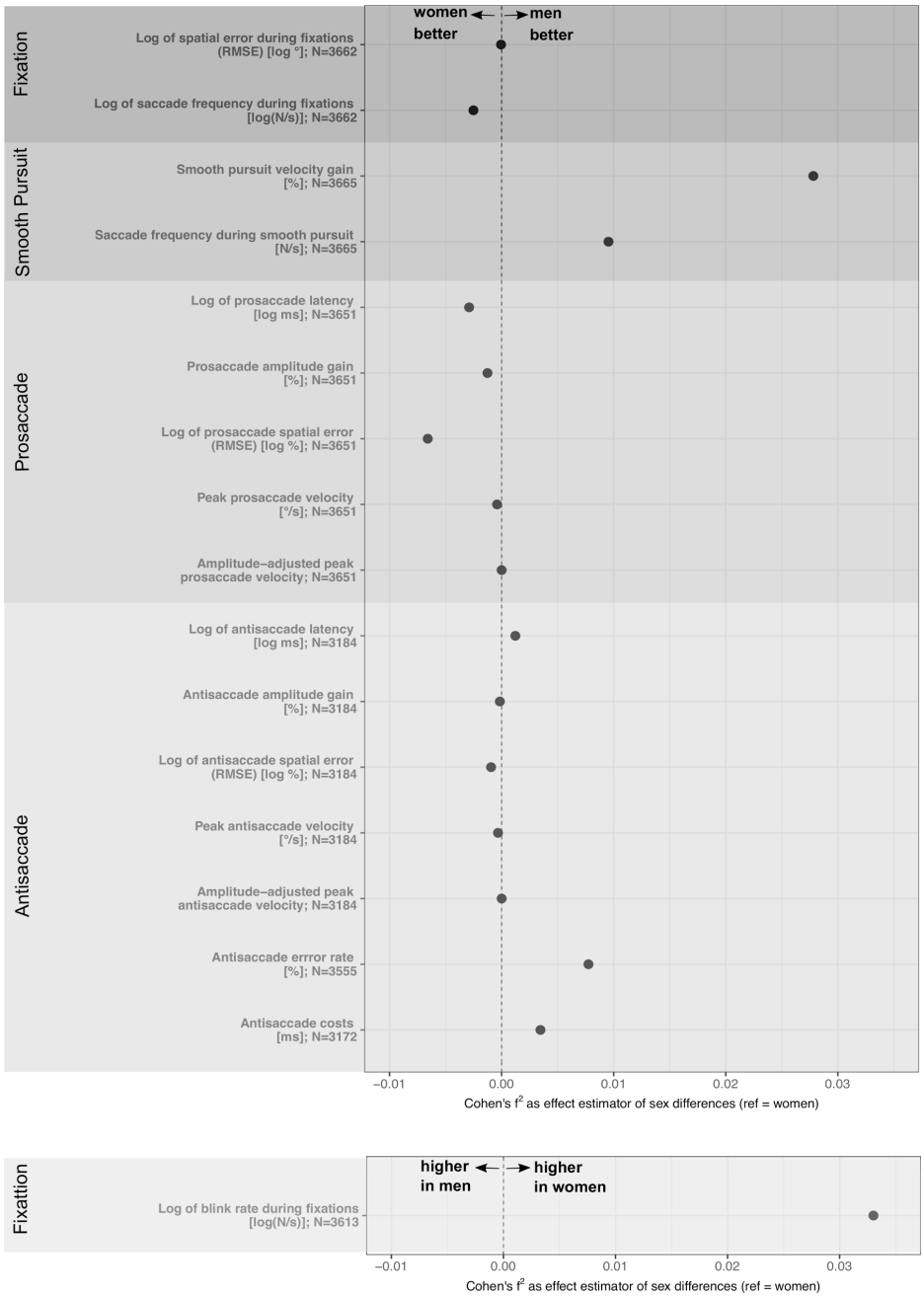

Fig. 2. Effect Sizes (Cohen's f^2) for Sex Differences in Eye Movement Performance. Cohen's f^2 indicates the effect size of change for sex for different eye movement outcomes (see y-axis). Reference group for sex: women. Cohen's f^2 for sex expresses the effect size of the difference in eye movement outcome between men and women. Outcomes in which women performed better where multiplied by −1 so that all points that lie on the left side of the vertical line indicate that women outperformed men in this outcome and vice versa. For blink rate during fixation, performance cannot be classified as good or bad and therefore the trend (higher or lower in men) is indicated.

is associated especially with activity in the medial superior temporal area (Krauzlis, 2004) and the frontal pursuit area in the frontal eye field (Tanaka & Lisberger, 2001). Thus, small sex differences in these areas might account for our finding. The finding of higher SPEM velocity gain in men is also in line with the results reported in Bargary et al. (2017). However, we could not confirm their finding of considerable sex differences in antisaccade error rate even if the trend was the same (higher error rates in women) (Bargary et al., 2017). The spontaneous blink rate is a marker of striatal dopamine (the more dopamine, the higher the blink rate) (Taylor et al., 1999) and is, for example, reduced in Parkinson's disease (Deuschl & Goddemeier, 1998). Our finding of lower blink rate in men is, therefore, compatible with evidence that women have higher striatal dopamine levels (Mozley et al., 2001).

4.3. Interaction effects between age and sex

Aging affected EM performance differently in men and women for six EM outcomes. Inspection of scatterplots revealed that performance declined more strongly in women than men in amplitude-adjusted and unadjusted prosaccade velocity and antisaccade error rate. The reverse pattern was observed for prosaccade latency. Additionally, performance declines earlier in women than men in SPEM velocity gain and amplitude-adjusted antisaccade velocity. These findings correspond to the presence of interactions between age and sex in brain metabolism (Kakimoto et al., 2016) and atrophy (Xu et al., 2000). Further, sex differences in aging are also known to exist in cognition, for example in reaction times (Der & Deary, 2006).

4.4. Potential of EMs as biomarkers of neurodegeneration

The importance of the current findings extends beyond characterising age and sex effects in the general, healthy population. Individuals with early signs of cognitive decline have impaired EM performance (Levy et al., 2018), making EMs a candidate preclinical biomarker of neuropathological changes. Since it is difficult to distinguish between normal age-related and pathological changes, an EM outcome that is unaffected by aging but impaired in neurodegenerative diseases would be an ideal biomarker of pathological cognitive decline.

In the antisaccade task, most participants corrected 100% of their errors, and in those who corrected less than 100% of their errors, age did not predict the amount of corrected errors. Interestingly, the percentage of corrected errors is decreased in Alzheimer's disease (Crawford et al., 2013; Garbutt et al., 2008; Noiret et al., 2018), making a low percentage of corrected antisaccade errors a suitable indicator of pathological brain changes as it cannot solely be explained by aging processes. However, it may be argued that its use as biomarker might be more applicable to those participants with higher visual acuity. Even if best-corrected visual acuity did not impact EM performance, 75% of the missings in our sample occurred due to technical issues during data acquisition or post-processing of the data and technical failures were more likely for participants wearing high dioptric glasses and for participants with artificial lenses or eye diseases. Nevertheless, calibration and validation did also succeed in some participants with low and medium visual acuity.

Correcting antisaccade errors requires error monitoring, which has been associated with activity in anterior cingulate cortex (Botvinick et al., 2004) and supplementary eye fields (Stuphorn et al., 2000). The ability to correct antisaccade errors has also been linked to general cognitive functioning and spatial working memory capacity in Alzheimer's disease patients (Crawford et al., 2013). Slow saccade latencies are unlikely to account for the low percentage of corrected antisaccade errors because healthy participants are able to initiate both an initial as well as a corrective saccade within less than one second (Crawford et al., 2013; Noiret et al., 2017). Individuals with dementia, however, have longer saccade latencies (Crawford et al., 2013) and, therefore, the possibility that participants with cognitive impairment do not correct antisaccade errors due to time constraints cannot be ruled out. A related limitation of the current work is that the measure of corrected errors in this study is based on a lower number of trials, requiring replication with a larger number of trials. Also, further work is needed to compare the sensitivity of EM performance in detecting pathological changes with the sensitivity of traditional cognitive tasks.

5. Conclusions

This study demonstrated that (i) EM performance declines with age in two thirds of outcomes, (ii) small sex differences exist in SPEM velocity gain and blink rate during fixation, and (iii) interindividual differences and intraindividual variability in EM performance increase with age. Although still requiring further validation, the best EM candidate preclinical biomarker of neurodegeneration may be the percentage of corrected antisaccade errors.

CRediT authorship contribution statement

Annabell Coors: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization, Writing - review & editing. **Natascha Merten:** Validation, Investigation, Writing - review & editing. **David D. Ward:** Visualization, Writing - review & editing. **Matthias Schmid:** Formal analysis, Writing - review & editing. **Monique M.B. Breteler:** Conceptualization, Methodology, Validation, Resources, Data curation, Supervision, Project administration, Funding acquisition, Writing - review & editing. **Ulrich Ettinger:** Conceptualization, Methodology, Validation, Investigation, Supervision, Project administration, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.visres.2020.10.004.

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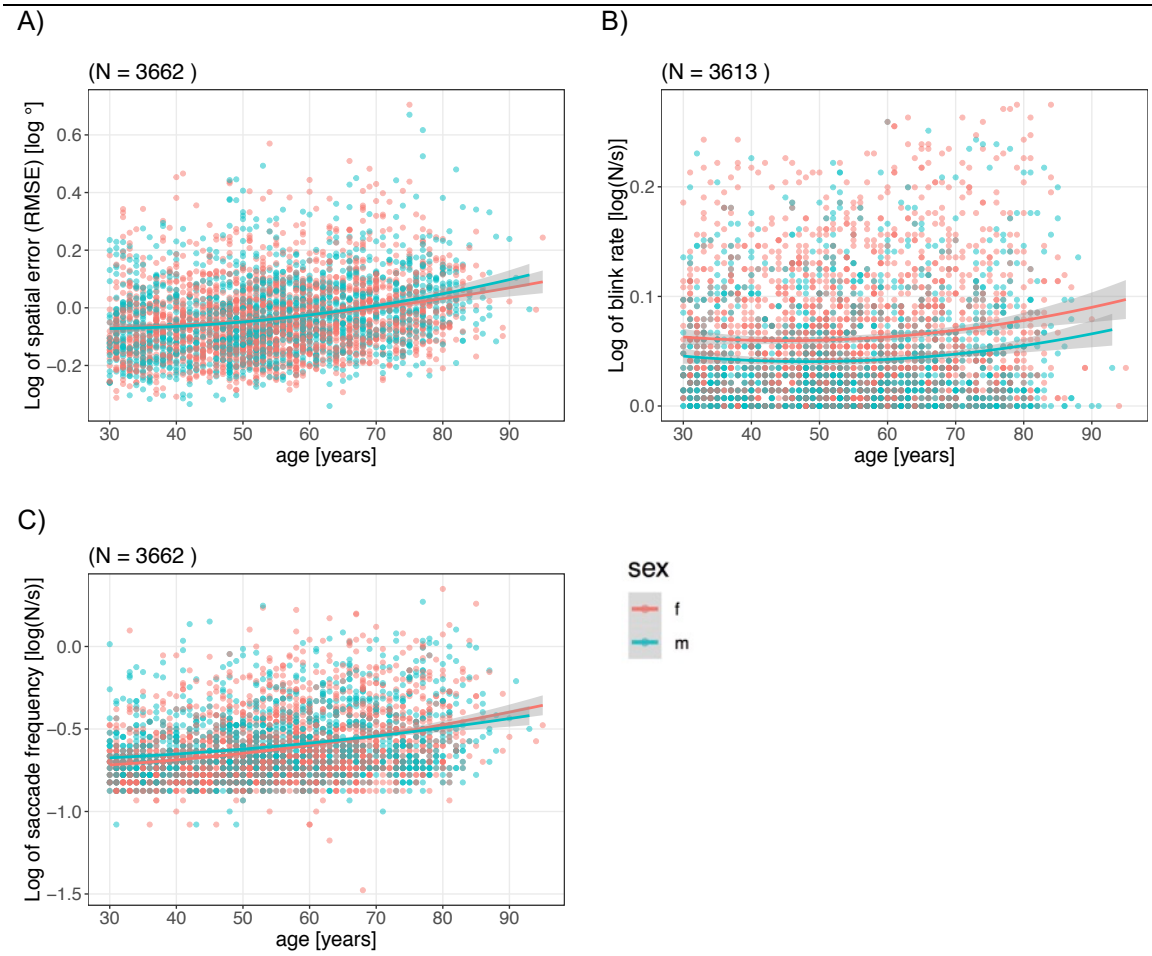
Supplementary Material – Strong Age but Weak Sex Effects in Eye Movement Performance in the General Adult Population: Evidence from the Rhineland Study

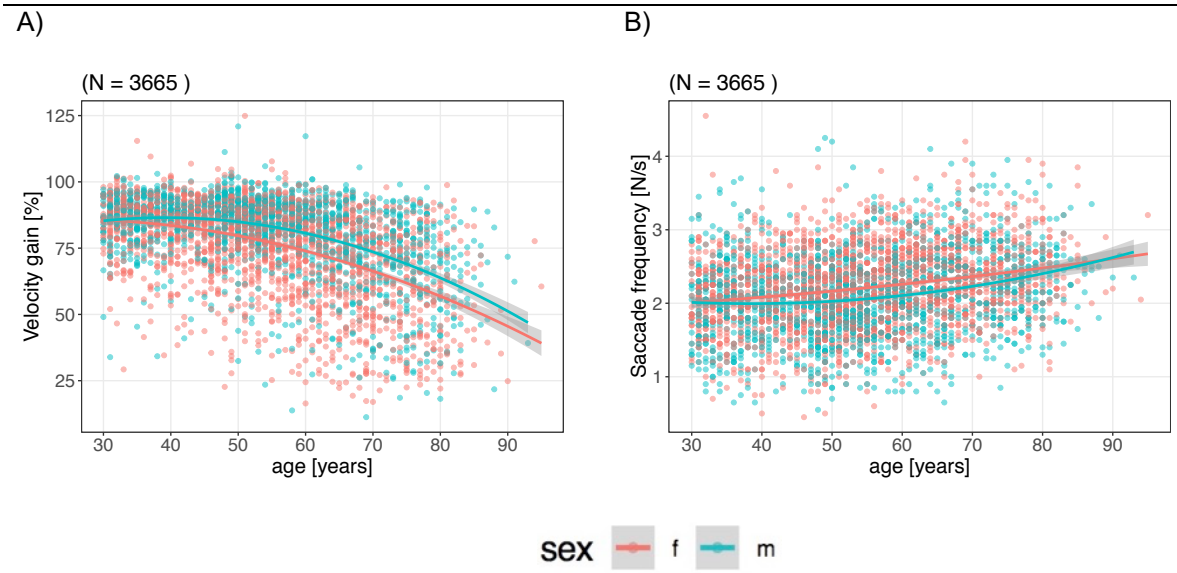
Supplement A: Scatterplots for the Associations between Age, Sex and Eye Movement Performance

Figure A.1 Scatterplots for Outcomes of the Fixation Task



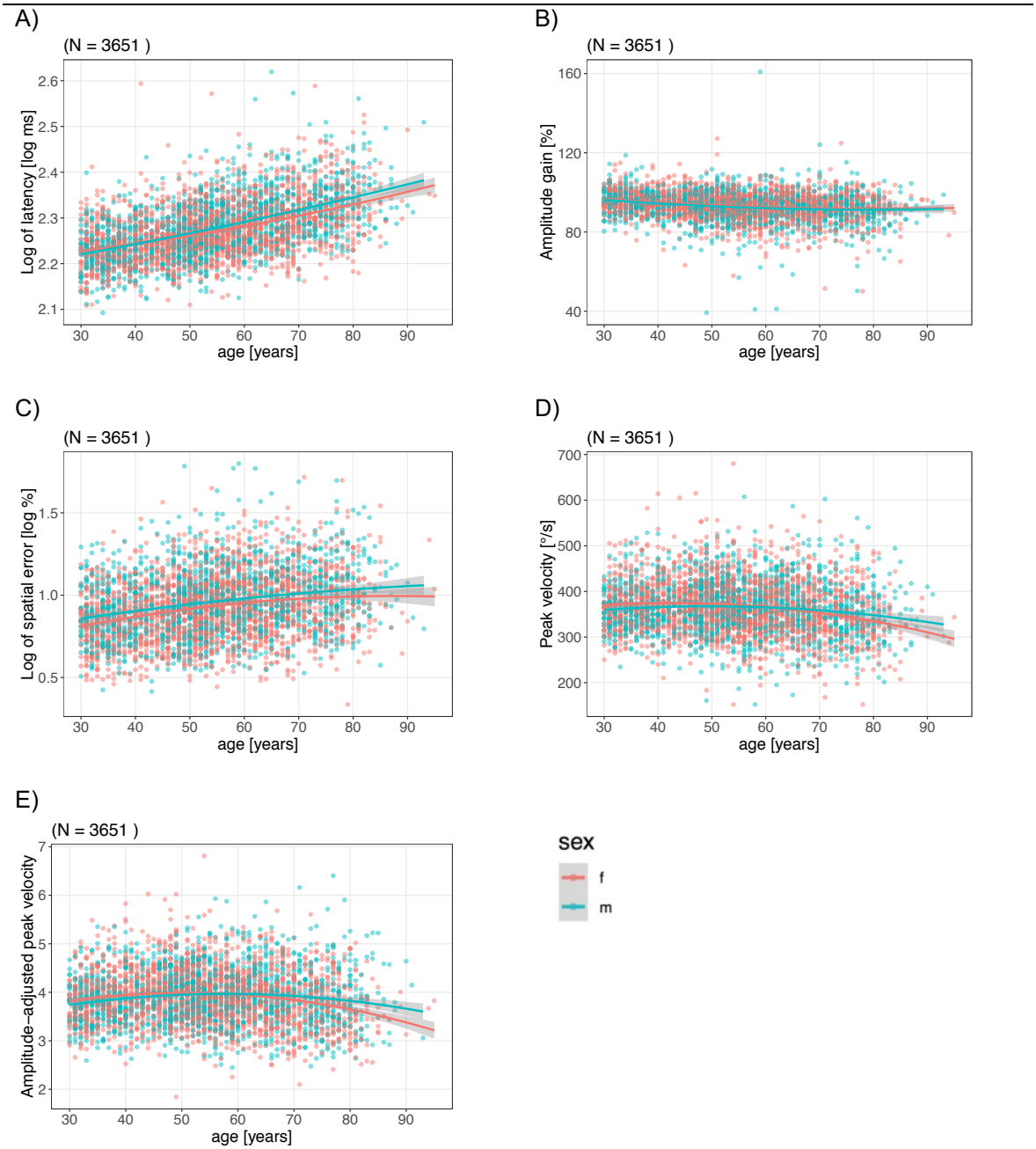
Note. Scatterplots with the eye movement outcome on the y-axis and the age (in years) on the x-axis. The heading indicates the number of participants (N= number). Each data point represents one participant. Since the data points of some participants overlap, some data points have a darker colour than others. Data of men and women are depicted in different colours, red for women and turquoise for men. For each sex there exists one superimposed function for the development of the outcome across the adult life span. The functions were obtained from a multivariable regression model with the following formula: Eye movement outcome ~ b_0 + age*b_1 + age^2*b_2 + residual error. A) Log of mean spatial error during fixations; B) Log of blink rate during fixations; C) Log of saccade frequency during fixations.

Figure A.2 Scatterplots for Outcomes of the Smooth Pursuit Task



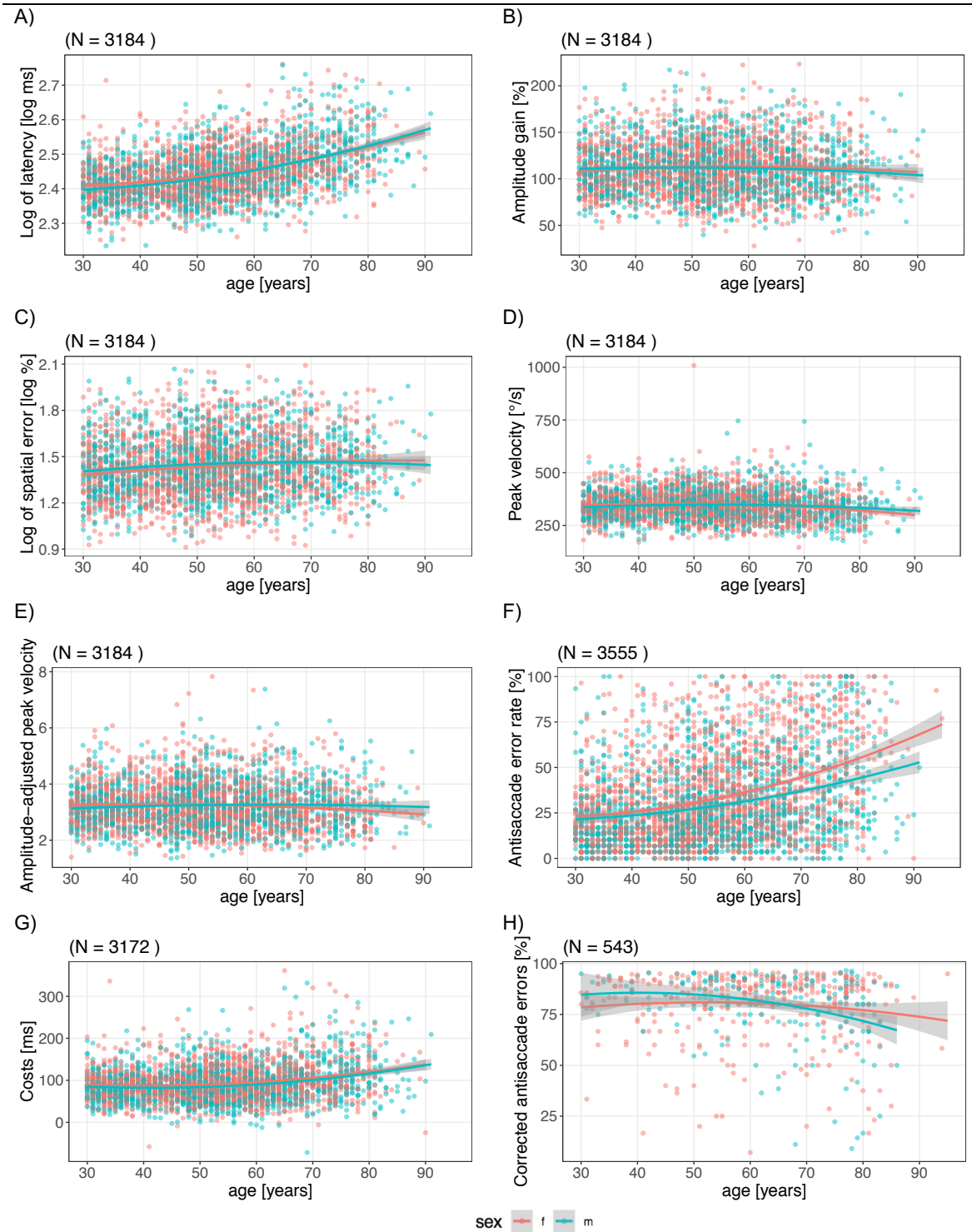
Note. Scatterplots with the eye movement outcome on the y-axis and the age (in years) on the x-axis. The heading indicates the number of participants (N= number). Each data point represents one participant. Since the data points of some participants overlap, some data points have a darker colour than others. Data of men and women are depicted in different colours, red for women and turquoise for men. For each sex there exists one superimposed function for the development of the outcome across the adult life span. The functions were obtained from a multivariable regression model with the following formula: Eye movement outcome ~ b_0 + age*b_1 + age^2*b_2 + residual error. A) Mean velocity gain; B) Saccade frequency during the smooth pursuit task.

