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Published in:
Journal of Vestibular Research

2008

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Running title:
Sleep deprivation and oculomotor function

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Abstract

Sleep restrictions and sleep deprivation have become common in modern society, as many people report daily sleep below the recommended 8 hours per night. This study aimed to examine the effects of sleep deprivation on oculomotor performance by recording smooth pursuit and saccadic eye movements after 24 and 36 hours of sleep deprivation. Another objective was to determine whether detected changes in oculomotor performance followed fluctuations according to a circadian rhythm and/or subjective Visuo-Analogue sleepiness Scale scores. Oculomotor responses were recorded from 18 subjects using electronystagmography, and comprised measurements of accuracy (i.e., the percentage of time the eye movement velocity was within the target velocity boundaries), velocity and latency. Continuous EEG recordings were used to validate that subjects had remained awake throughout the 36-hour period.

Our findings showed that sleep deprivation deteriorated smooth pursuit gain, smooth pursuit accuracy and saccade velocity. Additionally, the ratio between saccade velocity and saccade amplitude was significantly decreased by sleep deprivation. However, as the length of sleep deprivation increased, only smooth pursuit gain deteriorated further, whereas there were signs of improvement in smooth pursuit accuracy measurements. The latter observation suggests that smooth pursuit accuracy might be affected by the circadian rhythm of alertness. Surprisingly, high subjective scores of sleepiness correlated in most cases with better saccade performance, especially after 36 hours of sleep deprivation, suggesting that awareness of sleepiness might make subjects perform better during saccade assessments. To conclude, oculomotor function clearly decreased after sleep deprivation, but the performance deteriorations were complex and not necessarily correlated with subjectively felt sleepiness.

Key Words: Oculomotor; Smooth pursuit; Saccadic; Sleep deprivation.

1. Introduction

Sleep restrictions and sleep deprivation have become common in modern society, as many people report daily sleep below the recommended 8 hours per night [15]. As civilian and industrial endeavors become increasingly continuous with 24-hour operations, the potential for sleepiness-related accidents increases [3]. Sleep deprivation produces many of the effects associated with being drunk, such as a lack of coordination, judgment and reaction time [23, 24]. For example, people who drove after being awake for 17–19 hours performed worse than those with a blood alcohol level of 0.05 percent [23], which is the legal alcohol limit in many European countries. Hence, sleepiness is a factor that should not be ignored and knowledge about the effects of sleep deprivation is important for economical as well as for health and public safety reasons. Although levels of sleepiness and fatigue can be subjectively assessed, such evaluations may not reflect the objective physiological status of the tired person, mainly because subjective scores can be biased by motivation, personal factors, experience, training etc [2]. Therefore, there is a need to find a practical, noninvasive objective method to measure the effects of sleepiness, especially when reaching critical levels involving higher risks for accidents.

Investigations of sleep deprivation began as early as 1896 by Patrick and Gilbert, and their results are still valid today. Their investigations concluded that sleep deprivation results in the general deterioration of attention, alertness, reaction time and cognitive tasks [17]. More recent investigations have shown that sleep deprivation also deteriorates psychomotor responses such as electroencephalogram (EEG) and oculomotor function [7]. Research also suggests that deteriorated ocular measurements coincide with decreases of alertness [19] and attention [11, 12]. These two terms are used interchangeably, yet they have physiologically different functions. Alertness can be defined as a state of arousal in which the responses to all afferent stimuli are raised [22] and may fluctuate throughout the normal 24 hour day according to a cycle, known as the circadian rhythm [21]. Attention can be defined as selection of a particular afferent stimulus amongst other afferent stimuli, to which an appropriate response is produced [16].

Investigations of oculomotor performance during sleep deprivation have produced contrasting results. Porcu et al. (1998) found deterioration of smooth pursuit and saccadic accuracy at the highest point of fatigue after 24 hours of SDep in a study of circadian effects on oculomotor function [18]. Bocca et al. also found decreased saccade accuracy but no significant effects on peak saccade velocity after 24 hours of sleep deprivation [5]. Zils et al. found significantly decreased peak saccade velocity and decreased saccade accuracy but only for voluntary saccades (pro-saccades) [25]. Whereas, Crevits et al. found no significant effects after 20 hours of sleep deprivation on reflexive saccades, pro-saccades and anti-saccades [6]. In tests with 40 hours of sleep deprivation, De Gennaro et al. reported decreased smooth pursuit gain and decreased saccade velocity but no effects on saccade accuracy, suggesting that velocity measures were more affected after 40 hours of sleep deprivation than accuracy measures [7].

Based on prior research showing that both the saccadic and smooth pursuit eye movements might be affected by sleep deprivation, the aim of this study was to assess the effects of sleep deprivation on both saccadic and smooth pursuit eye movements after 24 (24SDep) and 36 (36SDep) hours of sleep deprivation. These assessments may provide information about whether the saccadic and smooth pursuit performance changes found under sleep deprivation are similar. Another objective was to study whether saccadic and smooth pursuit eye movement functions progressively deteriorate as the duration of sleep deprivation increases or whether there are indications of circadian rhythm effects. A third objective was to determine whether the oculomotor performance were associated with subjective scores of sleepiness.