Figure A.3 Scatterplots for Outcomes of the Prosaccade Task



Note. Scatterplots with the eye movement outcome on the y-axis and the age (in years) on the x-axis. The heading indicates the number of participants (N= number). Each data point represents one participant. Since the data points of some participants overlap, some data points have a darker colour than others. Data of men and women are depicted in different colours, red for women and turquoise for men. For each sex there exists one superimposed function for the development of the outcome across the adult life span. The functions were obtained from a multivariable regression model with the following formula: Eye movement outcome ~ b_0 + age*b_1 + age^2*b_2 + residual error. A) Log of latency; B) Amplitude gain; C) Log of spatial error; D) Peak velocity; E) Amplitude-adjusted peak velocity.

Figure A.4 Scatterplots for Outcomes of the Antisaccade Task



Note. Scatterplots with the eye movement outcome on the y-axis and the age (in years) on the x-axis. The heading indicates the number of participants (N= number). Each data point represents one participant. Since the data points of some participants overlap, some data points have a darker colour than others. Data of men and women are depicted in different colours, red for women and turquoise for men. For each sex there exists one superimposed function for the development of the outcome across the adult life span. The functions were obtained from a multivariable regression model with the following formula: Eye movement outcome ~ b_0 + age*b_1 + age^2*b_2 +residual error. A) Log of latency; B) Amplitude gain; C) Log of spatial error; D) Peak saccade velocity; E) Amplitude-adjusted peak velocity; F) Antisaccade error rate; G) Antisaccade costs; H) Percentage of corrected antisaccade errors for subgroup of participants who corrected less than 100% of their errors.

Supplement B: Statistical Analysis

Checking the Assumptions of Linear Regression Models

Multicollinearity was checked with the variance inflation factor (R package car (Fox & Weisberg, 2011), vif-function). The other assumptions of the multivariable regression analyses were checked with diagnostic plots. We inspected the "scale-location plot" (which is sometimes also referred to as "spread-location plot") to check the homoscedasticity assumption and the quantile-quantile-plot (Q-Q-plot) to check the normality assumption of the residuals. If the normality assumption of the residuals was questionable, we log-transformed the outcome variable and inspected the Q-Q-plot again. Finally, we inspected for each outcome the "residuals vs leverage plot" to see if there were any influential cases that determine the regression line. If there were any influential cases, we calculated the regression models with and without these cases. If the results were similar in both cases, we did not remove them.

Cohen's f^2

We calculated Cohen's f^2 to indicate the effect size of the associations between age and sex with eye movement performance. For this we first constructed three regression models for each eye movement outcome. The first model was the full model and included age, age^2, sex, best-corrected visual acuity and education. In the second model we removed the sex term from the full model and in the third model we removed both age terms from the full model. We then extracted R^2 (the proportion of variance accounted for) from each of these models. Finally, we calculated f^2 by using the following formula (Cohen, 1988; Selya et al., 2012): $f^2 = \frac{R^2_{included} - R^2_{excluded}}{1 - R^2_{included}}$

In this formula $R^2_{included}$ refers to the proportion of variance accounted by the full model (first model). $R^2_{excluded}$ is extracted from the model that includes all terms of the model except for the term for which the effect size is calculated (model 2 if the effect size of sex differences is calculated and model 3 if the effect size of aging effects is calculated).

Correction for Multiple Testing

We did not correct for multiple testing since this was an exploratory analysis on the associations between age, sex and eye movement performance (Althouse, 2016;

Perneger, 1998; Rothman, 1990; Sinclair et al., 2013). Thus, only additional confirmatory studies can rule out the possibility of false discoveries (Althouse, 2016).

Supplement C: Descriptive Results and Interindividual Variability in Eye Movement Performance

Table C.1: Descriptive Results for Outcomes of the Fixation and Smooth Pursuit Tasks – for the Total Sample and Stratified by Age

Age Group	Fixation – spatial error (RMSE) [°], N=3662	Fixation – Saccade frequency [N/s], N=3662	Fixation – Blink rate [N/s], N=3613	SPEM – Velocity gain [%], N=3665	SPEM – Saccade Frequency [N/s], N=3665
30-39	0.82 (0.70-1.00)	0.20 (0.17-0.20)	0.08 (0.03-0.20)	85.0 (10.6)	2.0 (0.5)
40-49	0.85 (0.72-1.00)	0.20 (0.17-0.30)	0.08 (0.03-0.20)	83.4 (12.4)	2.0 (0.6)
50-59	0.88 (0.75-1.10)	0.22 (0.18-0.30)	0.08 (0.03-0.20)	81.0 (13.6)	2.1 (0.5)
60-69	0.90 (0.79-1.20)	0.20 (0.18-0.40)	0.10 (0.03-0.20)	72.3 (17.9)	2.3 (0.6)
70-79	1.00 (0.84-1.20)	0.30 (0.20-0.40)	0.10 (0.03-0.20)	65.4 (18.9)	2.4 (0.5)
80+	1.10 (0.92-1.30)	0.30 (0.23-0.50)	0.10 (0.03-0.30)	58.8 (18.6)	2.4 (0.5)
Total	0.90 (0.75-1.10)	0.20 (0.18-0.30)	0.10 (0.03-0.20)	77.6 (16.7)	2.2 (0.6)

Note. N = Number of participants, SPEM = smooth pursuit eye movements. Values represent mean (SD) for all outcomes except for spatial error, saccade frequency and blink rate during fixation because these outcomes were severely skewed and therefore values represent median (interquartile range).

Table C.2: Descriptive Results for Outcomes of the Prosaccade Task – for the Total Sample and Stratified by Age

Age Group	Latency [ms]	Amplitude gain [%]	Spatial error [%],	Peak velocity [°/s]	Ad. peak velocity
30-39	170 (159-183)	95.8 (5.4)	7 (5-9)	366.1 (50.1)	3.8 (0.5)
40-49	177 (164-191)	93.9 (6.4)	8 (6-11)	375.3 (59.1)	4.0 (0.6)
50-59	186 (173-202)	92.7 (7.0)	9 (6-12)	365.7 (57.8)	3.9 (0.5)
60-69	197 (179-215)	92.2 (7.0)	9 (7-13)	362.9 (59.7)	3.9 (0.6)
70-79	207 (192-230)	91.8 (8.1)	10 (7-14)	349.8 (63.2)	3.8 (0.6)
80+	215 (198-242)	91.2 (7.2)	10 (8-14)	338.6 (59.5)	3.7 (0.6)
Total	186 (171-206)	93.2 (7.0)	8 (6-12)	364.0 (58.6)	3.9 (0.6)

Note. Ad. = amplitude-adjusted. Values represent mean (SD) for all outcomes except for latency and spatial error because these outcomes were severely skewed and therefore values represent median (interquartile range). Number of participants = 3651.

Table C.3: Descriptive Results for Outcomes of the Antisaccade Task – for the Total Sample and Stratified by Age

Age Group	Latency [ms], N=3184	Amplitude gain [%], N=3184	Spatial error [%], N=3184	Peak velocity [°/s], N=3184	Ad. peak velocity N=3184	Error rate [%], N=3555	Costs [ms], N=3172	Percentage of corrected errors, N=543
30-39	254	110.3	24	342.4	3.2	23.1	86.4	86.7
	(234-279)	(25.7)	(19-34)	(59.9)	(0.7)	(18.6)	(34.6)	(81.8-91.3)
40-49	261	113.1	25	354.1	3.3	26.1	86.2	84.0
	(239-289)	(27.1)	(19-36)	(65.5)	(0.8)	(20.5)	(38.1)	(71.4-91.5)
50-59	273	112.0	28	346.4	3.2	31.6	90.1	87.5
	(250-304)	(28.2)	(20-37)	(69.6)	(0.8)	(22.2)	(39.9)	(77.8-91.7)
60-69	290	112.6	28	347.8	3.2	36.9	94.8	86.0
	(262-321)	(29.7)	(21-40)	(70.0)	(0.8)	(24.6)	(47.8)	(80.0-90.9
70-79	315	108.4	28	334.0	3.2	45.0	112.6	85.0
	(283-356)	(27.8)	(20-38)	(73.3)	(0.8)	(28.3)	(55.3)	(70.6-91.3)
80+	325	110.3	28	330.1	3.1	50.3	117.7	80.0
	(296-383)	(29.4)	(21-42)	(68.0)	(0.8)	(27.4)	(59.1)	(59.3-88.9)
Total	274	111.6	27	345.8	3.2	32.4	92.5	85.7
	(247-307)	(27.8)	(20-37)	(67.6)	(0.8)	(24.1)	(43.5)	(75.0-91.1)

Note. N = Number of participants. Ad. = amplitude-adjusted. Values represent mean (SD) for all outcomes except for latency, spatial error and percentage of uncorrected errors because these outcomes were severely skewed and therefore values represent median (interquartile range). Percentage of uncorrected errors was calculated only for those participants who corrected less than 100% of their errors.

Supplement D: Intraindividual Variability in Eye Movement Performance

Table D.1: Associations between Age, Sex and Intraindividual Variability in Selected Eye Movement Outcomes

EM Outcome	Predictor	b (95%-CI)	p-value	Cohen's f^2
SD smooth pursuit velocity gain (N=3665)	Age	0.73 (0.62, 0.85)	**< 0.001**	0.04
	Sex	-1.11 (-1.42, -0.80)	**< 0.001**	0.01
Log of SD prosaccade latency (N=3651)	Age	0.05 (0.05, 0.05)	**< 0.001**	0.14
	Sex	0.03 (0.01, 0.04)	**< 0.001**	0.00
Log of SD prosaccade amplitude gain (N=3651)	Age	0.03 (0.02, 0.03)	**< 0.001**	0.04
	Sex	0.04 (0.03, 0.06)	**< 0.001**	0.01
Log of SD prosaccade spatial error (N=3651)	Age	0.03 (0.03, 0.04)	**< 0.001**	0.04
	Sex	0.04 (0.03, 0.06)	**< 0.001**	0.01
Log of SD prosaccade peak velocity (N=3651)	Age	0.01 (0.00, 0.01)	**0.004**	0.00
	Sex	0.08 (0.07, 0.10)	**< 0.001**	0.04
Log of SD antisaccade latency (N=3184)	Age	0.04 (0.03, 0.04)	**< 0.001**	0.10
	Sex	-0.00 (-0.02, 0.01)	0.588	0.00
Log of SD antisaccade amplitude gain (N=3184)	Age	0.01 (0.01, 0.02)	**< 0.001**	0.01
	Sex	0.03 (0.02, 0.04)	**< 0.001**	0.01
Log of SD antisaccade spatial error (N=3184)	Age	0.01 (0.01, 0.02)	**< 0.001**	0.01
	Sex	0.02 (0.01, 0.03)	**0.002**	0.00
Log of SD antisaccade peak velocity (N=3184)	Age	0.00 (-0.00, 0.00)	0.092	0.00
	Sex	0.06 (0.05, 0.07)	**< 0.001**	0.02

Note. Multivariable regression analyses were conducted to examine associations of age and sex with intraindividual variability in performance. N = number of participants, b = unstandardized regression coefficient, 95%-CI = 95%-confidence interval. Regression model: dependent variable ~ b_0 + age*b_1 + sex*b_2 + educational level + best-corrected visual acuity + residual error. Regression coefficients for age indicate the change in EM outcome per one year increase in age. Cohen's f^2 indicates for each dependent variable the effect size of the aging effect (linear and nonlinear aging effects considered together) and of the sex difference. The full model for the calculation of Cohen's f^2 was the following regression model: dependent variable ~ b_0 + age*b_1 + age^2*b_2 + sex*b_3 + educational level + best-corrected visual acuity + residual error.

Supplement E: Age(2)*Sex-Interaction Effects

Table E.1: Testing for Different Relations between Age and Eye Movement Performance for Men and Women (age$^{(2)}$*sex-interactions)

Eye movement outcome	F-value	p-value	Age$^{(2)}$*Sex-Interaction
Log of spatial error during fixations (RMSE) [log °]	1.85	0.16	no
Log of saccade frequency during fixations [log(N/s)]	2.67	0.070	no
Log of blink rate during fixations [log(N/s)]	0.26	0.771	no
Smooth pursuit velocity gain [%]	8.13	**< 0.001**	**yes**
Saccade frequency during smooth pursuit [N/s]	2.57	0.077	no
Log of prosaccade latency [log ms]	5.53	**0.004**	**yes**
Prosaccade amplitude gain [%]	0.04	0.964	no
Log of prosaccade spatial error (RMSE) [log %]	0.67	0.511	no
Peak prosaccade velocity [°/s]	5.54	**0.004**	**yes**
Amplitude-adjusted peak prosaccade velocity	7.94	**< 0.001**	**yes**
Log of antisaccade latency [log ms]	2.42	0.089	no
Antisaccade amplitude gain [%]	0.97	0.379	no
Log of antisaccade spatial error (RMSE) [log %]	0.94	0.392	no
Peak antisaccade velocity [°/s]	2.09	0.124	no
Amplitude-adjusted peak antisaccade velocity	4.00	**0.018**	**yes**
Antisaccade error rate [%]	4.53	**0.011**	**yes**
Antisaccade costs [ms]	0.23	0.792	no

We ran a likelihood-ratio test to test whether the relation between age and eye movement performance differs for men and women. We took a significant result as indicator of a different relation between age and eye movement performance for men and women. The test compares the model fit of model 1 (dependent variable ~ b_0 + age*b_1 + age^2*b_2 + sex*b_3 + educational level + best-corrected visual acuity + residual error) with the model fit of model 2 (dependent variable ~ b_0 + age*b_1 + age^2*b_2 + sex*b_3 + age*sex*b_4 + age^2*sex*b_5 + educational level + best-corrected visual acuity + residual error). The null hypothesis says that the data is equally likely under both models and can be rejected if the p-value is ≤0.05; otherwise it cannot be rejected. Interpretation of the F-value: the data is "F-value" times more likely if the interaction terms "age*sex" and "age^2*sex" are included in the model (model 2) than if they are not included in the model (model 1).

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4. Polygenic Risk Scores for Schizophrenia Are Associated with Oculomotor Endophenotypes

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Original Article

Polygenic risk scores for schizophrenia are associated with oculomotor endophenotypes

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Abstract

Background. Schizophrenia is a heterogeneous disorder with substantial heritability. The use of endophenotypes may help clarify its aetiology. Measures from the smooth pursuit and antisaccade eye movement tasks have been identified as endophenotypes for schizophrenia in twin and family studies. However, the genetic basis of the overlap between schizophrenia and these oculomotor markers is largely unknown. Here, we tested whether schizophrenia polygenic risk scores (PRS) were associated with oculomotor performance in the general population.
Methods. Analyses were based on the data of 2956 participants (aged 30–95) of the Rhineland Study, a community-based cohort study in Bonn, Germany. Genotyping was performed on Omni-2.5 exome arrays. Using summary statistics from a recent meta-analysis based on the two largest schizophrenia genome-wide association studies to date, we quantified genetic risk for schizophrenia by creating PRS at different p value thresholds for genetic markers. We examined associations between PRS and oculomotor performance using multivariable regression models.
Results. Higher PRS were associated with higher antisaccade error rate and latency, and lower antisaccade amplitude gain. PRS showed inconsistent patterns of association with smooth pursuit velocity gain and were not associated with saccade rate during smooth pursuit or performance on a prosaccade control task.
Conclusions. There is an overlap between genetic determinants of schizophrenia and oculomotor endophenotypes. Our findings suggest that the mechanisms that underlie schizophrenia also affect oculomotor function in the general population.

Background

Schizophrenia is a severe mental disorder with a lifetime prevalence of just under 1% (McGrath, Saha, Chant, & Welham, 2008). There is substantial evidence for a genetic basis of schizophrenia, with recurrence risk in families of about 8.6% (Lichtenstein et al., 2006) and heritability estimates of up to 81% (Sullivan, Kendler, & Neale, 2003). Genome-wide association studies (GWASs) thus far have identified 145 single nucleotide polymorphisms (SNPs) that are associated with schizophrenia (Pardiñas et al., 2018). However, the genetic variance of schizophrenia explained by these SNPs is low (Pardiñas et al., 2018). On the one hand, there are many SNPs that do not reach the genome-wide significant threshold in a GWAS (5×10^{-8}) but that could explain in sum a substantial proportion of genetic variance (International Schizophrenia Consortium, 2009). Another reason for this may be that schizophrenia is highly heterogeneous and encompasses a multitude of different syndromes that do not necessarily have a common biological basis (Braff, Freedman, Schork, & Gottesman, 2007).

Endophenotypes have been proposed as an approach to better understand the aetiology of schizophrenia (Braff et al., 2007). They are considered to link a disorder to its genetic basis and to be closer to the actions of genes than disease symptoms are (Gottesman & Gould, 2003). Furthermore, as current disease classification might not well reflect aetiology (The Brainstorm Consortium, 2018), endophenotypes may help identify more homogenous subgroups of patients with shared biological basis (Braff et al., 2007).

Deficits in antisaccade and smooth pursuit eye movement (SPEM) tasks are amongst the best replicated endophenotypes for schizophrenia (Calkins, Iacono, & Ones, 2008; Holzman, 2000).

In the antisaccade task, participants are required to make a saccade in the opposite direction to a sudden-onset, peripheral target (Hallett, 1978). Individuals with schizophrenia make more antisaccade errors (trials in which the initial saccade is erroneously executed towards the peripheral target) compared to controls (Clementz, McDowell, & Zisook, 1994; Ettinger et al., 2004; Fukushima et al., 1988; Radant et al., 2010; Reilly et al., 2014; Reuter, Rakusan, &

Kathmanna, 2005; Sereno & Holzman, 1995). Higher antisaccade latencies (time needed to initiate the first saccade after the appearance of the peripheral target) have also been reported in some (Curtis, Calkins, Grove, Feil, & Iacono, 2001; Ettinger et al., 2004; Fukushima et al., 1988; Fukushima et al., 1990b; Fukushima, Fukushima, Morita, & Yamashita, 1990a; Karoumi et al., 2001; Mazhari et al., 2011; Sereno & Holzman, 1995) but not all studies (Radant et al., 2007, 2010; Reuter et al., 2005). Antisaccade amplitude gain (a measure of spatial accuracy of directionally correct antisaccades) was found to be reduced in individuals with schizophrenia in some (Ettinger et al., 2004; Karoumi et al., 2001; Radant et al., 2010) but not all studies (Ettinger et al., 2018; Fukushima et al., 1990a). However, impaired antisaccade performance has been reported in patients with psychosis across different diagnostic categories (Reilly et al., 2014) and, therefore, may have relatively low specificity for schizophrenia.

In the SPEM task, participants follow a slowly moving target with their eyes. Patients with schizophrenia have long been known to have SPEM impairments (Diefendorf & Dodge, 1908; Holzman, Proctor, & Hughes, 1973), characterised primarily by lower velocity gain (ratio of eye and target velocity) and higher saccade rates than controls (O'Driscoll & Callahan, 2008; Sereno & Holzman, 1995). Greater deficits in antisaccade error rate have also been observed in patients with schizophrenia who have impaired SPEM performance compared to patients with schizophrenia without SPEM impairments (Sereno & Holzman, 1995).

Antisaccade and pursuit measures have moderate-to-high heritability (Bell, Abel, Li, Christian, & Yee, 1994; Greenwood et al., 2007; Hong et al., 2006; Katsanis, Taylor, Iacono, & Hammer, 2000; Litman et al., 1997; Macare, Meindl, Nenadic, Rujescu, & Ettinger, 2014; Malone & Iacono, 2002) and temporal stability (Calkins, Iacono, & Curtis, 2003; Campion et al., 1992; Crevits, De Clerck, & Van Maele, 2000; Ettinger et al., 2003; Flechtner, Steinacher, Sauer, & Mackert, 2002; Gooding, Mohapatra, & Shea, 2004; Light et al., 2012; Sweeney et al., 1999). Clinically unaffected first-degree relatives of schizophrenia patients show impairments similar to those seen in patients with schizophrenia, albeit with smaller effect sizes (Calkins et al., 2008).

In contrast, performance in prosaccade tasks is typically preserved in schizophrenia (Damilou, Apostolakis, Thrapsanioti, Theleritis, & Smyrnis, 2016; Ettinger et al., 2018; Fukushima et al., 1988; Fukushima et al., 1990a; Fukushima et al., 1990b), although some studies have observed reduced spatial accuracy (Schmid-Burgk, 1984; Schreiber et al., 1995).

Overall, these findings suggest an overlap in the genetic determinants of specific oculomotor endophenotypes and schizophrenia. However, schizophrenia candidate gene studies have revealed only limited and inconsistent associations with oculomotor endophenotypes (Gatt, Burton, Williams, & Schofield, 2015; Greenwood, Light, Swerdlow, Radant, & Braff, 2012; Haraldsson et al., 2009, 2010; Kattoulas et al., 2012; Rybakowski, Borkowska, Czerski, & Hauser, 2002; Thaker, Wonodi, Avila, Hong, & Stine, 2004). Thus, despite evidence of genetic overlap between eye movements and schizophrenia from family studies (Calkins et al., 2003, 2008; Levy, Sereno, Gooding, & O'Driscoll, 2010), evidence from molecular genetic studies is largely missing.

Here, we used SNPs previously associated with schizophrenia in GWASs to investigate whether genetic determinants of schizophrenia are associated with oculomotor endophenotypes in a large population-based cohort study. To this end, we calculated polygenic risk scores (PRS) based on the summary statistics of the largest schizophrenia GWAS to date (Pardiñas et al., 2018). The SNPs that have been identified so far in the schizophrenia GWAS have been associated with, inter alia, voltage-gated calcium channels, synaptic transmission, membrane depolarisation during action potentials, and fragile X mental retardation protein (FMRP) (Pardiñas et al., 2018). We hypothesised that higher PRS would be associated with worse antisaccade and SPEM performance (higher error rate, latency and saccade frequency during SPEM but lower amplitude gain and SPEM velocity gain) but unrelated to performance in the prosaccade control task (latency and amplitude gain).

Material and methods

Participants

We used data from participants of the Rhineland Study, a community-based cohort study in Bonn, Germany. All inhabitants of two geographically defined areas in Bonn who are 30 years or older are invited to participate in the Rhineland Study. Names and addresses were provided by the municipality. Study participation is possible upon invitation only and irrespective of health status. The only exclusion criterion is not having sufficient command of the German language to provide written informed consent. There are no financial incentives for study participation. The ethics committee of the Medical Faculty of the University of Bonn approved the study, which was carried out in accordance with the recommendations of the International Council for Harmonisation Good Clinical Practice standards (ICH-GCP). We restricted our sample to the first 4000 participants of the Rhineland Study. Since study recruitment is ongoing, we cannot provide information on general response rates, but 3523 participants (88.1%) of those first 4000 participants provided blood samples between March 2016 and July 2019. Of those, 3217 (91.3%) remained after quality control of genetic data (see section 'Genetic data and polygenic risk scores'). Of those, 250 participants (7.8%) had no SPEM and antisaccade data. Missing data were primarily due to technical issues during data acquisition and post-processing (74%), exclusion after visual inspection of data (9.6%), contraindications (8.8%), non-compliance (5.2%), refusal (0.8%), timeout (0.4%) or multiple of these reasons (1.2%). Finally, we excluded two individuals with a diagnosis of schizophrenia and nine individuals with a diagnosis of psychosis. Thus, we based our analysis on 2956 participants without schizophrenia or psychosis aged between 30 and 95 years.

Genetic data and polygenic risk scores

Genotyping of 3523 blood samples was performed using Illumina's Omni-2.5 exome arrays containing 2 612 357 SNPs. We processed genotype data with GenomeStudio (version 2.0.5), and performed quality control of the genotypes with PLINK (version 1.9) (Purcell et al., 2007). SNPs were excluded based on Hardy–Weinberg disequilibrium ($p < 1 \times 10^{-6}$), minor allele frequencies (<0.01) and poor genotyping rate (<99%) (Marees et al., 2018). Further, we removed participants with poor DNA samples as identified by poor call rate (<95%) ($N = 8$, 0.2%), abnormal heterozygosity ($N = 47$, 1.3%), cryptic relatedness ($N = 143$, 4.1%) and gender mismatch ($N = 7$, 0.2%). We used EIGENSTRAT (version 16000), which uses principal components to detect and correct for variation in population structure as this can cause systematic differences in allele

frequencies (Price et al., 2006) [exclusion of $N = 101$ (2.9%) non-Caucasian participants]. Finally, missing SNPs were imputed using IMPUTE version 2 software (Howie, Donnelly, & Marchini, 2009) based on the 1000 Genomes reference panel (Auton et al., 2015). Imputation quality of the SNPs was checked using the info score metric [values of >0.3 are considered to indicate reliable imputation quality (Verma et al., 2014)].

PRS for schizophrenia were created using summary statistics from the largest schizophrenia GWAS to date, which included a discovery sample of 40 675 schizophrenia cases and 64 643 controls and an independent replication sample of 5762 cases and 154 224 controls (Pardiñas et al., 2018; Ripke et al., 2014). The results are publicly available (https://walters.psycm.cf.ac.uk; last retrieved at: 2021/05/31). We calculated PRS using PLINK (version 1.9) (Purcell et al., 2007) by first multiplying the number of risk alleles by the known effect size of each individual SNP locus and then aggregating the weighted effects of all SNPs under consideration (International Schizophrenia Consortium, 2009). We first created PRS based on the pre-specified SNPs from the GWAS, i.e. the 145 SNPs that reached genome-wide significance in the GWAS (Pardiñas et al., 2018). Then, we applied clumping to identify the most significant SNPs per linkage disequilibrium (LD) block (kilo base pair window: 250, LD $r^2 < 0.1$) (Chasioti, Yan, Nho, & Saykin, 2019) and created PRS at p value threshold (p_T) for SNP inclusion of 0.01 and 0.05, since PRS at those thresholds were reported to have improved prediction accuracy (Jonas et al., 2019; Ripke et al., 2014; Toulopoulou et al., 2019; Zhang et al., 2019). For sensitivity analysis, we created two additional PRS. We created one PRS by first applying clumping and then using the genome-wide significant threshold for SNP inclusion ($p_T = 5 \times 10^{-8}$), and another PRS using a more lenient threshold ($p_T = 0.1$), as in previous studies (Toulopoulou et al., 2019; Zhang et al., 2019).

Eye movement data

A detailed description of oculomotor data acquisition and processing has been published (Coors et al., 2021). In brief, eye movements were recorded using video-based infrared oculography (EyeLink 1000 and EyeLink 1000 Plus; SR Research Ltd, Ottawa, Canada) at 1000 Hz. After a horizontal-vertical five-point calibration task, participants performed fixation (not reported here), SPEM, prosaccade and antisaccade tasks in fixed order. SPEM outcomes were velocity gain (in %) and saccade rate (given in N/s, across the entire task duration). Prosaccade outcomes were latency (in ms) and amplitude gain (saccade amplitude divided by target step amplitude). Antisaccade outcomes were error rate (in %), latency and amplitude gain. Prosaccade and antisaccade outcomes were only calculated if there were at least seven valid trials. In case of more than four antisaccade errors, there also had to be at least one corrective saccade to ensure that participants understood the instructions. Additionally, latency and amplitude gain were only calculated if there were at least seven valid trials with directionally correct initial saccades. Before applying those criteria, we performed sensitivity analysis to rule out the possibility that they led to the exclusion of the participants with the highest PRS as this could have explained invalid or poor performance. Since we found no systematic pattern, we excluded those cases (51 participants for antisaccade error rate, 305 participants for antisaccade latency and amplitude gain and 14 participants for prosaccade latency and amplitude gain).

Statistical analyses

We hypothesised that high genetic risk for schizophrenia would be associated with worse antisaccade and SPEM performance but not with prosaccade outcomes (Calkins et al., 2008).

First, linear regression model assumptions were tested with diagnostic plots (scale-location plot and quantile-quantile plot) and by calculating the variance inflation factor [R package car (Fox & Weisberg, 2019), vif-function]. For prosaccade and antisaccade latency, the normality assumption was violated and therefore we log-transformed those variables.

Then, we assessed the associations between PRS and oculomotor outcomes with separate multivariable linear regression models for SPEM, antisaccade and prosaccade outcomes. Regression models included z-standardised PRS as a predictor and were adjusted for age, age^2, sex and population stratification. For the latter, we calculated six principal components that we included as covariates in the model (Price et al., 2006). We used mean-centred age to reduce collinearity (Iacobucci, Schneider, Popovich, & Bakamitsos, 2016). Missing covariate data were imputed using predictive mean matching [Hmisc package, 10 bootstrap replicates (Harrell & Dupont, 2020)].

We did not correct for multiple testing as we had very specific *a priori* hypotheses regarding associations between the schizophrenia PRS and eye movement outcomes based on work that goes back decades (Diefendorf & Dodge, 1908; Fukushima et al., 1988; Holzman et al., 1973; Sereno & Holzman, 1995). Further, we included the prosaccade task as control condition and created additional PRS for sensitivity analysis. As argued elsewhere, correction for multiple testing is strongly context-dependent and can lead to misinterpretation of results if incorrectly applied (Rothman, 1990; Streiner & Norman, 2011). Multiple testing is considered inappropriate for a limited set of pre-specified hypotheses and becomes especially problematic if the statistical tests are not independent, which is clearly the case in our analyses where our predictors (i.e. PRS scores at different p value thresholds) are highly correlated (Streiner & Norman, 2011).

Given the large age range of our sample, we additionally tested whether the associations between PRS and eye movement outcomes varied with age using a likelihood ratio test. We also repeated the analyses in an age-truncated sample (participants aged 30–70 years).

Statistical analyses were performed in RStudio (version 1.1.447, R-base version 3.5.0), using an α level of 0.05.

Results

Study sample

Sample characteristics are presented in Table 1.

Associations between PRS for schizophrenia and oculomotor performance

The main results from the multivariable regression models are listed in Table 2 and the results of the PRS that we created for sensitivity analysis are in online Supplementary Table A.1. PRS was positively associated with antisaccade error rate irrespective of inclusion criteria (pre-specified SNPs, $p_T = 0.01$, $p_T = 0.05$), but sensitivity analysis at $p_T = 5 \times 10^{-8}$ and $p_T = 0.1$ was not significant. For antisaccade latency and amplitude gain, PRS at $p_T = 0.01$ and $p_T = 0.05$ were associated with those outcomes but PRS including the pre-specified SNPs and PRS created for

Table 1. Sample characteristics

Number of participants, *N* (%)	2956 (100)
30–39 years	506 (17.1)
40–49 years	559 (18.9)
50–59 years	782 (26.5)
60–69 years	565 (19.1)
70–79 years	421 (14.2)
80+ years	123 (4.2)
Age, *M* (s.d.) in years	55.1 (14.2)
Sex, *N* (%) women	1665 (56.3)
Education level, *N* (%)	2935 (99.3)
High	1578 (53.8)
Middle	1309 (44.6)
Low	48 (1.6)
Best-corrected visual acuity, *N* (%)	2956 (100)
High (⩾0.8)	2536 (85.8)
Middle (0.32–0.63)	381 (12.9)
Low (<0.32)	39 (1.3)
Antisaccade error rate [%], *M* (s.d.)	32.1 (24.1)
Antisaccade latency [ms], median (interquartile range)	273.9 (59.2)
Antisaccade amplitude gain [%], *M* (s.d.)	111.6 (27.9)
Smooth pursuit velocity gain [%], *M* (s.d.)	77.8 (16.7)
Saccade frequency during smooth pursuit [N/s], *M* (s.d.)	2.2 (0.6)
Prosaccade latency [ms], median (interquartile range)	186.4 (35.8)
Prosaccade amplitude gain [%], *M* (s.d.)	93.3 (6.9)

N = number of participants, *M* = mean, s.d. = standard deviation. Education level was determined using the International Standard Classification of Education 2011 (ISCED) and was coded as low (lower secondary education or below), middle (upper secondary education to undergraduate university level) and high (postgraduate university study). Assessment of best-corrected visual acuity was based on visual scores from the right eye and was measured using an automated refractometer (Ark-1s, NIDEK CO., Tokyo, Japan). Categorisation of the visual acuity values was based on the guidelines of the International Council of Ophthalmology.

sensitivity analysis were not. The associations were positive for antisaccade latency and negative for antisaccade amplitude gain. PRS at $p_T = 0.01$ was positively associated with SPEM velocity gain but the other two PRS (pre-specified SNPs, $p_T = 0.05$) were not significantly associated with it. In addition, sensitivity analysis revealed a positive association between PRS at $p_T = 0.1$ and SPEM velocity gain. None of the PRS was associated with saccade rate. Regarding the prosaccade control task, none of the PRS was significantly associated with latency or amplitude gain.

Effects for all associations were small, with at most 0.22% of variance in oculomotor outcomes explained by the PRS.

We found no interaction effects between age and PRS. Effect estimates in the age-truncated analysis were highly comparable to those in the whole sample, but given the smaller sample size ($N = 2636$), some confidence intervals were wider. For the association between PRS at $p_T = 0.05$ and antisaccade amplitude gain, this resulted in the inclusion of zero in the confidence interval, but the regression coefficient remained comparable (full sample: $b = -1.279$; 95% CI −2.491 to −0.067; age-truncated sample: $b = -1.228$; 95% CI −2.520 to 0.064).

Discussion

We investigated genetic determinants of schizophrenia in relation to oculomotor endophenotypes in a large, population-based cohort. We found that genetic variants that are associated with schizophrenia are also involved in the fine-regulation of particular aspects of oculomotor function. Schizophrenia-related genetic risk variants specifically affected antisaccade outcomes, but not saccade rate during SPEM or outcomes from the prosaccade control task. PRS showed inconsistent patterns of association with SPEM velocity gain. Whilst collectively these findings thus support the use of specific oculomotor endophenotypes as markers of those syndromes that are currently classified as schizophrenia, it should be noted that the effect sizes of the observed associations are small.

Our findings suggest that SNP inclusion thresholds of $p_T = 0.01$ and $p_T = 0.05$ were optimal for the detection of PRS correlates of eye movements, in line with the findings of previous studies (Jonas et al., 2019; Ripke et al., 2014; Toulopoulou et al., 2019; Zhang et al., 2019). Of the PRS that we calculated for sensitivity analysis, the $p_T = 0.1$ cut-off might have been less optimal for this study as a more lenient threshold implies the inclusion of more uninformative SNPs and, therefore, an increase in noise (Chasioti et al., 2019). On the contrary, PRS at the genome-wide significant p_T ($p_T = 5 \times 10^{-8}$) might have excluded too many informative SNPs (Pardiñas et al., 2018). Our sensitivity analysis showed that applying clumping and then applying the genome-wide significant threshold obscured the association between PRS and antisaccade error rate that we found when we used PRS based on only the pre-specified SNPs.

Supporting the endophenotype status of antisaccade latency and error rate, we found both to be positively associated with genetic risk for schizophrenia which is in line with the reports of deficits in these measures in patients with schizophrenia and their clinically unaffected relatives (Calkins et al., 2008; Curtis et al., 2001; Ettinger et al., 2004; Fukushima et al., 1988; Fukushima et al., 1990a; Fukushima et al., 1990b; Karoumi et al., 2001; Mazhari et al., 2011; Radant et al., 2007, 2010; Reilly et al., 2014; Reuter et al., 2005; Sereno & Holzman, 1995). Antisaccade latency depends on cognitive processes such as attention, response-related decision-making and response execution (Hutton, 2008) and has been linked to activity in saccade neurons in the frontal eye fields and superior colliculus (Munoz & Everling, 2004). Reduced activation of frontal eye fields in individuals with schizophrenia compared to controls during eye movement tasks has been reported (Keedy, Ebens, Keshavan, & Sweeney, 2006) and may, therefore, partly account for the association between PRS and latency. Successful inhibition of antisaccade errors has been associated with activity in the dorsolateral prefrontal cortex (Munoz & Everling, 2004). Since individuals with schizophrenia have been found to have lower activity in prefrontal areas during antisaccade task performance than controls (McDowell et al., 2002), the association between genetic risk for schizophrenia and antisaccade error rate may be partly mediated by prefrontal areas, although the striatum may also play a role (Raemaekers, Ramsey, Vink, van den Heuvel, & Kahn, 2006, 2002).

The negative association between genetic risk for schizophrenia and antisaccade amplitude gain is also in line with studies reporting lower antisaccade amplitude gain in schizophrenia patients (Ettinger et al., 2004; Karoumi et al., 2001; Radant et al., 2010) and their biological relatives (Ettinger et al., 2018,

Table 2. Associations between polygenic risk scores (PRS) for schizophrenia at different *p* value thresholds for SNP inclusion and eye movement outcomes

Eye movement outcome	*p* value threshold for SNP inclusion	*b* (95% CI) for PRS	*p* value	R^2 (%)
Antisaccade error rate (%)	Pre-specified SNPs	**0.897 (0.058 to 1.737)**	**0.036**	**0.1**
Antisaccade error rate (%)	0.01	**1.007 (0.056 to 1.958)**	**0.038**	**0.1**
Antisaccade error rate (%)	0.05	**1.104 (0.169 to 2.039)**	**0.021**	**0.1**
Log of antisaccade latency (log ms)	Pre-specified SNPs	0.002 (0.000 to 0.005)	0.092	–
Log of antisaccade latency (log ms)	0.01	**0.004 (0.001 to 0.007)**	**0.012**	**0.2**
Log of antisaccade latency (log ms)	0.05	**0.003 (0.000 to 0.006)**	**0.039**	**0.1**
Antisaccade amplitude gain (%)	Pre-specified SNPs	−0.193 (−1.295 to 0.909)	0.731	–
Antisaccade amplitude gain (%)	0.01	**−1.489 (−2.723 to −0.254)**	**0.018**	**0.2**
Antisaccade amplitude gain (%)	0.05	**−1.279 (−2.491 to −0.067)**	**0.039**	**0.1**
Smooth pursuit velocity gain (%)	Pre-specified SNPs	−0.005 (−0.536 to 0.525)	0.984	–
Smooth pursuit velocity gain (%)	0.01	**0.647 (0.048 to 1.247)**	**0.034**	**0.1**
Smooth pursuit velocity gain (%)	0.05	0.476 (−0.113 to 1.066)	0.113	–
Saccade frequency during smooth pursuit (*N*/s)	Pre-specified SNPs	−0.005 (−0.025 to 0.015)	0.632	–
Saccade frequency during smooth pursuit (*N*/s)	0.01	−0.013 (−0.035 to 0.010)	0.262	–
Saccade frequency during smooth pursuit (*N*/s)	0.05	0.001 (−0.021 to 0.023)	0.941	–
Prosaccade amplitude gain (%)	Pre-specified SNPs	−0.153 (−0.397 to 0.091)	0.218	–
Prosaccade amplitude gain (%)	0.01	−0.122 (−0.399 to 0.154)	0.385	–
Prosaccade amplitude gain (%)	0.05	−0.033 (−0.304 to 0.238)	0.813	–
Log of prosaccade latency (log ms)	Pre-specified SNPs	0.000 (−0.002 to 0.002)	0.937	–
Log of prosaccade latency (log ms)	0.01	−0.002 (−0.004 to 0.000)	0.112	–
Log of prosaccade latency (log ms)	0.05	−0.002 (−0.004 to 0.001)	0.153	–

The table displays the change in performance per one standard deviation increase in PRS for schizophrenia for different eye movement outcomes. *b*, unstandardised regression coefficient; 95% CI, 95% confidence interval. Unstandardised regression coefficients were obtained from the following multivariable linear regression model: Eye movement outcome $\sim b_0 + \text{PRS} \times b_1 + \text{age} + \text{age}^2 + \text{sex} + \text{population stratification} + \text{residual error}$. R^2 refers to the variance explained in eye movement performance by PRS in per cent. In bold are those associations with a *p* value below 0.05.

2006, 2004; Karoumi et al., 2001), again supporting the endophenotype candidacy of this measure. However, in those studies, the mean antisaccade accuracy of controls typically ranged between 95% and 100% and, therefore, lower amplitude gain in patients or relatives indicated lower spatial accuracy. Instead, participants in our study on average tended to make hypermetric (overshooting) antisaccades ($M = 111.7\%$, S.D. = 29.1; Table 1), implying that participants with higher PRS had in fact greater absolute spatial accuracy, as their scores were closer to 100%. Antisaccade amplitude gain values above 100% are not unusual and values comparable to ours have been reported by others (Sweeney, Rosanao, Berman, & Luna, 2001). Antisaccade spatial accuracy requires complex, non-standard sensorimotor transformations in the posterior parietal cortex (Herweg et al., 2014) and frontal eye fields (Moon et al., 2007). Together, these findings confirm a genetic overlap between schizophrenia and antisaccade amplitude gain and point to a general tendency of people with a higher genetic risk of schizophrenia to make antisaccades with lower amplitudes.

However, since schizophrenia is a highly heterogeneous disease (Braff et al., 2007) and antisaccade performance has rather a low specificity for schizophrenia (Reilly et al., 2014), we cannot exclude the possibility that the associations between PRS for schizophrenia and antisaccade outcomes may also be partly accounted for by genetic risk variants of other psychiatric disorders.

The finding of *higher* SPEM velocity gain and, therefore, better performance in participants with higher PRS at $p_T = 0.01$ was unexpected, given the highly consistent reports of *lower* velocity gain in patients with schizophrenia and their relatives compared to healthy controls (Calkins et al., 2008; O'Driscoll & Callahan, 2008). An explanation might be that higher genetic risk for schizophrenia but not having schizophrenia may be advantageous for performance in SPEM velocity gain. Genes associated with schizophrenia were found to be favoured by evolution which implies that they might be advantageous for (cognitive) functioning, at least to a certain degree (Banerjee et al., 2018; Srinivasan et al., 2016). However, it is unclear why those advantages should only exist in performance in one but not other oculomotor endophenotypes. In contrast to antisaccade outcomes, SPEM velocity gain was associated with PRS at $p_T = 0.01$ and $p_T = 0.1$ but unrelated to PRS at $p_T = 0.05$. Since this is the only eye movement outcome for which the pattern was inconsistent for PRS at $p_T = 0.01$ and $p_T = 0.05$, and since the inclusion of more than the genome-wide significant SNPs in PRS creation may also increase the level of noise (Chasioti et al., 2019), an alternative explanation is that the relation of higher PRS with higher SPEM velocity gain may have been a false-positive finding.

Despite previous findings of higher saccade rate during SPEM in schizophrenia patients (O'Driscoll & Callahan, 2008) and their relatives (Calkins et al., 2008), we did not find an association of

PRS with that measure. This could be due to either a limited overlap in the genetic determinants between schizophrenia and saccade rate, or the current PRS capturing only a small proportion of those shared genetic factors.

Our sensitivity analysis of the prosaccade control task showed that PRS were not associated with prosaccade latency and amplitude gain. Since performance in these outcomes is largely unaffected in patients with schizophrenia and their relatives (Calkins et al., 2008; Reilly et al., 2014), this finding corroborates our explicit *a priori* hypothesis that associations between PRS for schizophrenia and oculomotor outcomes are limited to those oculomotor outcomes that have been established as endophenotypes of schizophrenia.

From a genetic perspective, it is noteworthy that those SNPs that we included in the PRS were found to be associated with, *inter alia*, voltage-gated calcium channels and the FMRP (Pardiñas et al., 2018). Voltage-gated calcium channels play a key role in visual perception (Pangrsic, Singer, & Koschak, 2018) and mutations have been linked to visual deficits such as involuntary eye movements (Cain & Snutch, 2011). In mice, FMRP has been associated with prefrontal cortex dysfunction (Siegel et al., 2017), which is also a critical brain structure for successful antisaccade performance (Kaufman, Pratt, Levine, & Black, 2010). This suggests that shared mechanisms may underlie schizophrenia and oculomotor performance and that those shared mechanisms may become particularly evident in some specific oculomotor outcomes. However, we also know from genetics that there are systematic differences in allele frequencies between populations (Price et al., 2006). Thus, our findings may not be generalisable to non-Caucasian populations as we based our PRS on SNPs derived from a study including predominantly Caucasians (Wand et al., 2021).

Our sample included individuals aged between 30 and 95 years. Previous studies found that the majority of patients with schizophrenia develop the disease during adolescence and early adulthood (men: between age 10 and 25, women: between age 25 and 35) (Rajji, Ismail, & Mulsant, 2009). Approximately one-quarter of patients with schizophrenia, and particularly women, experience their first episode after the age of 40 and very few patients are diagnosed after age 60 (Rajji et al., 2009). Thus, the probability that our population-based sample included individuals that are about to develop schizophrenia but have not yet been diagnosed is very low. Our large sample size and the young typical age of onset benefit our research aim as these factors lower the risk that the observed associations between schizophrenia PRS and eye movement performance were due to individuals about to develop schizophrenia. We found no evidence that the observed associations varied with age, yet we lacked the statistical power to run age group-specific analyses in more narrow age ranges. Further research, conducted in large samples with narrower age ranges, is needed to confirm the associations we found between genetic liability for schizophrenia and oculomotor measures.

The observation that only small amounts of variance in established oculomotor endophenotypes could be explained by PRS needs critical examination. One possibility is that current PRS do not fully capture the genetic basis of schizophrenia. The estimate for the common-variant SNP heritability of schizophrenia calculated in the largest GWAS to date is 24.4% if all SNPs are considered, and only 6% for PRS at $p_T = 0.05$ (Pardiñas et al., 2018). These estimates are, therefore, well below family-based studies heritability estimates (Lichtenstein et al., 2009; Sullivan et al., 2003). Current PRS may capture only a fraction of genetic variance attributed to schizophrenia because even the largest GWASs to date were not sufficiently powered to detect all relevant common variants (Smeland, Frei, Dale, & Andreassen, 2020). In addition, part of the heritability results from copy number variants or rare variants that influence the boundaries of topologically associated domains (Halvorsen et al., 2020; Marshall et al., 2017), which are currently not tagged by conventional genotyping arrays and, therefore, not included in GWAS (Auer & Lettre, 2015).

Further, it should be remembered that heritability estimates for both schizophrenia (Lichtenstein et al., 2009; Sullivan et al., 2003) and eye movements (Bell et al., 1994; Greenwood et al., 2007; Hong et al., 2006; Katsanis et al., 2000; Litman et al., 1997; Macare et al., 2014; Malone & Iacono, 2002) are well below 100%. This, and the only modest sized oculomotor impairments in first-degree relatives of schizophrenia patients (Calkins et al., 2008), implies that oculomotor impairments in schizophrenia reflect not only genetic but also environmental factors as well as the interplay between genes and environment (Chakravarti & Little, 2003).

It should also be noted that our inclusion criterion of a minimum age of 30 years, combined with the typically rather early onset for schizophrenia (Rajji et al., 2009), may have led to the exclusion of some participants with very high genetic risk for schizophrenia, thereby reducing the variance in schizophrenia risk and oculomotor performance in our sample.

Taken together, the schizophrenia PRS alone is unlikely to fully account for differences in oculomotor performance. This also fits with our finding that current schizophrenia PRS do not have any predictive power for eye movement performance. Still, the molecular genetic confirmation implies that the role of those brain regions that are critically involved in antisaccade performance should be investigated more closely in the aetiology of schizophrenia. Thus, combining knowledge from eye movement and schizophrenia research could be beneficial to propel the field forward.

Conclusions

Using a molecular genetic approach, we confirm and extend previous findings from behavioural genetic studies, showing that antisaccade error rate, latency and amplitude gain have genetic overlap with schizophrenia. For SPEM outcomes, we found no association between PRS and saccade rate and inconsistent associations between PRS and velocity gain. As schizophrenia PRS based on currently available GWAS findings only accounted for <0.25% of variance in oculomotor endophenotypes, they currently have no predictive power. However, we expect that future studies using PRS that also include rare risk variants are likely to uncover a larger proportion of shared genetic determinants of schizophrenia and oculomotor performance.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0033291721003251

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Supplementary Material – Polygenic Risk Scores for Schizophrenia Are Associated with Oculomotor Endophenotypes

Table A.1 Associations between Polygenic Risk Scores (PRS) for Schizophrenia at Different p-value Thresholds for SNP Inclusion and Eye Movement Outcomes

Eye Movement Outcome	**p-value Threshold for SNP Inclusion**	**b (95%-CI) for PRS**	**p-value**	**R^2 (%)**
Antisaccade error rate (%)	$5*10^{-8}$	0.743 (-0.639, 2.125)	0.292	/
Antisaccade error rate (%)	0.1	0.864 (-0.069, 1.796)	0.069	/
Log of antisaccade latency (log ms)	$5*10^{-8}$	0.003 (-0.001, 0.007)	0.153	/
Log of antisaccade latency (log ms)	0.1	0.002 (-0.001, 0.005)	0.154	/
Antisaccade amplitude gain (%)	$5*10^{-8}$	-1.667 (-3.457, 0.123)	0.068	/
Antisaccade amplitude gain (%)	0.1	-1.088 (-2.299, 0.124)	0.078	/
Smooth pursuit velocity gain (%)	$5*10^{-8}$	-0.014 (-0.884, 0.857)	0.976	/
Smooth pursuit velocity gain (%)	0.1	**0.687 (0.100, 1.273)**	**0.022**	**0.14**
Saccade frequency during smooth pursuit (N/s)	$5*10^{-8}$	0.004 (-0.028, 0.037)	0.790	/
Saccade frequency during smooth pursuit (N/s)	0.1	-0.001 (-0.023, 0.020)	0.902	/
Prosaccade amplitude gain (%)	$5*10^{-8}$	-0.095 (-0.497, 0.307)	0.643	/
Prosaccade amplitude gain (%)	0.1	-0.079 (-0.350, 0.191)	0.564	/
Log of prosaccade latency (log ms)	$5*10^{-8}$	0.000 (-0.003, 0.003)	0.979	/
Log of prosaccade latency (log ms)	0.1	-0.002 (-0.004, 0.000)	0.126	/

Note. The table displays the change in performance per one standard deviation increase in PRS for schizophrenia for different eye movement outcomes. b=unstandardized regression coefficient, 95%-CI=95%-confidence interval. Unstandardized regression coefficients were obtained from the following multivariable linear regression model: Eye movement outcome ~ b_0 + PRS* b_1 + age + age^2 + sex + population stratification + residual error. R^2 refers to the variance explained in eye movement performance by PRS in percent.

5. Associations of genetic liability for Alzheimer's disease with cognition and eye movements in a large, population-based cohort study

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Associations of genetic liability for Alzheimer's disease with cognition and eye movements in a large, population-based cohort study

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To identify cognitive measures that may be particularly sensitive to early cognitive decline in preclinical Alzheimer's disease (AD), we investigated the relation between genetic risk for AD and cognitive task performance in a large population-based cohort study. We measured performance on memory, processing speed, executive function, crystallized intelligence and eye movement tasks in 5182 participants of the Rhineland Study, aged 30 to 95 years. We quantified genetic risk for AD by creating three weighted polygenic risk scores (PRS) based on the genome-wide significant single-nucleotide polymorphisms coming from three different genetic association studies. We assessed the relation of AD PRS with cognitive performance using generalized linear models. Three PRS were associated with lower performance on the Corsi forward task, and two PRS were associated with a lower probability of correcting antisaccade errors, but none of these associations remained significant after correction for multiple testing. Associations between age and trail-making test A (TMT-A) performance were modified by AD genetic risk, with individuals at high genetic risk showing the strongest association. We conclude that no single measure of our cognitive test battery robustly captures genetic liability for AD as quantified by current PRS. However, Corsi forward performance and the probability of correcting antisaccade errors may represent promising candidates whose ability to capture genetic liability for AD should be investigated further. Additionally, our finding on TMT-A performance suggests that processing speed represents a sensitive marker of AD genetic risk in old age and supports the processing speed theory of age-related cognitive decline.

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BACKGROUND

Alzheimer's disease (AD) can roughly be divided into three clinical disease stages: a pre-symptomatic phase characterised by pathological brain changes, a prodromal phase characterised by subtle cognitive impairment and then lastly the dementia stage in which impairments occur in multiple domains and lead to loss of function [1]. As only 10–15% of individuals with amnestic mild cognitive impairment (MCI) develop AD each year [2], prediction of disease progression is of great interest to identify those individuals best suited for disease-delaying interventions, such as drug trials [3]. A meta-analysis found that particularly episodic verbal memory performance (e.g., delayed recall of a word list) and performance in language tasks that implicate semantic memory and executive function (e.g., the word fluency task) have high predictive accuracy for disease progression [4].

Eye movement assessment may be an alternative promising method to identify individuals at high risk for AD as it provides language-independent and culture-fair measures [5, 6] of multiple cognitive, perceptual and motor processes, including attention, processing speed, motion processing, working memory, learning and inhibition [7, 8]. In people with AD, instability of fixation [9–11] and deficits in the prosaccade [9, 12–14], antisaccade [12–16] and smooth pursuit tasks [16, 17] have been reported.

In the prosaccade task, participants are asked to perform a saccade, i.e., a rapid eye movement executed to bring an object of interest onto the fovea, towards a sudden-onset peripheral target. Prosaccade tasks measure overt attention and response speed [18], and individuals with AD were found to have longer latencies, i.e., longer reaction times for the initiation of a saccade towards the peripheral target, compared to healthy controls [12–14]. The antisaccade task has the same task design as the prosaccade task but participants are asked to execute their first saccade in each trial in the opposite direction of the peripheral target [19]. In this task, which is a good measure of inhibitory control [20], individuals with AD and MCI have consistently been found to make more direction errors compared to controls, i.e., a first saccade within a trial towards the target instead of towards its mirror position [12, 14]. Additionally, they are also less likely to correct their direction errors than controls [13, 15, 16]. For antisaccade latencies, the majority of studies reported higher latencies in AD [13, 14]. Moreover, in both saccade tasks, individuals with AD and amnestic MCI were found to perform hypometric saccades, which is reflected by a low value in the spatial accuracy measure called

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amplitude gain (amplitude of the eye movement divided by the amplitude of the target movement) and is accompanied by a high spatial error [9, 14, 21]. Research on whether saccade velocity and antisaccade costs (antisaccade latency minus prosaccade latency) differ between individuals with AD and healthy controls is still largely lacking [14], but some evidence suggests that performance in these measures may also be impaired in AD [13, 21].

Smooth pursuit eye movements (SPEMs) are performed to keep a slowly moving object on the fovea [22]. SPEMs have been found to have a lower velocity gain (ratio of eye velocity to target velocity) in AD [16, 17].

Importantly, performance in many oculomotor measures has been found to correlate with dementia severity, for example, instability of fixation [11, 23], prosaccade and antisaccade latency and amplitude gain [11, 21, 24], antisaccade direction error rate [25, 26] and correction rate [24]. Additionally, some studies have found that oculomotor performance may also help to differentiate between individuals with amnestic MCI and non-amnestic MCI [24, 27]. This may be relevant for predicting disease progression to AD, as individuals with amnestic MCI seem to be more likely to develop AD than individuals with non-amnestic MCI [28]. However, the usefulness of eye movements in identifying individuals at high risk for AD remains largely unexplored.

Genetic factors play a substantial role in the development of AD [29]. Polygenic risk scores (PRS) for AD, which represent the weighted sum of AD risk alleles that an individual carries, are well-suited to quantify genetic risk for AD as they account for the complex polygenic nature of AD [30].

Studies on the association between AD PRS and performance in classical cognitive tests have been conducted in samples including mainly older individuals without dementia or a mixture of individuals with MCI and individuals without dementia. AD PRS have been found to be significantly associated with both baseline episodic verbal memory performance [31–33] and longitudinal decline in episodic verbal memory [32–35], yet other studies could not confirm these findings in individuals with MCI or healthy participants [36, 37]. AD PRS were not associated with baseline working memory in most studies [33, 37], although working memory was found to deteriorate faster with higher PRS [34, 35]. Similarly, AD PRS were not associated with baseline performance in processing speed in several population-based studies [31, 33], except for a subgroup of 70 to 99-year-olds [31]. However, AD PRS were related to decline in processing speed [34, 35]. Studies examining the relation between AD PRS and baseline executive function either reported negative [31] or no associations[32, 37], whereas studies exploring the relation between AD PRS and longitudinal change in executive function were inconclusive [38, 39]. We are only aware of one study that investigated the relation between genetic risk for AD and eye movement performance [18]. That study found that antisaccade performance was similar between apolipoprotein E (*APOE*) ε4 carriers and non-carriers, yet APOE ε4 carriers performed worse on the prosaccade task. However, the sample size was small ($N = 97$), the participants were relatively young (17–35 years) and AD genetic risk was only based on *APOE* ε4 carrier status [18].

Here, we aimed to assess which, if any, cognitive measures are sensitive to genetic susceptibility for AD in a large, population-based sample including a wide age range. We investigated the relation with both classical tests of cognitive function and eye movement performance. Additionally, we investigated whether genetic liability for AD modifies the association between age and cognitive performance.

MATERIALS AND METHODS

Participants

We used baseline data from the Rhineland Study, a community-based cohort study that includes inhabitants aged ≥30 years (current age range: 30 to 95 years) from two geographically defined areas in Bonn, Germany. The only exclusion criterion is not having sufficient command of the German language to provide written informed consent. The ethics committee of the Medical Faculty of the University of Bonn approved the study that was carried out in accordance with the recommendations of the International Council for Harmonisation Good Clinical Practice standards (ICH-GCP). Of originally 5801 participants who provided blood samples between March 2016 and October 2021, 5189 remained after quality control of genetic data (see Section 2.2). Of those, 5182 had data in at least one cognitive task and were therefore included in the analyses.

Genetic data and polygenic risk scores

Blood samples were genotyped using Illumina Omni-2.5 exome arrays containing 2,612,357 single-nucleotide polymorphisms (SNPs). Genotype data were processed using GenomeStudio (version 2.0.5) and quality controlled using PLINK software (version 1.9). SNP exclusion criteria were Hardy-Weinberg disequilibrium ($p < 1*10^{-6}$), minor allele frequency, (<0.01) and poor genotyping rate (<99%). Participants with poor DNA samples were excluded, which comprised 41 cases with poor call rate (<95%), 86 cases with abnormal heterozygosity, 290 cases with cryptic relatedness and 30 cases with gender mismatch. Because variation in population structure can cause systematic differences in allele frequencies [40], we used EIGENSTRAT (version 16000), which uses principal components to detect and correct for variation in population structure [40] (exclusion of $N = 165$ participants). Finally, we imputed missing SNPs based on the 1000 Genomes reference panel [41] using IMPUTE (version 2) [42]. To include only SNPs with high imputation quality, we checked for an info score metric greater than 0.3 as this value is considered to indicate reliable imputation quality [43].

Using PLINK (version 1.9), we created three different weighted AD PRS scores based on the genome-wide significant SNPs (i.e., those SNPs that had a *p*-value below $5*10^{-8}$ in the respective genome-wide association study (GWAS)). One PRS (PRS_{Jansen}) was created based on 29 genome-wide significant SNPs that were found in the meta-analysis by Jansen et al. in 2019 [44] (https://ctg.cncr.nl/software/summary_statistics; retrieved on January 15, 2021). The two additional PRS scores were created based on the genome-wide significant SNPs identified in two more recent meta-analyses by Wightman et al. [45] ($PRS_{Wightman}$) and Schwartzentruber et al. [46] ($PRS_{Schwartzentruber}$). The study by Wightman et al. [45] is an extension of the study by Jansen et al. [44]. This study included a larger number of participants in one of the included cohorts as well as data from 12 additional cohorts (in total: $N = 1,126,563$ participants), and identified 38 risk loci. However, the authors could only provide us with the beta estimates from the summary statistics excluding the UK biobank ($N = 364,859$) and the 23andMe data ($N = 363,646$). Thus, we compared the signs of the z-scores they had reported for the original data set with those of the beta estimates they had provided and found that they were consistent. Additionally, one SNP (rs115186657) was missing in the summary statistics that they had provided and one SNP (rs2632516) was not available in our data. Therefore, we were able to include 36 SNPs in $PRS_{Wightman}$. To create $PRS_{Schwartzentruber}$, we used all 37 risk loci for AD that were identified in the meta-analysis by Schwartzentruber et al. [46]. This meta-analysis combined the data of the study by Kunkle et al. from 2019 [47] and the updated results of a GWAS study of UK Biobank participants with a family history of AD. Earlier results from the GWAS analysis of the UK Biobank AD proxy cases were also included in the Jansen et al. publication [44]. $PRS_{Wightman}$ and $PRS_{Schwartzentruber}$ were highly correlated with each other (Pearson's $r = 0.95$) but their correlations were lower with PRS_{Jansen} ($PRS_{Wightman}$: $r = 0.60$; $PRS_{Schwartzentruber}$: $r = 0.63$). This may be due to the fact that the two more recent GWAS only partially replicated the genome-wide significant loci reported by Jansen et al. [44] (Schwartzentruber: replication of 23 loci out of 29 from Jansen; Wightman: replication of 22 loci out of 29 from Jansen).

Outcome measures

We measured cognitive performance using classical tests of working memory, episodic verbal memory, processing speed, executive function and crystallized intelligence, along with an eye movement test battery. The examinations were administered following a standardized procedure by certified study technicians. Working memory was assessed with the forward and backward digit span task and the forward and backward Corsi block-tapping test (Corsi), adapted from the PEBL battery [48]. The Auditory Verbal Learning and Memory Test (AVLT) was used to assess episodic verbal memory (immediate recall: sum of correctly recalled nouns

in the first five trials, delayed recall: number of correctly recalled words after a time delay of 20 to 30 min) [49]. Processing speed was measured with a numbers-only trail-making test (TMT-A: time to completion). Executive function was assessed with a 60 s categorical word fluency task (number of uniquely named animals) and a number-and-letters trail-making test (TMT-B: time to completion). Crystallized intelligence was measured with the 37-item Mehrfachwahl-Wortschatz-Intelligenztest (MWT-B), a vocabulary test in which participants had to select an existing German word among four non-words in each of 37 trials [50].

The eye movement test battery consisted of fixation, SPEM, prosaccade and antisaccade tasks. For recording of eye movements, we used video-based infrared oculography (EyeLink 1000 and EyeLink 1000 Plus; SR Research Ltd) at 1000 Hz. Fixations were defined as periods of at least 100 ms duration without blinks or saccades directed toward the target (a white circle 0.35° in diameter on black background). The target appeared first in the centre ($x = 0°$, $y = 0°$) for 5 s and then in a random order for 10 s each at the top ($x = 0°$, $y = 9.63°$), bottom ($x = 0°$, $y = -9.63°$), left ($x = -9.63°$, $y = 0°$), or right ($x = 9.63°$, $y = 0°$), always returning to the centre after each of these four eccentric locations. Thus, the central position had to be fixated four times in total. To obtain measures for fixation stability, we calculated the mean spatial error of gaze position (in degree of visual angle), mean saccade rate (saccades/second) and mean blink rate (blinks/second) during fixation. In the 21 s long SPEM task, the target started in the centre and then moved horizontally for ten full cycles in a sinusoidal waveform between ±9.63° at a frequency of 0.5 Hz. All eye movements with velocity <30°/s and duration ≥50 ms were classified as SPEMs. We determined the mean SPEM gain for the middle two quarters of each half-cycle of target motion (left to right or right to left) separately and then took the average of these values to calculate the mean velocity gain (in %). In all tasks, saccades were defined as eye movements with an amplitude >1° and either a velocity ≥60°/s or a velocity ≥22°/s and an acceleration ≥3800°/s^2. We calculated the mean saccade rate (in saccades/second) during smooth pursuit. Prosaccade and antisaccade tasks consisted of 30 trials each (plus six antisaccade practice trials). In each trial, the target appeared first in the centre for a random duration of 1–2 s (average 1.5 s) and then stepped randomly to the left or right ($x = \pm 9.63°$, $y = 0°$, 15 times per side), where it remained for 1 s before returning to the centre for the next trial. For both saccade tasks, we calculated mean latencies (in ms), the two spatial accuracy measures amplitude gain and spatial error (both in %), and amplitude-adjusted and unadjusted peak velocities (in degree of visual angle/s) for valid trials with a directionally correct initial saccade. For the antisaccade task, we additionally calculated costs (in ms), direction error rate (in %), and correction rate (the percentage of direction errors corrected by participants who made at least 5 antisaccade direction errors by performing a saccade toward the mirror position that crossed at least the midline) for valid trials. Trials were defined as valid when the fixation on the central fixation point started at least 100 ms before peripheral target onset and was no more than 3° off the fixation point. Further, the initial saccade had to end before the peripheral target timed out and saccades with amplitude <1° or latency <80 ms were excluded. Additionally, no saccade or blink was allowed to occur during this period. To obtain reliable data, there had to be ≥7 valid and correct trials for prosaccade and antisaccade outcomes, except for the direction error and correction rate, for which only the criterion of valid but not correct trials applied. Additionally, for all antisaccade outcomes, at least one corrective saccade had to occur in case of ≥5 direction errors. A more detailed description of the oculomotor data acquisition can be found in a previous publication [51].

Statistical analyses

Statistical analyses were performed in RStudio (version 1.3.959, R-base version 4.0.3) using a two-sided significance test with an alpha level of 0.05. We assessed the associations between genetic risk for AD and cognitive performance separately for the three different AD PRS scores using multivariable linear or one-inflated beta regression models for each cognitive outcome. Models included z-standardized AD PRS as the predictor variable and were adjusted for age, age^2 and sex, using mean-centred age to reduce collinearity between the main and quadratic term [52]. In order to correct for population stratification, we additionally adjusted for the first six genetic principal components [40]. We imputed missing covariate data using predictive mean matching (Hmisc package, 10 bootstrap replicates). We report unadjusted and false discovery rate adjusted (FDR-adjusted, $N = 28$ comparisons) p-values. We were particularly interested in cognitive outcomes that were consistently associated with all three different PRS to identify the most robust cognitive indicators of genetic risk for AD.

As age is a key risk factor for AD [53], we further examined whether genetic risk for AD modified the associations between age and cognitive outcomes by including PRS*age and PRS*age^2 in the models and comparing the model fit with a likelihood ratio test. In case of significant interactions, we plotted the association between age and the respective cognitive outcome for three different PRS groups (low: z-standardised PRS score below −1; medium: z-standardised PRS score between −1 and 1; high: z-standardized PRS score above 1) separately to visualize how age interacts with genetic susceptibility to influence cognitive decline. Additionally, we tested differences in the slopes between the three PRS groups using Tukey post-hoc tests (pairs-function of the emmeans package [54] in R).

All models were checked for multicollinearity (variance inflation factor, R package car, vif-function), homoscedasticity (scale-location plot) and normality of residuals (quantile-quantile-plot). Because the normality assumption was violated for performance in TMT-A and TMT-B, prosaccade and antisaccade spatial error, and the three fixation outcomes spatial error, saccade rate and blink rate, we log-transformed those outcome variables. Because severe skewness of performance in antisaccade correction rate could not be reduced by log-transformation, we used a one-inflated beta regression model (gamlss package) instead, which is a mixture model consisting of a logistic regression model and a beta regression model. The logistic regression part of the one-inflated beta regression models whether or not AD PRS is associated with the probability of correcting all versus not correcting all antisaccade direction errors. In a second step, the beta regression model part tests whether AD PRS is associated with the percentage of uncorrected antisaccade direction errors in those individuals who did not correct all of their antisaccade direction errors.

We additionally performed a post-hoc power analysis using G-Power (version 3.1) [55] to evaluate which effect sizes for the associations between AD PRS and cognitive outcomes we would be able to detect with our sample size with a statistical power of between 80% to 90%. For this, we performed an F-test with one predictor, setting the sample size to 5182 participants, and the type I error rate to 0.05.

RESULTS

Study sample

Sample characteristics are displayed in Table 1. Participants were overall highly educated and only 0.1% reported a diagnosis of AD. The eye movement measure antisaccade correction rate was computed for 3053 participants, representing the number of participants who made at least 5 antisaccade direction errors and corrected at least one of these direction errors. Of these 3053 participants, 677 participants did not correct all of their antisaccade direction errors.

Associations between AD PRS and cognitive performance

The associations between AD PRS_{Jansen}, $PRS_{Wightman}$, $PRS_{Schwartzentruber}$ and cognitive outcomes are displayed in Table 2.

Higher genetic risk for AD was significantly associated with lower performance in the Corsi forward task across all three AD PRS scores but these associations did not remain significant after correction for multiple testing. Additionally, before correcting for multiple testing, a higher PRS_{Jansen} score was associated with lower saccade frequency in the smooth pursuit task, higher prosaccade latency, and a lower probability of correcting all antisaccade direction errors (odds ratio and 95% confidence interval (OR and 95%-CI): 0.884 (0.810–0.964); $p = 0.005$; FDR-adjusted $p = 0.140$), but not with the proportion of uncorrected errors in those participants who did not correct all of their direction errors (OR and 95%-CI: 0.997 (0.943–1.054); $p = 0.908$). The uncorrected p-value also indicated that $PRS_{Schwartzentruber}$ was associated with a lower probability of correcting all antisaccade direction errors (OR and 95%-CI: 0.916 (0.840–0.999); $p = 0.047$; FDR-adjusted $p = 0.235$), but not with the percentage of uncorrected direction errors in those who did not correct all of their antisaccade direction errors (OR and 95%-CI: 0.990 (0.935–1.048); $p = 0.727$). As for the other two PRS, only the

Table 1. Sample characteristics.

Number of participants, *N*	5182
Age [years], M (SD)	55.5 (13.8)
30–39 years	800 (15.4)
40–49 years	928 (17.9)
50–59 years	1470 (28.4)
60–69 years	1071 (20.7)
70–79 years	684 (13.2)
80+ years	229 (4.4)
Sex, *N* (%) women	2890 (55.8)
Education level, *N* (%)	5134 (99.1)
High	2789 (54.3)
Middle	2260 (44.0)
Low	85 (1.7)
Diagnosis of Alzheimer's Disease, *N* (%)	5 (0.1)
APOE ε4-carriers (ε4/ε4, ε2/ε4, ε3/ε4), *N* (%)	1326 (25.8)
Working memory	
Digit span forward [number of digits], mean (SD) for $N = 5109$; max = 9	6.4 (1.2)
Digit span backward [number of digits], mean (SD) for $N = 5102$; max = 9	4.8 (1.2)
Corsi forward [number of blocks], mean (SD) for $N = 4972$; max = 9	4.9 (1.1)
Corsi backward [number of blocks], mean (SD) for $N = 4937$; max = 9	4.8 (1.0)
Episodic verbal memory	
AVLT - immediate recall [sum of recalled words over recall 1 to 5], mean (SD) for $N = 5160$; max = 75	51.3 (10.1)
AVLT - delayed recall [number of recalled words], mean (SD) for $N = 5152$ max = 15	10.3 (3.3)
Processing speed	
Trail-making test A [completion time in s], median (IQR) for $N = 5109$	33.2 (15.1)
Executive function	
Trail-making test B [completion time in s], median (IQR) for $N = 5089$	43.9 (26.7)
Word fluency task [number of unique words], mean (SD) for $N = 5132$	26.4 (6.9)
Crystallized intelligence	
MWT-B [sum of correctly recognized words], mean (SD) for $N = 4886$; max = 37	30.6 (3.4)
Fixation performance	
Spatial error (RMSE) [°], median (IQR) for $N = 4744$	0.9 (0.3)
Saccade frequency [N/s], median (IQR) for $N = 4744$	0.2 (0.1)
Blink rate [N/s], median (IQR) for $N = 4676$	0.1 (0.2)
Smooth pursuit performance	
Velocity gain [%], mean (SD) for $N = 4761$	78.1 (16.3)
Saccade rate [N/s], mean (SD) for $N = 4762$	2.2 (0.6)
Prosaccade performance	
Prosaccade latency [ms], mean (SD) for $N = 4747$	190.6 (28.4)
Amplitude gain [%], mean (SD) for $N = 4747$	93.8 (6.7)
Spatial error [%], median (IQR) for $N = 4747$	8.2 (5.3)
Peak velocity [°/s], mean (SD) for $N = 4747$	364.8 (57.6)
Amplitude-adjusted peak velocity, mean (SD) for $N = 4747$	3.9 (0.6)
Antisaccade performance	
Latency [ms], mean (SD) for $N = 4178$	282.0 (50.6)
Amplitude gain [%], mean (SD) for $N = 4178$	112.0 (27.8)
Spatial error [%], median (IQR) for $N = 4178$	26.7 (17.4)
Peak velocity [°/s], mean (SD) for $N = 4178$	346.6 (67.3)
Amplitude-adjusted peak velocity, mean (SD) for $N = 4178$	3.2 (0.8)
Antisaccade costs [ms], mean (SD) for $N = 4165$	91.8 (43.1)
Antisaccade error rate [%], mean (SD) for $N = 4622$	31.6 (23.6)
Antisaccade correction rate [%], median (IQR) for $N = 3053$	100.0 (0)

We indicated the mean and standard deviation for almost normally distributed variables and the median and interquartile range for non-normally distributed variables. Education level was determined using the International Standard Classification of Education 2011 (ISCED) and was coded as low (lower secondary education or below), middle (upper secondary education to undergraduate university level) and high (postgraduate university study).
N number of participants, *SD* standard deviation, *IQR* interquartile range, *max* maximum, *AVLT* Auditory Verbal Learning and Memory Test, *MWT-B* Mehrfachwahl-Wortschatz-Intelligenztest.

uncorrected *p*-values indicated that a higher $PRS_{Wightman}$ score was associated with better performance in the digit span backward and lower TMT-A performance. $PRS_{Wightman}$ was neither associated with the probability of correcting all versus not all antisaccade direction errors (OR and 95%-CI: 0.920 (0.844–1.003); $p = 0.058$), nor with the percentage of uncorrected errors in those who did not correct all of their antisaccade errors (OR and 95%-CI: 0.988 (0.933–1.047); $p = 0.685$).

Exclusion of AD cases ($N = 5$, Table 1) from the sample, or adding educational level as an additional covariate, did not materially change the results (data not shown).

Interaction effects

We found significant interactions between the three different PRS and age and age^2 for TMT-A performance that remained significant after correcting for multiple testing (Table 3). For AVLT (immediate and delayed recall), the interactions between PRS and age and age^2 were also significant for all three different PRS, but only the interactions between PRS_{Jansen} and age and age^2 for AVLT immediate recall remained significant after correcting for multiple testing. In addition, we found significant interaction effects between $PRS_{Wightman}$, and $PRS_{Schwartzentruber}$ and age and age^2 for saccade frequency during smooth pursuit, but they did not survive correction for multiple testing. Visualisation of the interaction effects showed that individuals with the highest genetic risk for AD showed the strongest age-related decline in AVLT (immediate and delayed recall) and TMT A performances (Fig. 1). For saccade frequency during smooth pursuit, the scatterplot did not reveal a clear pattern (Fig. 1). Post-hoc comparisons using the Tukey test revealed that delayed recall performance in individuals at highest genetic risk, based on $PRS_{Wightman}$ and $PRS_{Schwartzentruber}$ was worse compared to those in the medium ($PRS_{Wightman}$ model: $p = 0.001$, $PRS_{Schwartzentruber}$ model: $p = 0.025$) and low ($PRS_{Wightman}$ model: $p = 0.017$, $PRS_{Schwartzentruber}$ model: $p = 0.031$) genetic risk groups. Additionally, high genetic risk individuals performed worse than medium ($PRS_{Wightman}$ model: $p = 0.018$, $PRS_{Schwartzentruber}$ model: $p = 0.016$) and low ($PRS_{Wightman}$ model: $p < 0.001$, PRS_{Jansen} model: $p = 0.003$)

Table 2. Associations between three different Alzheimer's disease polygenic risk scores (PRS) and cognitive test scores and eye movement outcomes.

Cognitive outcome	b (95%-CI) for AD PRS Jansen	*p*-value	FDR-adjusted p-value	b (95%-CI) for AD PRS Wightman	*p*-value	FDR-adjusted *p*-value	b (95%-CI) for AD PRS Schwartzentruber	*p*-value	FDR-adjusted *p*-value
Outcomes - classical cognitive tasks									
Digit span forward [number of digits]	−0.017 (−0.048, 0.013)	0.273	0.588	0.006 (−0.025, 0.037)	0.693	0.808	0.001 (−0.030, 0.031)	0.968	1.000
Digit span backward [number of digits]	0.016 (−0.016, 0.049)	0.323	0.595	**0.034 (0.001, 0.067)**	**0.041**	**0.383**	0.027 (−0.006, 0.059)	0.105	0.368
Corsi forward [number of blocks]	**−0.031 (−0.058, −0.005)**	**0.022**	**0.252**	**−0.031 (−0.058, −0.004)**	**0.025**	**0.383**	**−0.038 (−0.065, −0.012)**	**0.005**	**0.140**
Corsi backward [number of blocks]	−0.018 (−0.044, 0.008)	0.167	0.585	−0.015 (−0.041, 0.010)	0.242	0.522	−0.014 (−0.040, 0.012)	0.281	0.564
AVLT – immediate recall [sum of recalled words]	−0.147 (−0.371, 0.076)	0.197	0.588	−0.077 (−0.301, 0.147)	0.499	0.665	−0.114 (−0.338, 0.110)	0.318	0.564
AVLT – delayed recall [number of words]	−0.037 (−0.112, 0.039)	0.340	0.595	−0.034 (−0.110, 0.041)	0.375	0.665	−0.038 (−0.114, 0.037)	0.320	0.564
Word fluency task [number of animals]	−0.137 (−0.316, 0.043)	0.137	0.548	−0.104 (−0.283, 0.076)	0.258	0.522	−0.133 (−0.313, 0.047)	0.146	0.409
Trail-making test A [log s]	0.002 (−0.001, 0.006)	0.217	0.588	**0.004 (0.000, 0.007)**	**0.029**	**0.383**	0.003 (−0.001, 0.006)	0.097	0.368
Trail-making test B [log s]	0.003 (−0.001, 0.008)	0.123	0.548	0.004 (−0.001, 0.008)	0.084	0.470	0.004 (−0.001, 0.008)	0.096	0.368
MWT-B [sum of correctly recognised words]	−0.007 (−0.098, 0.085)	0.887	0.920	−0.033 (−0.125, 0.058)	0.474	0.665	−0.037 (−0.129, 0.054)	0.423	0.564
Outcomes - eye movement tasks									
Log of spatial error during fixation [log °]	0.002 (−0.001, 0.006)	0.250	0.588	0.002 (−0.002, 0.006)	0.259	0.522	0.002 (−0.001, 0.006)	0.248	0.564
Log of saccade rate during fixation [log N/s]	−0.001 (−0.006, 0.005)	0.845	0.910	0.001 (−0.004, 0.007)	0.692	0.808	0.002 (−0.003, 0.008)	0.416	0.564
Log of blink rate during fixation [log N/s]	−0.001 (−0.003, 0.000)	0.085	0.548	−0.001 (−0.003, 0.000)	0.143	0.500	−0.001 (−0.002, 0.001)	0.422	0.564
Smooth pursuit velocity gain [%]	−0.145 (−0.553, 0.264)	0.487	0.620	−0.389 (−0.797, 0.019)	0.061	0.427	−0.393 (−0.801, 0.016)	0.059	0.368
Saccade frequency during smooth pursuit [N/s]	**−0.017 (−0.033, −0.002)**	**0.027**	**0.252**	−0.001 (−0.017, 0.014)	0.857	0.908	−0.003 (−0.018, 0.013)	0.715	0.834
Prosaccade latency [ms]	**0.889 (0.177, 1.601)**	**0.014**	**0.252**	0.407 (−0.303, 1.117)	0.261	0.522	0.594 (−0.117, 1.305)	0.102	0.368
Prosaccade amplitude gain [%]	−0.085 (−0.272, 0.101)	0.370	0.599	−0.143 (−0.329, 0.043)	0.132	0.500	−0.178 (−0.364, 0.009)	0.062	0.368
Log of prosaccade spatial error [log %]	0.003 (−0.003, 0.008)	0.300	0.595	0.001 (−0.004, 0.007)	0.669	0.808	0.003 (−0.003, 0.008)	0.335	0.564
Prosaccade peak velocity [°/s]	−0.925 (−2.549, 0.698)	0.264	0.588	0.559 (−1.060, 2.178)	0.499	0.665	0.077 (−1.544, 1.699)	0.926	0.997
Amplitude-adjusted peak prosaccade velocity	−0.006 (−0.022, 0.009)	0.419	0.599	0.011 (−0.005, 0.026)	0.179	0.522	0.007 (−0.009, 0.023)	0.387	0.564
Antisaccade latency [ms]	0.563 (−0.830, 1.956)	0.428	0.599	−0.110 (−1.493, 1.273)	0.876	0.908	0.072 (−1.314, 1.458)	0.919	0.997
Antisaccade amplitude gain [%]	−0.175 (−1.022, 0.672)	0.686	0.768	−0.517 (−1.357, 0.324)	0.228	0.522	−0.654 (−1.496, 0.188)	0.128	0.398
Log of antisaccade spatial error [log %]	0.005 (−0.001, 0.011)	0.133	0.548	−0.002 (−0.008, 0.004)	0.462	0.665	−0.002 (−0.008, 0.004)	0.499	0.635
Antisaccade peak velocity [°/s]	−0.597 (−2.641, 1.447)	0.567	0.690	−0.184 (−2.213, 1.845)	0.859	0.908	−0.506 (−2.539, 1.527)	0.626	0.762
Amplitude-adjusted peak antisaccade velocity	0.010 (−0.014, 0.034)	0.405	0.599	0.018 (−0.006, 0.042)	0.133	0.500	0.020 (−0.004, 0.044)	0.101	0.368
Antisaccade costs [ms]	−0.474 (−1.760, 0.812)	0.470	0.620	−0.531 (−1.806, 0.744)	0.414	0.665	−0.526 (−1.804, 0.751)	0.419	0.564
Antisaccade error rate [%]	0.152 (−0.491, 0.794)	0.644	0.751	0.243 (−0.398, 0.885)	0.457	0.665	0.298 (−0.344, 0.941)	0.363	0.564

The table displays the change in cognitive performance per one standard deviation increase in Alzheimer's disease PRS for the three different PRS scores separately. The regression coefficients for each PRS were obtained from the following multivariable linear regression model: Cognitive outcome ~ b0 + PRS* b1 + age + age^2 + sex + population stratification + residual error. The FDR-correction is based on 28 comparisons (27 in the table plus antisaccade correction rate) and was conducted for each PRS score separately. None of the association between PRS and cognitive performance remained significant after excluding *APOE* from the PRS. In bold are those associations with an unadjusted *p*-value below 0.05.

AD Alzheimer's disease, *b* unstandardized regression coefficient, *FDR* false discovery rate, *AVLT* Auditory Verbal Learning and Memory Test, *MWT-B* Mehrfachwahl-Wortschatz-Intelligenztest, 95%-CI = 95%-confidence interval.

Table 3. Testing for interactions between Alzheimer's Disease PRS and age and age^2 on cognitive performance.

Cognitive outcome	*F*-value in model with PRS Jansen	*p*-value	FDR-adjusted *p*-value	*F*-value in model with PRS Wightman	*p*-value	FDR-adjusted *p*-value	*F*-value in model with PRS Schwartzentruber	*p*-value	FDR-adjusted *p*-value
Outcomes - classical cognitive tasks									
Digit span forward [number of digits]	2.174	0.114	0.579	2.344	0.071	0.320	2.116	0.096	0.432
Digit span backward [number of digits]	1.898	0.150	0.579	1.304	0.271	0.617	1.280	0.279	0.731
Corsi forward [number of blocks]	1.164	0.312	0.782	1.295	0.274	0.617	1.028	0.379	0.731
Corsi backward [number of blocks]	1.222	0.295	0.782	1.071	0.360	0.730	1.101	0.347	0.731
AVLT – immediate recall [sum of recalled words]	**5.780**	**0.003**	**0.042**	**4.256**	**0.005**	**0.070**	**4.125**	**0.006**	**0.084**
AVLT – delayed recall [number of words]	**5.091**	**0.006**	**0.056**	**3.526**	**0.014**	**0.097**	**3.507**	**0.015**	**0.099**
Word fluency task [number of animals]	0.501	0.606	0.877	0.684	0.562	0.844	0.541	0.654	0.921
Trail-making test A [log s]	**10.165**	**<0.001**	**0.001**	**7.046**	**<0.001**	**0.003**	**6.976**	**<0.001**	**0.003**
Trail-making test B [log s]	2.163	0.115	0.579	1.631	0.180	0.540	1.634	0.179	0.589
MWT-B [sum of correctly recognised words]	1.423	0.241	0.782	1.029	0.379	0.730	1.060	0.365	0.731
Outcomes - eye movement tasks									
Log of spatial error during fixation [log °]	0.266	0.766	0.877	0.288	0.834	0.925	0.274	0.844	0.925
Log of saccade rate during fixation [log N/s]	0.700	0.496	0.877	0.584	0.625	0.844	0.779	0.505	0.853
Log of blink rate during fixation [log N/s]	0.388	0.679	0.877	0.632	0.594	0.844	1.076	0.358	0.731
Smooth pursuit velocity gain [%]	0.026	0.974	0.974	0.129	0.943	0.943	0.158	0.925	0.925
Saccade frequency during smooth pursuit [N/s]	2.000	0.135	0.579	**3.733**	**0.011**	**0.097**	**3.551**	**0.014**	**0.099**
Prosaccade latency [ms]	1.144	0.319	0.782	2.393	0.067	0.320	1.876	0.131	0.507
Prosaccade amplitude gain [%]	1.018	0.361	0.813	0.679	0.565	0.844	0.710	0.546	0.867
Log of prosaccade spatial error [log %]	0.445	0.641	0.877	0.619	0.603	0.844	0.393	0.758	0.925
Prosaccade peak velocity [°/s]	0.159	0.853	0.886	1.348	0.257	0.617	0.870	0.456	0.820
Amplitude-adjusted peak prosaccade velocity	0.820	0.440	0.877	1.965	0.117	0.395	1.562	0.196	0.589
Antisaccade latency [ms]	0.331	0.718	0.877	0.618	0.603	0.844	0.500	0.682	0.921
Antisaccade amplitude gain [%]	0.403	0.669	0.877	0.290	0.833	0.925	0.386	0.763	0.925

Table 3. continued

Cognitive outcome	*F*-value in model with PRS Jansen	*p*-value	FDR-adjusted *p*-value	*F*-value in model with PRS Wightman	*p*-value	FDR-adjusted *p*-value	*F*-value in model with PRS Schwartzentruber	*p*-value	FDR-adjusted *p*-value
Log of antisaccade spatial error [log %]	0.227	0.797	0.877	2.184	0.088	0.338	2.229	0.083	0.432
Antisaccade peak velocity [°/s]	0.208	0.812	0.877	0.257	0.856	0.925	0.174	0.914	0.925
Amplitude-adjusted peak antisaccade velocity	0.270	0.764	0.877	0.168	0.918	0.943	0.179	0.911	0.925
Antisaccade costs [ms]	0.397	0.672	0.877	0.305	0.821	0.925	0.302	0.824	0.925
Antisaccade error rate [%]	0.757	0.469	0.877	0.515	0.672	0.864	0.529	0.662	0.921

The table displays the results of the likelihood ratio test, which compares the model fit of model 1 (cognitive variable ~ $b_0/\beta_0 + PRS^* b_1/\beta_1 + age + age^2 + sex +$ residual error) with the model fit of model 2 (cognitive variable ~ $b_0/\beta_0 + PRS^*b_1/\beta_1 + age^*PRS^*b_2/\beta_2 + age + age^2 + sex +$ residual error). The null hypothesis says that the data are equally likely under both models and can be rejected if the p-value is <0.05; otherwise, it cannot be rejected. Interpretation of the *F*-value: the data are "*F*-value" times more likely if the interaction terms "age*PRS" and "age^2*PRS" are included in the model (model 2) than if they are not included in the model (model 1). We conducted the likelihood ratio tests for each PRS score separately and present the resulting *F*-values and corresponding *p*-values and FDR-adjusted *p*-values next to each other. The FDR-correction is based on 27 comparisons. In bold are those associations with an unadjusted *p*-value below 0.05.
PRS polygenic risk score, *SNP* single-nucleotide polymorphism, *FDR* false discovery rate, *AVLT* Auditory Verbal Learning and Memory Test.

genetic risk individuals in TMT-A performance. Further, in the $PRS_{Wightman}$ model, medium genetic risk individuals differed from low genetic risk individuals in TMT-A performance ($p = 0.016$). All other post-hoc comparisons were non-significant.

Statistical power analysis

Our post-hoc analysis showed that we could detect effect sizes (Cohen's f^2) of 0.0020, 0.0015 and 0.0010 with a statistical power of 90%, 80% and 62%, respectively. To illustrate the magnitude of the effect sizes that we were able to detect, we calculated the effect sizes for the associations between the three different AD PRS scores and Corsi forward performance. The effect sizes were $f^2 = 0.0011$ for PRS_{Jansen}, $f^2 = 0.0010$ for $PRS_{Wightman}$ and $f^2 = 0.0016$ for $PRS_{Schwartzentruber}$.

DISCUSSION

We found that genetic risk for AD was not significantly associated with any cognitive or oculomotor measure from our test battery after correcting for multiple testing. However, prior to correcting for multiple testing, all three AD PRS were significantly associated with lower performance in the Corsi forward working memory task, and two AD PRS with a lower probability of correcting versus not correcting all antisaccade errors. Further, the association between age and TMT-A performance varied with genetic risk for AD and was strongest in those individuals at highest genetic risk for AD.

The unadjusted *p*-values indicated an association between all three AD PRS and visuo-spatial working memory performance as measured by the Corsi forward task, which has not been found in previous studies [33, 37]. We found that the associations between AD PRS and Corsi forward performance did not vary with age, suggesting that the discrepancy between our and previous studies is unlikely to be due to differences in the age distribution. One reason for this finding may be that we created the PRS based on more recent genome-wide association studies and, therefore, included at least seven more SNPs in our PRS than previous studies. However, a previous study comparing visuo-spatial working memory performance between homozygous *APOE* ε4 carriers and non-ε4 carriers reported lower Corsi performance (combined score for forward and backward performance) in homozygous *APOE* ε4 carriers [56]. Still, the associations between AD PRS and Corsi forward performance did not remain significant after correcting for multiple testing. FDR-correction probably was too conservative as discussed further below this. Nevertheless, our results should be considered as suggestive until validated in independent studies. Concerning the other tests for visuo-spatial working memory, we found that a higher $PRS_{Wightman}$ score was associated with a *better* digit span backward performance. However, this association was not found using PRS_{Jansen} and $PRS_{Schwartzentruber}$, thus likely representing a false positive observation.

Regarding associations between genetic risk for AD and episodic verbal memory performance, previous studies have reported significant associations [31–33], which we could not confirm across the entire range of 30+ year-olds. However, our results suggest that the association between age and AVLT immediate recall varied with genetic risk for AD (Table 3), and was strongest in those individuals at highest genetic risk for AD (Fig. 1). For PRS_{Jansen}, this interaction effect remained significant after correction for multiple testing. Additionally, the association between age and AVLT delayed recall was also modified by genetic risk for AD, with AVLT delayed recall declining strongest in those individuals at highest genetic risk for AD, as indicated by Fig. 1 and the results of Tukey post-hoc tests for $PRS_{Wightman}$ and $PRS_{Schwartzentruber}$. However, these interactions did not remain significant after correction for multiple testing. Thus, our findings overall suggest that differences in episodic verbal memory

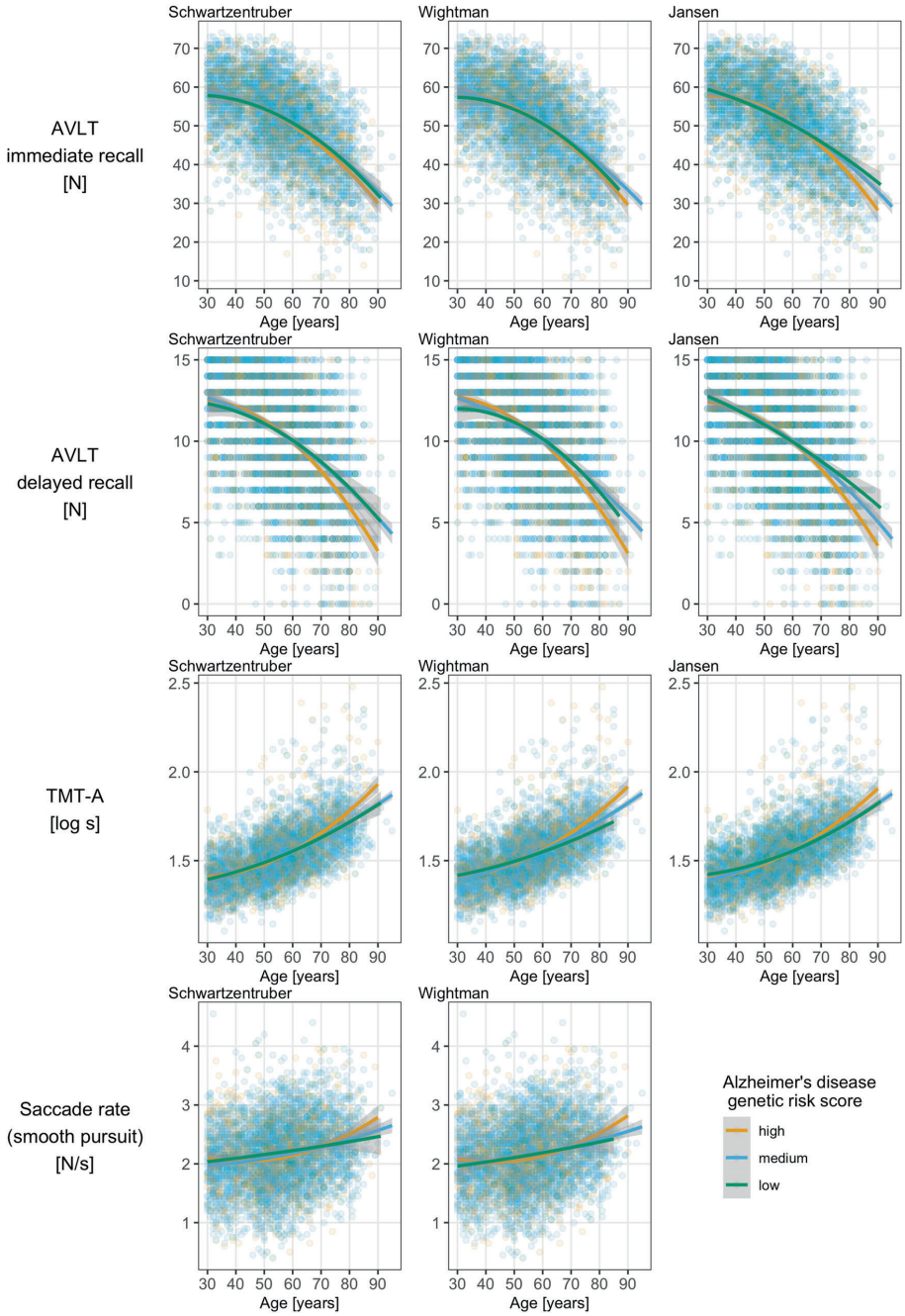
Schwartzentruber
Wightman
Jansen
AVLT
immediate recall
[N]
AVLT
delayed recall
[N]
TMT-A
[log s]
Saccade rate
(smooth pursuit)
[N/s]
Age [years]
Alzheimer's disease
genetic risk score
high
medium
low

Fig. 1 Scatterplots for interaction effects between age and Alzheimer's disease polygenic risk scores (PRS). The scatterplots show how the associations between age and different cognitive outcomes vary with genetic risk for Alzheimer's disease. Each column represents a different polygenic risk score and each row represents a cognitive outcome. The colours represent three different genetic risk groups for Alzheimer's disease (orange = high risk/ z-standardized PRS score above 1; blue = medium risk/ z-standardised PRS score between −1 and 1; green = low risk/ z-standardised PRS score below −1). For each genetic risk group there exists one superimposed function for the development of the cognitive outcome across the adult life span. The functions were obtained from a multivariable regression model with the following formula: cognitive outcome ~ b_0 + age*b_1 + age^2*b_2 + residual error. The grey area around the risk group-specific regression lines indicates the 95% confidence interval in each case. AVLT Auditory Verbal Learning and Memory Test; TMT-A Trail-making test A, N = number.

performance among the three genetic risk groups may become more pronounced at older ages, which is compatible with the finding that age is a major risk factor for AD [53].

Associations between all three PRS scores and TMT-A performance varied robustly with age, as the interaction terms remained significant even after correcting for multiple testing. We observed that differences in TMT-A performance among the three PRS groups were strongest at older ages (Fig. 1). Across the entire age range, only $PRS_{Wightman}$ was associated with lower TMT-A performance, but this association did not remain significant after correction for multiple testing. A previous population-based study reported no association between AD PRS and processing speed across the sample but only in the 70- to 99-year-olds [31]. Thus, our results support the previous finding that associations between AD PRS and TMT-A performance are more likely to emerge in old age. Additionally, our robust finding that genetic risk for AD gradually affects the magnitude of age-related decline in processing speed, but not in other cognitive domains across the adult lifespan, supports Salthouse's processing speed theory of age-related cognitive decline [57]. According to this theory, slowing of processing speed is the global mechanism underlying age-related cognitive decline [57]. This suggests that AD partly results from individuals at high genetic risk for AD experiencing a stronger age-related decline in processing speed compared to individuals at low genetic risk for AD, resulting in lower cognitive performance across all domains in the long term. Our finding is also in line with previous reports of AD PRS being related to longitudinal decline in processing speed [34, 35].

Consistent with some previous studies [32, 37], we found no associations between AD PRS and executive function, as measured by performance in TMT-B and the word fluency task. Associations between AD PRS and oculomotor measures had not been assessed before. Using AD PRS_{Jansen}, we found that higher genetic risk was associated with higher prosaccade latency, lower saccade frequency during pursuit, and lower antisaccade error correction probability. However, as neither of those associations could be found with the other two AD PRS scores and as none of the findings survived correction of multiple testing, they should be interpreted with caution as they may represent false positive findings.

Our finding of a lower probability of correcting antisaccade errors in individuals with higher genetic risk for AD may be more robust as it was found using both PRS_{Jansen} and $PRS_{Schwartzentruber}$. Still, neither association remained significant after correction for multiple testing. The association between genetic risk for AD and a lower probability of correcting antisaccade errors agrees with previous reports of lower antisaccade error correction probabilities in individuals with AD and MCI [13, 15, 16]. Moreover, scores in dementia screening tests have also been found to correlate with the probability of correcting antisaccade errors [24]. Still, the association between genetic risk for AD and antisaccade corrections probability requires further investigation as it was not found using $PRS_{Wightman}$ and did not survive correction for multiple testing.

A potential limitation of our study is lack of longitudinal data, precluding assessment of the associations between AD PRS and change in cognitive outcomes. However, our sample included a wide age range, which allowed us to investigate associations between AD PRS and cognitive outcomes across the adult lifespan, and how the associations between age and cognitive tests vary between different AD genetic risk groups. Further, we employed an extensive cognitive test battery including eye movement outcomes that were not part of the cognitive test batteries in previous large population studies.

Another potential limitation of our study relates to statistical power. The associations between AD PRS and cognitive measures did not remain significant after FDR-correction. However, our approach for correcting for multiple comparisons may have been too conservative as it is only appropriate in case of disjunction testing [58]. On the one hand, we wanted to infer from the individual cognitive measures which cognitive domain is most sensitive to capturing genetic risk for AD. In this scenario, one could argue that our testing approach represents disjunction testing, as a significant association between genetic risk and a cognitive measure would be taken as an indication that the represented cognitive domain in general is especially sensitive to capturing genetic risk for AD [58]. On the other hand, we aimed to identify the most sensitive *individual* cognitive measure of genetic risk for AD without making a general statement about the associated cognitive domain. This approach would clearly fall into the category of individual testing, for which correction for multiple testing is not appropriate [58]. Thus, as long as we do not overgeneralize from single tests to cognitive domains, correction for multiple testing may be too conservative. Importantly, we also conducted the analyses using three different AD PRS scores to identify the most consistent associations. Moreover, it should be noted that current AD PRS only explain about 7% of AD heritability at optimal *p*-value threshold for SNP inclusion [44], despite heritability estimates for AD ranging from 58% to 79% [59]. This may be due to PRS being based on summary statistics of GWAS that rely on conventional genotyping arrays that capture common variants but not rare or structural variants [29]. Finally, AD is a heterogeneous disease and influenced by a complex interplay between both genetic and environmental factors [60]. Thus, very strong associations between AD PRS and cognitive outcome are unlikely to occur, exemplified by the small effect sizes (Cohen's $f^2 \leq 0.0016$) for the associations between the three different AD PRS scores and Corsi forward performance. Therefore, Corsi forward performance and the likelihood of correcting antisaccade errors may be promising candidate measures whose ability to capture the genetic predisposition to AD should be investigated further in future studies. Lastly, the suitability of TMT-A performance in detecting genetic liability for AD in old age also requires further investigation.

CONCLUSION

PRS for AD were not robustly associated with any of our cognitive and oculomotor measures. Of all our cognitive measures, Corsi forward performance and the probability of correcting antisaccade errors may be the most suitable candidates for capturing genetic liability for AD across the adult lifespan, but these associations require confirmation in independent samples. In addition, TMT-A, which measures processing speed performance,

may be a sensitive marker of genetic susceptibility to AD in old age. Lastly, our finding of a stronger association between age and processing speed performance in individuals at high genetic risk for AD supports the processing speed theory of age-related cognitive decline by Salthouse, suggesting a decline in processing speed as the global mechanism underlying general cognitive decline.

DATA AVAILABILITY

The datasets for this manuscript are not publicly available because of data protection regulations. Access to data can, however, be provided to scientists in accordance with the Rhineland Study's Data Use and Access Policy. Requests to access the datasets should be directed to Dr Monique Breteler, RS-DUAC@dzne.de.

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AUTHOR CONTRIBUTIONS

AC: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing – original draft, writing – review & editing. M-AI: conceptualization, methodology, writing – review & editing. MMB: investigation, writing – review & editing. NAA: conceptualization, methodology, writing – review & editing. MMBB: conceptualization, methodology, validation, resources, writing – review & editing, supervision, project administration, funding acquisition. UE: conceptualization, methodology, validation, writing – review & editing, supervision.

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ADDITIONAL INFORMATION

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6. Processing speed, but not working memory or global cognition, is associated with pupil diameter during fixation

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ORIGINAL ARTICLE

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Processing speed, but not working memory or global cognition, is associated with pupil diameter during fixation

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Abstract

Mean pupil size during fixation has been suggested to reflect interindividual differences in working memory and fluid intelligence. However, due to small samples with limited age range (17–35 years) and suboptimal light conditions in previous studies, these associations are still controversial and it is unclear whether they are observed at older ages. Therefore, we assessed whether interindividual differences in cognitive performance are reflected in pupil diameter during fixation and whether these associations are age-dependent. We analyzed pupillometry and cognition data of 4560 individuals aged 30–95 years of the community-based Rhineland Study. Pupillometry data were extracted from a one-minute fixation task. The cognitive test battery included tests of oculomotor control, working memory, episodic verbal memory, processing speed, executive function, and crystallized intelligence. For data analysis, we used multivariable regression models. Working memory and global cognition were not associated with pupil diameter during fixation. Better processing speed performance was associated with larger pupil diameter during fixation. Associations between cognition and pupil diameter during fixation hardly varied with age, but pupil diameter during fixation declined linearly with age (adjusted decline: 0.33 mm per 10 years of age). There were no significant sex differences in pupil size. We conclude that interindividual differences in mean pupil diameter during fixation may partly reflect interindividual differences in the speed of processing and response generation. We could not confirm that interindividual differences in working memory and fluid intelligence are reflected in pupil size during fixation; however, our sample differed in age range from previous studies.

KEYWORDS

age, cognition, cohort studies, intelligence, pupillometry

Monique M. B. Breteler and Ulrich Ettinger contributed equally to this work.

1 | INTRODUCTION

The pupil dilates and constricts in response to changes in lighting, known as the pupillary light reflex (Lowenstein & Loewenfeld, 1950). Non-luminance-driven pupil dilations have been linked to activity in superior colliculus and locus coeruleus (Joshi & Gold, 2020). The locus coeruleus is one of the first sites showing Alzheimer's disease pathology (Mather & Harley, 2016) and is the main source of norepinephrine in the central nervous system (Joshi & Gold, 2020). Norepinephrine is a major neuromodulator that mediates arousal (Joshi & Gold, 2020) and has been linked to cognitive performance (Wang et al., 2013). Specifically, pupil dilations have been associated with arousal (Joshi & Gold, 2020) and mental effort (van der Wel & van Steenbergen, 2018). However, it is controversial whether interindividual differences in cognitive abilities are reflected in pupil diameter during fixation (Tsukahara et al., 2016; Tsukahara & Engle, 2021; Unsworth et al., 2020). Evidence of a relationship between interindividual differences in cognitive abilities and pupil diameter during fixation would further support the importance of the locus coeruleus-norepinephrine system in cognitive performance (Joshi & Gold, 2020).

Some studies have reported that individuals with higher working memory capacity (Heitz et al., 2008; Tsukahara et al., 2016; Tsukahara & Engle, 2021) and higher fluid intelligence (Bornemann et al., 2010; Tsukahara et al., 2016; Tsukahara & Engle, 2021; van Der Meer et al., 2010) have larger pupil diameter during a passive baseline condition than individuals with lower working memory capacity and fluid intelligence. However, a recent study and meta-analysis found no association between working memory capacity and baseline pupil diameter (Unsworth et al., 2020). Only three of the 26 studies included in the meta-analysis reported effect sizes that were statistically significantly different from zero for the association between working memory capacity and mean pupil size during fixation, with one study reporting a negative association (Sibley et al., 2018) and two studies reporting a positive association (Tsukahara et al., 2016; Tsukahara & Engle, 2021). Since the variability in effect sizes in the meta-analysis was large, the authors performed a moderator analysis and concluded that the primary moderator for heterogeneity across studies was the laboratory in which the study was conducted (laboratory of Tsukahara versus other) (Unsworth et al., 2020).

In response, Tsukahara and Engle (2021) argued that one major reason for negative findings from other laboratories may have been sub-optimal experimental light conditions. They argued that because of the pupillary light reflex (Lowenstein & Loewenfeld, 1950), bright experimental light conditions reduce the variance in pupil diameter, which in turn reduces the chances to detect statistically significant associations with cognitive performance across individuals (Tsukahara & Engle, 2021). They then systematically varied the light conditions in their own experiments and found that a reduction of the mean-variance in pupil diameter to a similar level as in the study by Unsworth et al. (2020) completely eliminated the association between working memory capacity and pupil diameter and decreased the association with fluid intelligence (Tsukahara & Engle, 2021).

However, Unsworth et al. (2021) reanalyzed the data of Tsukahara and Engle (2021) and found that controlling for the additional confounder race also eliminated the association between fluid intelligence and baseline pupil diameter. This finding is in line with previous studies that reported differences in baseline pupil size between races (Tsukahara et al., 2016). In response to the re-analysis of their data, Tsukahara et al. (2021) combined the data of their different samples ($N = 831$), controlled for age and race and reported that there is an association between fluid intelligence and pupil diameter.

Given these controversies and given that the meta-analysis was only based on 4356 persons in total with more than half of the studies originating from two laboratories, the association between cognitive performance and pupil diameter during fixation needs further investigation in a large, independent sample, and under comparable experimental conditions as in the study by Tsukahara et al. (2016). According to the findings reported in the study by Tsukahara et al. (2016) and their follow-up studies (Tsukahara et al., 2021; Tsukahara & Engle, 2021), the chances of detecting associations between cognition and mean pupil size during fixation can be maximized by measuring each cognitive domain with multiple tests, and by ensuring that the experimental room is dark enough to allow for large variation in pupil size across participants (standard deviation across participants in Tsukahara et al. (2016): 1.1 mm). Further, it should be controlled for interindividual differences in genetic background and age as these represent important confounders (Unsworth et al., 2021).

Linear age-related decline in pupil size and more restricted range in pupil dilation have also been reported in older adults as a result of a degenerating dilation muscle in the iris (Van Gerven et al., 2004). If age also reduced interindividual variation in pupil size, then the chances to detect statistically significant associations between cognitive performance and pupil diameter during fixation across individuals might be extremely low in older age (Tsukahara & Engle, 2021). As all studies included in the meta-analysis by Unsworth et al. (2020) and the additional studies cited before were conducted in individuals aged 17 to 35 years, it remains an open question whether associations between cognitive performance and pupil diameter during fixation are age-dependent.

We therefore investigated, in a large population-based cohort under experimental conditions deemed optimal (Tsukahara et al., 2016; Tsukahara & Engle, 2021), whether interindividual differences in cognitive performance (including global cognition and working memory performance) are reflected in pupil diameter during fixation and whether these associations are age-dependent.

2 | METHOD

2.1 | Participants

We used data from the first 5000 participants of the Rhineland Study, a community-based cohort study in Bonn, Germany. Inclusion criteria are living in one of two geographically defined areas in Bonn and being at least 30 years or older. Participation is only possible upon invitation and there are no financial incentives. The only exclusion criterion is not having sufficient comprehension of the German language to provide written informed consent. Eligibility is irrespective of health status. The ethics committee of the Medical Faculty of the University of Bonn approved the study, which was carried out in accordance with the recommendations of the International Council for Harmonisation Good Clinical Practice standards (ICH-GCP).

Participants underwent 8 h of examinations, including a one-hour cognitive and eye movement test battery. The eye movement tests included fixation, smooth pursuit, prosaccade, and antisaccade tasks (Coors et al., 2021). Pupillometry data were taken from the fixation task (see below) and were available in 4568 participants out of the first 5000 participants. Missing values ($N = 432$) were primarily due to technical issues during data acquisition and post-processing (71.1%), exclusion after visual inspection of data (14.8%), contraindications (8.1%), non-compliance (5.1%), refusal (0.7%), or timeout (0.2%). An additional 8 participants did not have pupil data available for all fixation positions and were therefore excluded. This left 4560 participants for our analysis.

2.2 | Pupillometry data

We recorded eye movements using video-based infrared oculography (EyeLink 1000 and EyeLink 1000 Plus; SR Research Ltd) at 1000 Hz. We extracted the mean pupil diameter during a one-minute fixation task. In the fixation task, participants had to fixate the target at the center ($x = 0°$, $y = 0°$), the left ($x = -9.63°$, $y = 0°$), the right ($x = 9.63°$, $y = 0°$), the top ($x = 0°$, $y = 9.63°$) and the bottom ($x = 0°$, $y = -9.63°$). The order within which the target was presented in these positions was randomized across participants but eccentric locations were always followed by the central location. The central position thus had to be fixated four times in total. The target appeared at each eccentric location for 10 s and at the central location for 5 s each time. The target was a white (RGB 255, 255, 255) circle 0.35° in diameter presented on black (0, 0, 0) background. A PCE-172 light meter (PCE Instruments, Meschede, Germany) was used to measure luminance. During the examination, the overall room luminance value was about 1 Lux (light meter placed on the table at a distance of 70 cm from the monitor screen, faced up) and the screen luminance value was about 4 Lux (light meter placed at a distance of about 1 cm from the fixation point displayed on the monitor screen, faced toward the screen). A detailed description of oculomotor data acquisition can be found in Coors et al. (2021).

Pupil diameter values typically vary between 1.5 and 8 mm (McDougal & Gamlin, 2008) with more extreme values possibly representing measurement artifacts. Based on a visual inspection of a plot of pupil diameter values against age, we decided to consider all participants with pupil diameter values above 9 mm as outliers ($N = 6$, Figure 1). We performed all analyses including and excluding outlier values and compared the results.

Pupil diameter values were highly correlated across fixation positions (Pearson $r \geq 0.97$), suggesting high internal consistency of measurement. Therefore, we calculated the mean pupil diameter across all positions and used this value for subsequent analyses.

2.3 | Cognitive tests

The cognitive test battery measured working memory, episodic verbal memory, processing speed, executive function, and crystallized intelligence. We assessed working memory with the orally performed Digit Span forward (sequence length 3 to 9) and backward (sequence length 2 to 9) tasks and the touchpad-based Corsi block-tapping test (Corsi) forward and backward (sequence lengths 2 to 9), adapted from the PEBL battery (Mueller & Piper, 2014). In both tasks, the sequence was increased until the participant made two errors within one sequence. The length of the last successfully completed sequence was then taken as a measure of the working memory span. Episodic verbal memory was assessed with the Auditory Verbal Learning and Memory test (AVLT) with a list length of 15 words (immediate recall: sum of correctly recalled nouns in the first five trials out of 75 words; delayed recall: number of correctly recalled words out of 15 words after a time delay of 20–30 min) (Boenniger et al., 2021). Results on a numbers-only trail-making test (TMT-A: time to connect 24 digits that were randomly distributed on a computer screen in ascending order) and prosaccade latency (time needed to initiate a saccade after

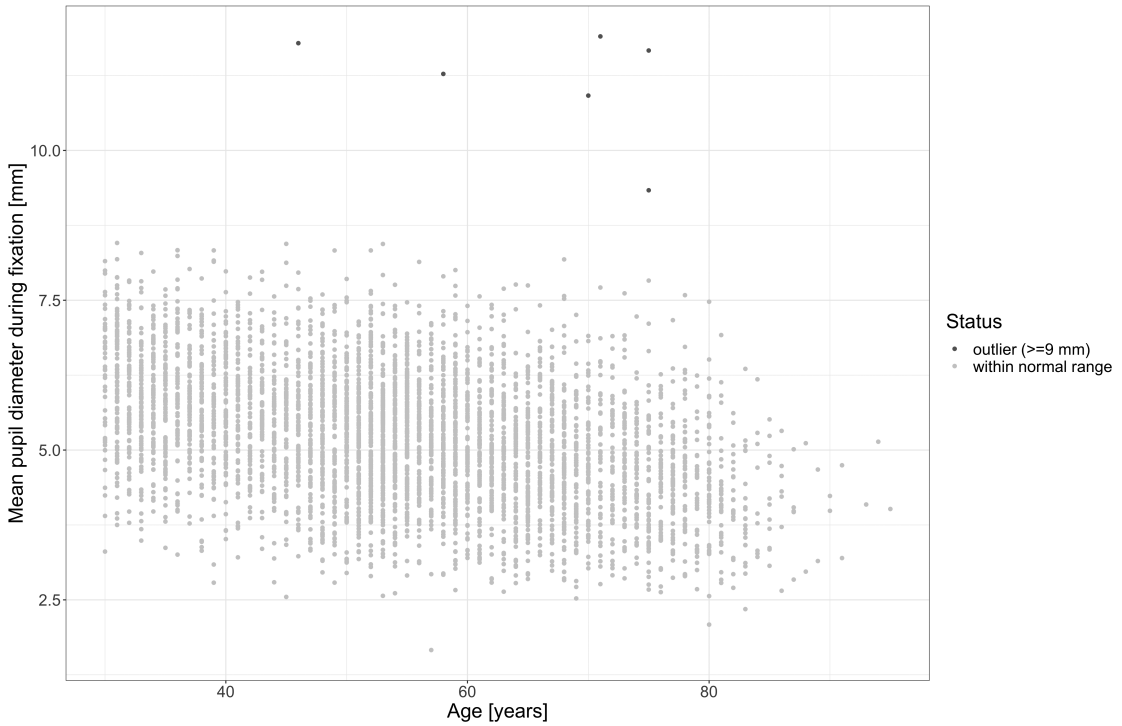

FIGURE 1 Mean pupil diameter during fixation across age – Outlier determination

the target has stepped to one side) were combined to assess processing speed. We assessed executive function with a 60s categorical word fluency task (number of uniquely named animals), a number-and-letters trail-making test (TMT-B: time to connect 12 digits and 12 letters in ascending order and in an alternating fashion [1-A-2-B]), and antisaccade error rate (percentage of trials in which the initial saccade was made toward the target instead of the opposite direction). Crystallized intelligence was measured with the 37-item Mehrfachwahl-Wortschatz-Intelligenztest (MWT-B), which is a vocabulary test in which participants select an existing German word among four non-words in each of 37 trials (Lehrl, 2005).

We calculated domain scores based on averaged z-scores for the separate test scores in that domain. The assignment of cognitive tests to domain scores was based on previous literature. Finally, we averaged the domain scores for working memory, episodic verbal memory, processing speed, and executive function to obtain a global cognition score. Individuals whose native language was not German or those with severe cognitive impairment as a result of traumatic brain injury or other non-aging-related diseases did not contribute to the mean and standard deviation statistics used in the standardization process. However, we computed z-scores for those participants based on the mean and standard deviation of the remaining sample, except for the crystallized intelligence score where German as a native language is a test requirement.

2.4 | Genetic ancestry

We used genome-wide single-nucleotide polymorphisms (SNP) arrays (Illumina Omni-2.5 exome array) to determine heterogeneity in genetic background. Genotype data were processed with GenomeStudio (version 2.0.5), and quality-controlled using PLINK (version 1.9) (Purcell et al., 2007). We calculated six principal components using EIGENSTRAT (version 16000) (Price et al., 2006) to represent differences in genetic background.

2.5 | Statistical analyses

To quantify associations between cognitive scores and pupil diameter during fixation, we calculated a separate regression model for each cognitive score that included the cognitive score as independent variable, pupil diameter during fixation as dependent variable, and age, sex,

best-corrected visual acuity, educational level, and six principal components to correct for population stratification as additional variables to adjust for. In addition, we added native language as additional variable to all models except for the two models that included prosaccade latency and antisaccade error rate as independent variables. To evaluate whether associations between cognitive performance and pupil diameter during fixation vary with age, we ran an additional model for each cognitive predictor that included an age*cognition interaction term in addition to the covariates mentioned above. In addition, we performed age-stratified analysis for the strata 30- to 49-year-olds, 50- to 60-year-olds, and 61+-year-olds. These age cut-offs were chosen to yield approximately equal stratum sizes, and to reflect age ranges with little or no age-related cognitive decline (30–49 years), an intermediate age range (50–60 years), and an age range where some participants might start showing age-related cognitive decline (≥61 years).

We then quantified how pupil diameter during fixation differs across age and between men and women using a linear regression model that included age and sex as independent variables, pupil diameter during fixation as dependent variable, and best-corrected visual acuity, educational level, and population stratification as covariates.

To make our results on the associations between cognitive performance and baseline pupil diameter comparable to previous studies, we additionally calculated zero-order Pearson correlations for the whole sample and for the three aforementioned age strata. For the correlation analyses, we used participants without missing values in any of the cognitive scores ($N = 3702$).

Statistical analyses were carried out in RStudio (version 1.3.959, R-base version 4.0.3). Missing covariate data in the linear regression models (<1% missing values for visual acuity, education and native language, and 16.2% missing values for population stratification) were imputed using predictive mean matching (Hmisc package, 10 bootstrap replicates). We present standardized point estimates of association with 95% confidence intervals. We did not correct for multiple testing as we conducted an exploratory analysis on the associations between cognitive and eye movement measures and mean pupil size during fixation (Althouse, 2016). Thus, only additional confirmatory studies can rule out the possibility of false discoveries (Althouse, 2016).

3 | RESULTS

3.1 | Study sample

Table 1 gives descriptive characteristics of the study sample. Our sample was on average highly educated and had

TABLE 1 Sample characteristics

Number of participants, *N* (%)	4560 (100)
30–49 years	1656 (36.3)
50–60 years	1346 (29.5)
61+ years	1558 (34.2)
Age, *M* (*SD*) in years	54.7 (13.9)
Sex, *N* (%) women	2604 (57.1)
Education level, *N*	4519
High	2377 (52.6)
Middle	2052 (45.4)
Low	90 (2.0)
Best-corrected visual acuity, *N*	4535
High (≥0.8)	3931 (86.7)
Middle (0.32–0.63)	571 (12.6)
Low (<0.32)	33 (0.7)
Mean pupil size during fixation, *M* (*SD*)	5.2 (1.1)
30- to 49-year-olds	5.7 (1.0)
50- to 60-year-olds	5.2 (1.0)
61+-year-olds	4.7 (1.0)
Digit span forward [number of digits], *M* (*SD*), max = 9	6.3 (1.2)
Digit span backward [number of digits], *M* (*SD*), max = 9	4.8 (1.2)
Corsi forward [number of blocks], *M* (*SD*), max = 9	4.9 (1.1)
Corsi backward [number of blocks], *M* (*SD*), max = 9	4.8 (1.0)
AVLT - immediate recall [sum of recalled words over recall 1 to 5], *M* (*SD*), max = 75	51.3 (10.0)
AVLT - delayed recall [number of recalled words], *M* (*SD*), max = 15	10.3 (3.3)
Trail-making test A [completion time in s], median [IQR]	32.9 (26.8, 41.7)
Mean prosaccade latency [ms], median [IQR]	185.8 (170.6, 205.5)
Trail-making test B [completion time in s], median [IQR]	43.5 (33.5, 60.3)
Word fluency [number of unique words], *M* (*SD*)	26.0 (7.0)
Antisaccade error rate [%], *M* (*SD*)	31.9 (23.9)
MWT-B [sum of correctly recognized words], *M* (*SD*), max = 37	30.5 (3.4)

Note: Education level was determined using the International Standard Classification of Education 2011 (ISCED) and was coded as low (lower secondary education or below), middle (upper secondary education to undergraduate university level), and high (postgraduate university study). Assessment of best-corrected visual acuity was based on visual scores from the right eye and was measured using an automated refractometer (Ark-1 s, NIDEK CO., Tokyo, Japan). Categorization of the visual acuity values was based on the guidelines of the International Council of Ophthalmology.

Abbreviations: AVLT, Auditory Verbal Learning and Memory test; *M*, mean; MWT-B = Mehrfachwahl-Wortschatz-Intelligenztest; *N*, number of participants; SD, standard deviation.

TABLE 2 Pearson correlations between different cognitive domain and test scores

	Global cognition score	Working memory	Episodic verbal memory	Processing speed	Executive function	Crystallized intelligence	Digit span forward
Working memory	0.728						
Episodic verbal memory	0.791	0.375					
Processing speed	0.729	0.396	0.413				
Executive function	0.784	0.521	0.498	0.411			
Crystallized intelligence	0.014	0.056	0.044	−0.149	0.096		
Digit span forward	0.415	0.674	0.184	0.175	0.287	0.123	
Digit span backward	0.511	0.718	0.288	0.223	0.377	0.127	0.424
Corsi forward	0.537	0.681	0.271	0.346	0.388	−0.075	0.220
Corsi backward	0.535	0.667	0.286	0.344	0.376	−0.026	0.191
AVLT-immediate recall	0.779	0.397	0.954	0.410	0.503	0.054	0.221
AVLT-delayed recall	0.732	0.321	0.956	0.380	0.449	0.030	0.132
Trail-making test A	−0.655	−0.402	−0.392	−0.709	−0.502	0.034	−0.188
Prosaccade latency	−0.436	−0.176	−0.229	−0.801	−0.110	0.170	−0.053
Trail-making test B	−0.647	−0.461	−0.413	−0.461	−0.658	−0.004	−0.225
Word fluency	0.505	0.270	0.360	0.220	0.705	0.194	0.178
Antisaccade error rate	−0.447	−0.322	−0.253	−0.122	−0.710	−0.017	−0.164

Note: The cognitive test scores and the corresponding domain score as well as the correlations between them are shown in the same color. Crystallized intelligence was only measured with one single test. The global cognition score is a composite measure consisting of the domain scores for working memory, episodic verbal memory, processing speed, and executive function.

high best-corrected visual acuity. The mean pupil diameter during fixation was 5.2 mm with a standard deviation of 1.1 mm. In each age group, the standard deviation in pupil diameter was 1.0 mm.

3.2 | Associations between cognition and pupil diameter

Correlations between the cognitive test and domain scores are shown in Table 2. In Table 3, we present the regression results for the associations between cognitive performance and mean pupil size during fixation for the cognitive domain scores and in Table 4 for the individual cognitive test scores. Across the sample, better performance in the domain score for processing speed (Table 3) and its subtest prosaccade latency (Table 4) were associated with larger pupil diameter during fixation.

When we included an age*cognition-interaction term in the models, it did not become statistically significant for any model ($p \geq .071$). In the age-stratified analyses, the processing speed domain score was significantly associated with pupil size during fixation in the 50- to 60-year-olds and in the 61+-year-olds. Moreover, prosaccade latency was significantly associated with mean pupil diameter during fixation in all three strata (Table 5). Digit span forward performance was significantly associated with mean pupil size during fixation in the 30–49 years old (p value = .048), but not in any of the other age strata (Table 5). Visualizations of the associations between cognitive domain scores and mean pupil diameter during fixation can be found in Figure 2.

Across the sample and before adjusting for covariates, some cognitive scores were correlated with baseline pupil diameter ($-0.25 \leq$ Pearson $r \leq 0.31$) (Table 6). However, when we age-stratified the correlation analyses, the correlations were largely diminished and very small ($r < 0.14$) for all cognitive scores except for the domain score for processing speed ($0.09 \leq r \leq 0.20$) and prosaccade latency ($-0.18 \leq r \leq -0.09$). For both cognitive scores, the strongest correlation emerged in the 61+-year-olds (Table 6).

Our sensitivity analysis revealed that the exclusion of the six participants with pupil diameter values above 9 mm did not materially change any of the results.

3.3 | Associations between age, sex, and pupil diameter

Pupil diameter during fixation declined linearly with increasing age (average adjusted decrease 0.33 [95% CI: −0.35 to −0.30] mm per 10 years of age). There were no sex differences in mean pupil diameter during fixation (adjusted difference between men and women −0.03 [95% CI: −0.09 to 0.03] mm).

Digit span backward	Corsi forward	Corsi backward	AVLT-immediate recall	AVLT-delayed recall	Trail-making test A	Prosaccade latency	Trail-making test B	Word fluency
0.269								
0.263	0.386							
0.316	0.270	0.280						
0.236	0.247	0.267	0.825					
−0.265	−0.327	−0.324	−0.391	−0.358				
−0.055	−0.186	−0.191	−0.224	−0.212	0.215			
−0.311	−0.374	−0.355	−0.408	−0.381	0.481	0.221		
0.228	0.166	0.166	0.367	0.321	−0.253	−0.074	−0.246	
−0.211	−0.258	−0.251	−0.258	−0.225	0.287	−0.090	0.281	−0.205

TABLE 3 Associations between cognitive domain scores and mean pupil diameter during fixation

Cognitive predictor	*β* (95% CI)	*p*-value	Variance explained (%)
Global cognition score	0.060 (−0.004, 0.124)	.065	16.3
Working memory	0.020 (−0.025, 0.065)	.377	16.3
Episodic verbal memory	0.006 (−0.029, 0.041)	.755	16.5
Processing speed	**0.125 (0.080, 0.169)**	**<.001**	**17.2**
Executive function	−0.020 (−0.064, 0.023)	.360	16.6
Crystallized intelligence	0.014 (−0.017, 0.044)	.389	16.6

Note: The table displays the change in standard deviations in mean pupil diameter during fixation per one standard deviation increase in the cognitive domain score (β and 95% CI). Regression coefficients were obtained from the following multivariable linear regression model: Mean pupil diameter during fixation ~ β_0 + cognitive domain score*β_1 + age + sex + population stratification + education + visual acuity + native language + residual error. Variance explained refers to the adjusted variance explained by the total model including the cognitive predictor. The adjusted variance explained in the model without cognitive predictor was 16.8%. In bold are those associations with a *p* value below .05.

Abbreviations: 95% CI, 95% confidence interval; β, standardized regression coefficient.

4 | DISCUSSION

Of all cognitive measures, only processing speed, and prosaccade latency were associated with pupil diameter during fixation across the sample. Associations between cognitive performance and pupil diameter during fixation did not significantly vary with age, yet age-stratified analyses showed larger effect sizes for processing speed and prosaccade latency at higher age. Mean pupil diameter during fixation declined about 0.33 mm per 10 years of age. Men and women did not differ in mean pupil size during fixation.

We could not confirm that working memory capacity and fluid intelligence are associated with mean pupil size during fixation (Tsukahara et al., 2016; Tsukahara & Engle, 2021; van Der Meer et al., 2010). Our finding for working memory capacity is in line with the result of the meta-analysis by Unsworth et al. (2020). However, as experimental conditions may play a major role, we compared our experimental conditions with the ones in the study by Tsukahara et al. (2016), in which associations were found, and present the comparison in Table 7. Based on this comparison we can rule out that our null findings

TABLE 4 Associations between cognitive test scores and mean pupil diameter during fixation

Cognitive predictor	*β* (95% CI)	*p*-value	Variance explained (%)
Digit span forward	0.023 (−0.005, 0.051)	.108	16.4
Digit span backward	0.007 (−0.022, 0.035)	.642	16.3
Corsi forward	0.007 (−0.024, 0.038)	.660	15.8
Corsi backward	−0.001 (−0.031, 0.029)	.948	16.0
AVLT-immediate recall	0.002 (−0.032, 0.035)	.920	16.6
AVLT-delayed recall	0.008 (−0.024, 0.040)	.643	16.6
Trail-making test A	0.002 (−0.030, 0.034)	.911	16.6
Prosaccade latency	**−0.099 (−0.129, −0.069)**	**<.001**	**17.5**
Trail-making test B	0.008 (−0.024, 0.041)	.616	16.6
Word fluency	−0.028 (−0.058, 0.001)	.062	16.5
Antisaccade error rate	−0.009 (−0.038, 0.020)	.541	16.4

Note: The table displays the change in standard deviations in mean pupil diameter during fixation per one standard deviation increase in the cognitive test score (*β* and 95% CI). Regression coefficients were obtained from the following multivariable linear regression model: Mean pupil diameter during fixation ~ β_0 + cognitive test score*β_1 + age + sex + population stratification + education + visual acuity + (native language) + residual error. Native language was not included in the models with prosaccade latency and antisaccade error rate as predictors. Variance explained refers to the adjusted variance explained by the total model including the cognitive predictor. The adjusted variance explained in the model without cognitive predictor was 16.7% for the model excluding native language and 16.8% for the model including native language as covariate. In bold are those associations with a *p* value below .05.

Abbreviations: 95% CI, 95% confidence interval; AVLT, Auditory Verbal Learning and Memory test; *β*, standardized regression coefficient.

TABLE 5 Associations between cognitive test scores and mean pupil diameter during fixation in age groups 30–49, 50–60, and 61+ years

Cognitive predictor	*β* (95% CI) in the 30- to 49-year-olds	*β* (95% CI) in the 50- to 60-year-olds	*β* (95% CI) in the 61+-year-olds
Global cognition score	0.068 (−0.050, 0.186)	0.075 (−0.049, 0.199)	0.057 (−0.044, 0.159)
Working memory	0.032 (−0.043, 0.106)	0.022 (−0.063, 0.107)	0.017 (−0.059, 0.093)
Episodic verbal memory	0.022 (−0.045, 0.088)	0.013 (−0.053, 0.079)	−0.014 (−0.069, 0.040)
Processing speed	0.076 (−0.006, 0.159)	**0.129 (0.042, 0.217)**	**0.150 (0.083, 0.216)**
Executive function	−0.016 (−0.098, 0.067)	−0.021 (−0.105, 0.064)	−0.010 (−0.078, 0.058)
Crystallized intelligence	0.033 (−0.020, 0.086)	−0.012 (−0.073, 0.048)	0.020 (−0.030, 0.070)
Digit span forward	**0.047 (0.000, 0.094)**	0.003 (−0.049, 0.054)	0.018 (−0.031, 0.066)
Digit span backward	0.006 (−0.039, 0.051)	0.010 (−0.042, 0.061)	0.011 (−0.042, 0.063)
Corsi forward	0.002 (−0.050, 0.053)	0.022 (−0.036, 0.079)	0.004 (−0.047, 0.056)
Corsi backward	−0.004 (−0.054, 0.045)	0.002 (−0.057, 0.060)	0.006 (−0.045, 0.056)
AVLT-immediate recall	0.012 (−0.050, 0.073)	0.007 (−0.056, 0.070)	−0.013 (−0.066, 0.041)
AVLT-delayed recall	0.025 (−0.037, 0.086)	0.015 (−0.045, 0.075)	−0.013 (−0.062, 0.036)
Trail-making test A	0.017 (−0.055, 0.089)	−0.017 (−0.102, 0.067)	0.002 (−0.037, 0.042)
Prosaccade latency	**−0.066 (−0.128, −0.005)**	**−0.098 (−0.158, −0.038)**	**−0.112 (−0.154, −0.069)**
Trail-making test B	0.007 (−0.099, 0.114)	−0.016 (−0.090, 0.059)	0.017 (−0.022, 0.056)
Word fluency	−0.033 (−0.082, 0.016)	−0.041 (−0.097, 0.014)	−0.004 (−0.056, 0.047)
Antisaccade error rate	−0.043 (−0.099, 0.013)	0.002 (−0.053, 0.056)	−0.008 (−0.051, 0.035)

Note: The table displays the change in standard deviations in mean pupil diameter during fixation per one standard deviation increase in the cognitive test or domain score (*β* and 95% CI) for three different age strata. Regression coefficients were obtained from the following multivariable linear regression model: Mean pupil diameter during fixation ~ β_0 + cognitive score*β_1 + age + sex + population stratification + education + visual acuity + (native language) + residual error. Native language was not included in the models with prosaccade latency and antisaccade error rate as predictors. In bold are those associations with a *p* value below .05.

Abbreviations: 95% CI, 95% confidence interval; AVLT, Auditory Verbal Learning and Memory test; *β*, standardized regression coefficient.

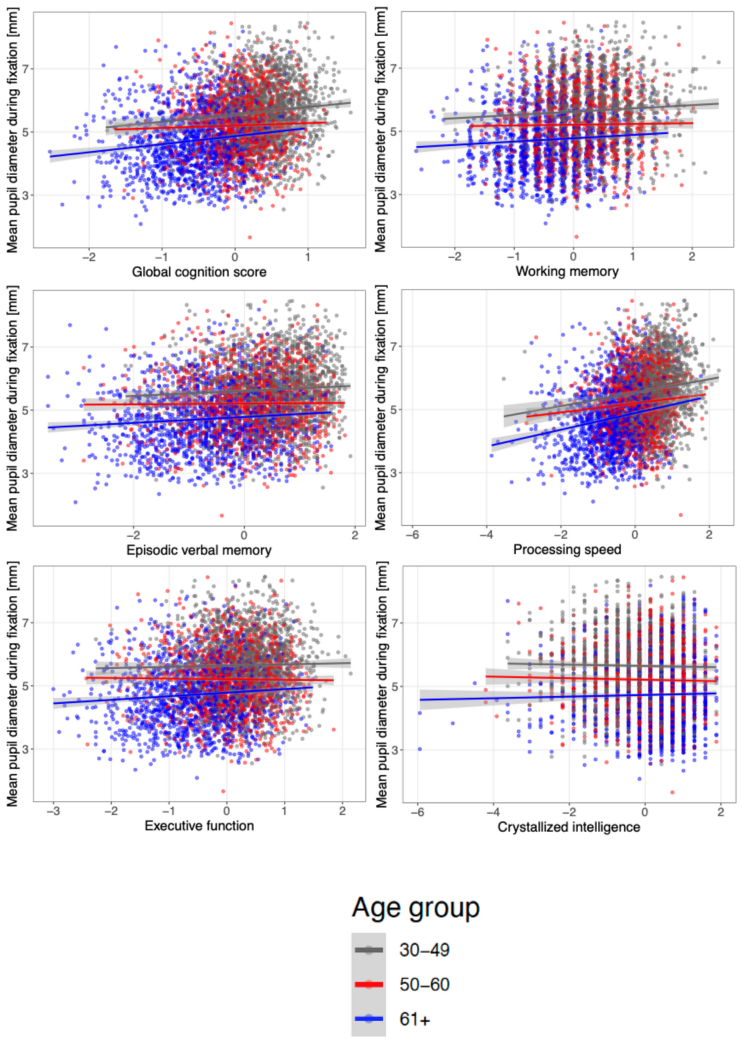

FIGURE 2 Associations between cognitive domain scores and mean pupil diameter during fixation. For each cognitive domain, there is a single scatterplot with the domain score (z-score) on the x-axis and the mean pupil diameter during fixation [in mm] on the y-axis. Each data point represents one participant. Since the data points of some participants overlap, some data points have a darker color than others. Data of three age groups are depicted in different colors, gray for the 30–49 years old, red for the 50–60 years old, and blue for the 61+-years old. For each age group there exists one superimposed linear function for the zero-order correlation between the cognitive domain score and mean pupil diameter during fixation

were due to differences in pupil size measurement leading to limited variation in pupil size as the standard deviation in pupil diameter across all participants was 1.1 mm, as in the study by Tsukahara et al. (2016), and more than double of the standard variation in pupil diameter reported in Unsworth et al. (2020). Tsukahara and Engle (2021) also highlighted that each domain should be measured with several different tasks to avoid that task-specific ability that are unrelated to the domain strongly influence the domain score. Since we measured working memory with four test scores from two tasks and built the global cognition score based on 11 outcomes from eight tasks, we also took this into account. Further, our regression models were also adjusted for interindividual differences in age and genetic background (see discussion in Tsukahara et al., 2021; Unsworth et al., 2021). It should be noted, however, that our participants deviate considerably in age range from the study by Tsukahara et al. (2016).

Age had a strong effect on pupil size, with the average pupil diameter during fixation decreasing by 0.33 mm per 10 years of increase in age. For comparison, the pupil dilates rarely more than 0.5 mm in response to non-luminance-driven changes (e.g., changes in mental effort) (Beatty & Lucero-Wagoner, 2000). Small correlations ($-0.32 \leq r \leq -0.17$) between age and pupil diameter have been reported in previous studies with more restricted age ranges (Tsukahara et al., 2016; Unsworth et al., 2020). Further, our correlation analyses showed that age largely accounted for correlations between cognitive performance and mean pupil diameter during fixation as the zero-order correlations were largely diminished in the age-stratified analysis compared to the analysis across the whole sample.

TABLE 6 Zero-order correlations between cognitive scores and mean pupil diameter during fixation across the sample and in different age strata

Cognitive score	Whole sample	30- to 49-year-olds	50- to 60-year-olds	61+-year-olds
Global cognition score	**0.29**	**0.10**	0.03	**0.14**
Working memory	**0.18**	**0.06**	0.02	**0.07**
Episodic verbal memory	**0.20**	**0.06**	0.01	**0.09**
Processing speed	**0.31**	**0.12**	**0.09**	**0.20**
Executive function	**0.18**	0.02	−0.01	**0.09**
Crystallized intelligence	**−0.11**	−0.02	−0.02	0.03
Digit span forward	**0.09**	**0.06**	0.00	**0.05**
Digit span backward	**0.09**	0.03	0.00	0.04
Corsi forward	**0.18**	0.04	0.03	**0.05**
Corsi backward	**0.14**	0.04	0.01	**0.05**
AVLT- immediate recall	**0.19**	0.05	0.00	**0.09**
AVLT-delayed recall	**0.19**	**0.06**	0.01	**0.08**
TMT-A	**−0.21**	−0.03	−0.02	**−0.07**
Prosaccade latency	**−0.25**	**−0.10**	**−0.09**	**−0.18**
TMT-B	**−0.20**	**−0.06**	−0.01	**−0.07**
Word fluency	**0.04**	−0.03	−0.04	**0.06**
Antisaccade error rate	**−0.12**	**−0.05**	0.00	−0.05

Note: The table displays Pearson correlations between cognitive scores and mean pupil diameter during fixation for the whole sample and for three different age strata. In bold are those zero-order correlations with a *p* value below .05.

However, we found that the age*cognition interaction term was not significant in any model, indicating that the associations between cognitive performance and mean pupil size during fixation were quite consistent across age. Additionally, the standard deviation in mean pupil size was highly comparable in all age groups (Table 1), which indicates that interindividual variation in pupil size remained consistent across age groups. This rules out the possibility that the associations between cognitive performance and baseline pupil diameter vary with age due to an age-related decrease in interindividual variation in pupil size (Tsukahara & Engle, 2021).

Nevertheless, we additionally performed age-stratified analyses. In the age-stratified analysis, better digit span forward performance was significantly associated with larger mean pupil diameter during fixation in the 30- to 49-year-olds (p value = .048). However, the effect size was small (ß = .0471) and the confidence interval was very close to zero (95% CI: 0.0003 to 0.0939). Moreover, we did not observe a consistent pattern across the age strata. Further, the three other working memory test scores and the domain score for working memory were not associated with mean pupil diameter during fixation in this age group. Thus, the association between digit span forward performance and pupil size in this age group should be interpreted with caution.

Investigating associations not only of working memory and global cognitive scores with mean pupil size during fixation but also of additional cognitive measures, we found that higher values in the processing speed domain score were associated with larger mean pupil diameters during fixation across the sample and most strongly in the 50- to 60-year-olds and the 61+-year-olds. In line with this, higher prosaccade latency, which indicates worse performance, was associated with lower mean pupil diameter during fixation across the sample and in all three age strata, with the strength of the effect increasing with increasing age. Since the processing speed domain score only consists of TMT-A and prosaccade latency, the association between processing speed and pupil diameter may have been mainly driven by the oculomotor outcome. Higher pre-saccadic pupil dilations have been found to precede lower saccade latencies (Jainta et al., 2011; Wang et al., 2015). In addition, there is evidence for a link between the pupil control circuit and the superior colliculus (Wang & Munoz, 2015). Thus, pupil size may reflect preparatory neural activity related to saccade generation (Jainta et al., 2011; Wang et al., 2015). This suggests that individuals with larger pupil diameter during fixation may generally have higher levels of preparatory neural activity and, therefore, on average also lower saccade latencies.

Despite known sex differences in the locus coeruleus–norepinephrine system (Bangasser et al., 2016), which has been linked to pupil size (Joshi & Gold, 2020), and sex differences in cognitive performance (Herrera et al., 2019), we did not find sex differences in pupil diameter during fixation. This may be due to the very simple nature of the fixation task as sex differences in arousal are more

TABLE 7 Method comparison with the study by Tsukahara et al. (2016)

Experimental condition	Study by Tsukahara et al. (2016)	Our study	Comparable conditions?
Sample characteristics	• Age range: 17 to 35 years • Analysis based on 337 participants • Data come in part from college students • No information on sex distribution	• Age range: 30 to 95 years with a mean age of 54.7 years (SD: 13.9) • Analysis based on 4560 participants • Data come from a population-based cohort study; women and individuals with high educational level are slightly over-represented	• Age range not comparable but sensitivity analyses conducted (age-stratified analyses; no interaction effects between age and cognition on mean pupil size during fixation) • Over-representation of women in our sample not detrimental as we found no sex effect on mean pupil size during fixation
Measurement of cognitive domains	• Working memory: z-score averaging of the cognitive outcomes from three complex span tasks • Fluid intelligence: z-score averaging of the outcomes from three fluid intelligence tasks (Raven advanced progressive matrices, Letter sets, Number series)	• Working memory: z-score averaging of four test scores derived from two cognitive tasks (digit span and Corsi forward and backward) • Fluid intelligence: z-score averaging of 11 cognitive test scores derived from eight tasks measuring four different domains (processing speed, working memory, episodic verbal memory, executive function)	Yes
Pupil size measurement	• Average pupil size during fixation on a gray fixation point on black background for 30s	• Average pupil size during fixation on a white fixation point on black background that appears at five different positions during a one-minute fixation task	Yes
Experimental light conditions that lead to sufficient variation in pupil size	• SD in pupil diameter across all participants: 1.1 mm	• SD in pupil diameter across all participants: 1.1 mm • SD in pupil diameter in the three separate age groups: 1.0 mm	Yes
Statistical models	• Self-reported ethnicity (three categories: Caucasian, African-American, other) was included in the regression models • All models were controlled for age, nicotine use in the past 10 h, and intake of medication in the past 24 h that may affect attention and memory (all variables were self-reported)	• Six principal components included in the regression models to control for differences in genetic background • All models were controlled for age, sex, best-corrected visual acuity, educational level, and native language (except for the two models with an eye movement predictor)	• In both studies, the models were controlled for the confounders age and race • Some confounders were not present in our study and vice versa

Abbreviations: SD, standard deviation.

pronounced under stress (Bangasser et al., 2011, 2016). In addition, the mean pupil size during fixation is not a pure marker of activity in the locus coeruleus-norepinephrine system but has also been linked to activity in other brain areas such as the superior colliculus (Joshi & Gold, 2020), for which there is no clear evidence of sex differences.

Some strengths and weaknesses of our study should be addressed. Our study tested the associations between cognitive performance and pupil diameter during fixation in a large sample under comparable experimental conditions as in the study by Tsukahara et al. (2016) except for a much wider age range. The wide age range allowed us to extend the findings from the literature to an older and broader age range and to examine whether any associations were age-dependent. However, the wide age range limits a direct comparison of our findings with previous findings (Table 7). We tried to address this limitation by calculating age-stratified analyses. Still, even the participants in our youngest age group (30- to 49-year-olds) were older than the participants in previous studies that mainly included individuals aged 17 to 35 years (Unsworth et al., 2020). We thus cannot entirely exclude on the basis of our data that there may be a relationship between working memory capacity and fluid intelligence with pupil size in adolescents and younger adults. However, given the absence of such a relation across the entire older age range, as well as the absence of any indication that associations between cognitive performance and pupil size strongly depended on age, we consider this unlikely. Another limitation of our study is that there was only one run per cognitive test and that we were not able to validate the internal consistencies of our cognitive measures. Further, the variance explained did not largely differ between the models including different cognitive predictors (Tables 3 and 4). This suggests that processing speed and prosaccade latency explained little variance in mean pupil size during fixation and that most of the variance in pupil outcome could be explained by the covariates included in the regression models (age, sex, best-corrected visual acuity, educational level, race, and native language).

Future research could help to clarify how far performance in non-oculomotor processing speed tasks is associated with pupil diameter during fixation. Further, the assumption that pupil diameter and processing speed are associated because of differences in the level of neural activation could also be investigated by testing whether resting-state network activity partly mediates the association between processing speed and pupil diameter during fixation.

5 | CONCLUSIONS

Working memory and global cognition were not associated with pupil diameter during fixation, which cannot be explained by limited interindividual variation in pupil diameter during fixation or a small number of tests per cognitive domain. Processing speed and prosaccade latency were associated with pupil diameter, suggesting that differences in pupil diameter may inform about differences in levels of preparatory neural activity for saccades. These associations were consistent across a large age range. Pupil diameter declined linearly with age but did not differ between men and women.

AUTHOR CONTRIBUTIONS

Annabell Coors: Conceptualization; data curation; formal analysis; investigation; methodology; validation; visualization; writing – original draft; writing – review and editing. **Monique M. B. Breteler:** Conceptualization; funding acquisition; methodology; project administration; resources; supervision; validation; writing – review and editing. **Ulrich Ettinger:** Conceptualization; methodology; supervision; writing – review and editing.

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CONFLICT OF INTEREST

None.

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7. Discussion and Conclusion

In this chapter, I will first discuss the implications of my findings on the correlates and determinants of eye movement performance in terms of whether it is worthwhile to include oculomotor tasks in population-based studies on aging and age-related diseases. Next, I will discuss the general limitations of this thesis and give directions for future research.

7.1 Added value of eye movements tasks in population-based studies

The inclusion of an eye movement battery in the study protocol of a population-based study on aging and age-related diseases has several advantages. First of all, eye movement performance can be used as biomarker for normal age-related changes in cognitive performance, such as processing speed (as measured by prosaccade latency) (Noiret et al., 2017) or executive function (as measured by antisaccade error rate) (Mirsky et al., 2011). The large sample size and the wide age range of participants of the Rhineland Study, have allowed me to show that aging differentially affects oculomotor measures (Chapter 4). This is relevant for distinguishing normal age-related changes from pathological age-related changes.

Second, eye movement performance may represent a sensitive biomarker for pathophysiological changes, as illustrated by the projects on schizophrenia (Chapter 4) and Alzheimer's disease (Chapter 5). Regarding psychiatric disorders, it has long been known that individuals with schizophrenia show oculomotor impairments (Diefendorf & Dodge, 1908). However, only recent advancements in genetics, particularly the conduction of a large genome-wide association study of schizophrenia (Pardiñas et al., 2018) and the introduction of PRS (International Schizophrenia Consortium, 2009), have allowed me in combination with our large oculomotor dataset to provide evidence for antisaccades being an endophenotype of schizophrenia (Chapter 4). Surprisingly, I found no evidence congruent with the endophenotype status of SPEMs for schizophrenia, which is discussed in more detail in Chapter 4. I could also show that the antisaccade correction rate may be sensitive in detecting genetic liability for Alzheimer's disease, however, this still requires further evaluation (Chapter 5).

Based on the findings from the first three studies, I conclude that several oculomotor measures are potential biomarkers for identifying individuals at risk for brain-related

disease. The most suitable biomarkers may be those eye movement measures, in which performance remained stable over the adult lifespan but which are known to be affected in diseases. For example, spatial accuracy in the antisaccade task was not associated with age (chapter 3) but was associated with genetic risk for schizophrenia (chapter 4). Spatial accuracy in the antisaccade task depends on non-standard sensorimotor transformations in posterior parietal cortex (Herweg et al., 2014) and frontal eye fields (Moon et al., 2007), brain regions which are also involved in the aetiology of schizophrenia (Keedy et al., 2006). However, oculomotor markers have to be selected disease-specific, taking into account the neuroanatomical correlates of the oculomotor measures. For example, patients with Parkinson's disease often have lesions in the cerebellum and therefore differ from controls in prosaccade accuracy but are not impaired in antisaccade accuracy (Mosimann et al., 2005; Optican, 2005; Wu & Hallett, 2013).

Third, there are some general advantages of eye movements tasks for cognitive testing. Eye movements have the advantage vis-à-vis traditional cognitive tasks that they allow for a culture-free and language-independent assessment of cognitive performance (Noiret et al., 2017). This is particularly relevant for population-based cohort studies, as these are likely to include participants with different cultural backgrounds and native languages, as well as for cross-cultural studies. Further, participants' technical abilities may influence performance in traditional cognitive tests when they are computerized or touchpad-based, but not oculomotor task performance. Moreover, sex differences have been reported in traditional cognitive tasks, especially language-dependent and visual-spatial memory tasks (Lewin et al., 2001; Voyer et al., 2017). I found, however, no sex differences in eye movement performance except for small sex differences in smooth pursuit velocity gain and blink rate during fixation (Chapter 3). Thus, another general advantage of eye-tracking tasks for cognitive assessment in population-based studies is that for most oculomotor measures the results of men and women can be considered together. This potentially increases statistical power. Additionally, an eye-tracking examination like ours takes on average 11 minutes per participant and is therefore a very time-efficient way of measuring performance in different cognitive domains such as processing speed (Noiret et al., 2017) or executive function (Mirsky et al., 2011).

Fourth, oculomotor performance also provides additional information of interest for population-based cohort studies. The SPEM task provides for example information on sensorimotor integration skills and movement prediction abilities (Sprenger et al., 2011), whereas peak saccadic velocity is related to arousal/ activity in the sympathetic nervous system (Di Stasi et al., 2013). The correction rate of antisaccade errors is an informative measure of error monitoring (Hutton & Ettinger, 2006), an ability that may be impaired in individuals at high genetic risk for Alzheimer's disease (Chapter 5). Further, a mixture of traditional cognitive tasks and oculomotor tasks in the cognitive test battery has the advantage that some cognitive domains can be measured with very different tasks to avoid performance being strongly influenced by task-specific abilities (Ackerman & Hambrick, 2020). Additionally, an oculomotor test battery provides investigators with pupillometry data. I could show that interindividual differences in processing speed, particularly in prosaccade latency, are reflected in pupil diameter during fixation (Chapter 6). Pupillometry data are a rich source of information about cognitive processes and I discuss further potential uses of these and other eye movement measures in section 7.3.

Although oculomotor tasks offer many advantages for measuring normal and pathophysiological changes in population-based cohort studies, I also experienced that data acquisition is particularly complicated in individuals with low visual acuity. The reason is that high dioptric glasses, eye diseases or artificial lenses often lead to technical failures during acquisition. We experienced that when technical problems arise due to varifocals, providing reading glasses with an appropriate dioptric number can solve the problem in many instances.

7.2 Limitations of the thesis

All projects were based on cross-sectional data, which is mainly a major limitation for the first project, in which I quantified aging effects on oculomotor performance (Chapter 3). In the other projects, longitudinal data could have been beneficial to answer additional questions such as whether genetic risk for Alzheimer's disease is related to a higher rate of change in cognitive performance. However, the Rhineland Study is planned longitudinally, so that these and other questions that could previously only be investigated in cross-sectional or small longitudinal samples can be studied in the future.

Another general limitation is that the oculomotor tasks included had only a limited number of trials. Thus, some findings such as aging effects on the probability of correcting antisaccade errors (Chapter 3) require replication in a study with a larger number of trials. However, given that the oculomotor tasks represent only one part of the cognitive battery in the Rhineland Study, an increased number of trials would probably not outweigh the disadvantages associated with a longer cognitive test duration (e.g. cognitive exhaustion or decreased motivation).

Moreover, there are three limitations related to our sample. First, the educational level of our sample was high (see Table 1 in all projects). Second, I observed a healthy volunteer bias as for example only 0.1% of the sample in Chapter 4 reported a diagnosis of schizophrenia compared to a prevalence rate of about 1% in the general population (J. McGrath et al., 2008). These two aspects potentially limit the generalizability of some results to the general population, and could have led to an over- or underestimation of the effects. For example, in our sample, age-related effects on oculomotor performance (Chapter 3) may have been underestimated, as high education represents a protective factor against cognitive decline (Stern, 2009). However, if variability in oculomotor performance was lower in our sample, this may have led to increased statistical power in the genetic projects (Chapters 4 to 5) to detect small associations between genetic risk scores and oculomotor performance (Cohen, 1988, p. 8). Third, the genetic projects were restricted to a Caucasian population and the findings are, therefore, not necessarily transferable to non-Caucasian populations (Wand et al., 2021).

7.3 Future research

With the projects on schizophrenia and Alzheimer's disease I selected two out of many diseases in which eye movement performance is altered (Anderson & MacAskill, 2013). Future population-based studies that include oculomotor tests could apply the idea of associating PRS with oculomotor measures and classical cognitive tests to other diseases. Cognitive measures that turn out to be sensitive to genetic liability for certain diseases may be helpful for differential diagnoses, e.g., in case of different dementia types (Braaten et al., 2006). Further, they may help to identify more homogeneous subgroups of diseased individuals, which could benefit clinical research on disease mechanisms (Braff et al., 2007). However, a prerequisite for creating PRSs is that there are large

disease-specific genome-wide association studies. A more general approach would be to investigate the potential of oculomotor measures as markers of neurodegeneration. Here, one possibility would be to associate oculomotor measures with neurofilament light concentration in blood, which represents a non-specific marker of neuro-axonal damage (Khalil et al., 2018). Next, it would be of interest to understand why oculomotor measures capture genetic liability for certain diseases. One approach would be to take advantage of the fact that eye movements have moderate to high heritability (Katsanis et al., 2000; Malone & Iacono, 2002) and therefore conduct a genome-wide association study and an epigenome-wide association study of eye movement measures with subsequent pathway analyses. Genome-wide association studies on eye movements are still largely lacking and have so far been limited to clinical samples (Kikuchi et al., 2018; Lencer et al., 2017). No epigenome-wide association study of eye movements has yet been conducted, although it seems promising, as not only genes but also environmental factors and gene-environment interactions are likely to account for oculomotor impairments in diseases (Chakravarti & Little, 2003). Another example that illustrates the influence of environmental factors is cultural differences in eye movements (Kelly et al., 2011).

Since motivation is known to influence cognitive performance, particularly in older individuals (Germain & Hess, 2007), another direction for future research would be to investigate whether oculomotor measures can be used to determine how motivated participants were during the cognitive assessment. It would be beneficial to have an objective marker of motivation, as self-reports of motivation may be susceptible to response bias (R. E. McGrath et al., 2010). Some participants may report that they had low motivation during the examination to excuse feared poor cognitive performance (negative impression management), while others may report high motivation to reply in a socially desirable manner (positive impression management) (R. E. McGrath et al., 2010). It has been suggested that the association between motivation and cognitive performance is partly mediated by striatal dopamine levels (Westbrook & Braver, 2016). Since the blink rate during fixation is a marker of striatal dopamine, with a higher blink rate reflecting higher dopamine levels (Jongkees & Colzato, 2016), it may be used as a proxy for motivational levels. Further, reward is known to affect some eye movement measures (Brielmann & Spering, 2015; Hayhoe & Ballard, 2005), suggesting that there may be other potential oculomotor markers for motivation. To evaluate how suitable those oculomotor

markers are in capturing motivational levels, it could be tested if they can explain a considerable amount of variance in cognitive performance. Also, correlations between these oculomotor measures and self-reported motivation (e.g., by asking the participants to indicate their level of motivation during the cognitive tests on a five-point Likert scale) and the personality dimension conscientiousness would be expected (Hart et al., 2007). Associations between personality and eye movement measures have been investigated in healthy subjects in only few studies to date (Nguyen et al., 2008; Taylor, 2016) and it would also be of general interest to analyse in how far oculomotor performance reflects differences in personality.

Additionally, it would be of interest to record the pupil size during the performance of classical cognitive tasks to get information on participants' cognitive load and mental effort (Joshi & Gold, 2020; van der Wel & van Steenbergen, 2018). The pupil dilates with increasing cognitive demand until the task demands exceed the participant's capacity (van der Wel & van Steenbergen, 2018). Pupil diameter values may, therefore, provide additional information about task processing, e.g., in memory tasks, pupil diameter may provide information about whether difficulties were already encountered during memory encoding or only during memory retrieval (Miller & Unsworth, 2020). Further, mental effort-driven pupil dilations have been linked to activity in the locus coeruleus, which is the main source of norepinephrine in the central nervous system (Joshi & Gold, 2020). Thus, the role of pupil dilation as a marker of norepinephrine levels in humans should be further explored across a wide age range, e.g., by linking it to neuronal density in the locus coeruleus (Murphy et al., 2014), which can be measured by neuromelanin-sensitive magnetic resonance imaging (Clewett et al., 2016).

7.4 General conclusion

This doctoral thesis shows that the recording of eye movements provides a variety of valuable markers for normal age-related and pathophysiological changes in brain activity beyond information obtained from classical cognitive tasks. Therefore, for population-based studies on aging and age-related diseases, I would recommend supplementing a traditional cognitive test battery with an eye movement battery consisting of fixation, smooth pursuit, prosaccade and antisaccade tasks. Since oculomotor measures are largely culture-free and language-independent, this will allow the study of different

cognitive abilities, including sensorimotor integration skills and movement prediction ability, even in culturally diverse samples spanning an age spectrum from infancy to old age.

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Supplement 1 – Eye Movement Measures in the Rhineland Study

Table 1. Overview of Eye Movement Outcomes

Outcome	Unit	Interpretation	Performance
Fixation Task			
Spatial error (RMSE)	°	Spatial error of gaze position during fixation	The lower the spatial error, the more accurate is a fixation, the better the performance.
Saccade frequency	N/s	Number of saccades during the fixation task	The less saccades, the less interruptions of periods of fixations, the better the performance.
Blink rate	N/s	Number of blinks during the fixation task	Performance cannot be classified as good or bad based on the blink rate.
Smooth Pursuit Task			
Velocity gain	%	Ratio between eye and target velocity	A value of 100% indicates perfect eye-target velocity match, whilst values below or above 100% indicate that the eye movements are slower or faster than the target, respectively.
Saccade frequency	N/s	Number of saccades per second in the smooth pursuit task	A lower saccade frequency indicates better performance.
Prosaccade Task			
Latency	ms	Time from target appearance to saccade initiation	The lower the latency, the faster the saccade initiation, the better the performance.
Spatial error	%	Mean deviation of the eye position from the target position	The lower the spatial error the more spatially accurate is a saccade, the better the performance.

Table 1 continued.

Amplitude gain	%	Average landing position of the eye relative to the target	A value of 100% indicates that the saccade perfectly landed on the target, whilst values below or above 100% indicate that the saccade amplitude was too low or high, respectively.
Peak velocity	°/s	Average of the peak saccade velocities from all valid trials with a correct initial saccade	Higher peak velocity indicates better performance.
Amplitude-adjusted peak velocity	/	Peak velocity divided by amplitude gain (reason: strong correlation between saccade amplitude and peak velocity)	Higher peak velocity indicates better performance.
Antisaccade Task			
Latency	ms	Time from target appearance to saccade initiation	The lower the latency, the faster the saccade initiation, the better the performance.
Spatial error	%	Mean deviation of the eye position from the target position	The lower the spatial error the more spatially accurate is a saccade, the better the performance.
Amplitude gain	%	Average landing position of the eye relative to the target	A value of 100% indicates that the saccade perfectly landed on the target, whilst values below or above 100% indicate that the saccade amplitude was too low or high, respectively.

Table 1 continued.

Peak velocity	°/s	Average of the peak saccade velocities from all valid trials with a correct initial saccade	Higher peak velocity indicates better performance.
Amplitude-adjusted peak velocity		Peak velocity divided by amplitude gain (reason: strong correlation between saccade amplitude and peak velocity)	Higher peak velocity indicates better performance.
Costs	ms	Antisaccade latency minus prosaccade latency	The lower the costs, the less additional time is needed to initiate an antisaccade compared to a prosaccade, which indicates better performance.
Error rate	%	Percentage of trials where the first saccade is erroneously made towards the target	The lower the error rate, the less saccades are first made towards the target, the better the performance.
Correction rate	%	Percentage of corrected antisaccade errors	The higher the correction rate the more antisaccade errors are corrected, the better the performance.