2. Methods and Materials

2.1. Subjects

Oculomotor tests were performed on eighteen (ten male and eight female) healthy subjects (mean age 23.8 years, range 17-38 years) with no history of dizziness or central nervous system disease. The subjects were instructed not to consume any alcohol, sleepiness-inducing or revitalizing products, such as caffeine, 24 hours before and during testing. At the time of experimentation no subject was taking any form of medication and signed consent from all subjects was obtained before testing. The experiments were performed in accordance to the Helsinki declaration of 1975 and approved by the local ethical committee.

2.2. Equipment

The visual target used in the oculomotor tests, a circular red dot with a diameter of 3mm, was projected on a dark canvas screen using a laser contained within a moveable over-head console, allowing optimal individual vertical positioning. Eye movements were recorded by electronystagmography (ENG) using a bipolar recording technique. Two Ag/AgCl ENG-electrodes were placed about 1 cm from the outer canthi of the eyes measuring horizontal eye movements. Two other electrodes were fixed above and below the left eye to measure vertical eye movements and blinking, and finally one ground electrode was attached on the mid-forehead. The eye movement data were initially filtered by an analogue 340 Hz low-pass filter, digitized by a 12-bit AD converter (PCI 6024E, National Instruments Inc.) and sampled on-line at 200 Hz. Inappropriate head movements were prevented by a custom-made headrest.

Prior to each test, a calibration procedure was performed to ensure that electrical ENG signals correctly corresponded to right and left eye movements within the range of 10-30 degree amplitude. The eye movements were calibrated in the horizontal direction in a separate saccade calibration program with amplitudes of 10, 20 and 30º to the right and left. In the vertical direction, a calibration amplitude was set with reference to the effects of eye blinking. A customized computer program Vestcon™ controlled the visual target projection, calibration and sampled the ENG data. Once collected, the computer program also rejected eye movements considered as artifacts and automatically analyzed the ENG data for each test.

2.3. Procedure

On day 1, subjects were asked to wake up at 7am or 8am (depending on the organized time of recordings) to begin their sleep deprivation tests and go about their daily routines as normal. The subjects came to the laboratory at 7pm or 8pm on day 1 (12 hours into their deprived state) to be attached with a portable EEG recoding device (Embletta™). The EEG device was used to record whether any of the subjects had fallen asleep prior and between the test sessions. The EEG equipment comprised 3 electrodes; an active electrode positioned on the upper temple; a reference electrode positioned on the upper mastoid bone on the opposite side to the active electrode; and a ground electrode positioned on the mid-forehead. Subjects returned on day 2 at 7am or 8am, 24 hours into sleep deprivation, then again that evening at 7pm or 8pm, 36 hours into sleep deprivation for oculomotor assessment. The EEG equipment was removed prior to oculomotor testing to avoid any interference with the ENG equipment and the EEG data was stored for off-line analysis before re-attachment. Prior to oculomotor testing, the subjects were also instructed to provide a subjective score using Visuo-Analogue sleepiness Scale (VAS) of alertness ranging from “completely alert” to “exhausted to near sleep”. The subjects analogue scores were converted into numbers ranging from 1 to 10, where 1 = “completely alert” and 10 = “exhausted to near sleep”. The subjective VAS score was collected before the oculomotor measurements in order to avoid experiences of poor performance during the oculomotor measurements from influencing the VAS score given.

The control test was performed on all subjects after a whole night of sleep, either one week before or one week after the sleep deprivation tests according to a randomized schedule.

2.3.1. Smooth pursuit eye movement recordings

Each subject was tested in a completely dark room and seated in an inclined chair directly in front of a large black canvas screen. Subjects were then instructed to fixate on a red target projected onto the screen and follow its movement as accurately as possible without turning their head or moving their eyes before the target had moved. The smooth pursuit target moved horizontally with a constant velocity from side to side, with ranges ±30º of the visual field, i.e. through distances of 60º to 30º to the right (+) and 30º to the left (-). Testing started after a 3 second period where the target was stationary straight forward (0º). Thereafter the target moved directly +30º to the right and this position was maintained for 1 second. Then, the visual target moved according to the following sequence of velocities: 10, 20, 30, 40, 40, 30, 20, 10 º/s, where the smooth pursuit eye movements were tested 4 times at each velocity step, two times for smooth pursuit movements directed from right to left, and two times for movements directed from left to right. When the visual target reached the maximum amplitude, i.e., ±30º either to the right or left, the position was maintained for 1 second before the next smooth pursuit movement commenced in the opposite direction. The total test time for the smooth pursuit test was 135 seconds.

2.3.2. Saccadic eye movement recordings

The conditions and calibration before testing were identical to smooth pursuit recordings as were the test instructions. In the pro-saccade assessment, testing started after a 5 second period where the target was stationary straight forward (0º). Thereafter, the visual target moved horizontally according to the following sequence of amplitudes: ±10, ±20, ±30º, yielding saccades of a total range of respectively 20, 40 and 60º amplitude. The visual target appeared for 1.5 second at each position. The saccades were tested 10 times at each amplitude, five times for saccades from right to left, and five times for saccades from left to right. Between each sequence step, the visual target was projected straight forward for 5 seconds. The total test time for the saccade test was 66 seconds.

2.4.1. Smooth pursuit data analysis

A customized computer program (Vestcon™) performed an automatic analysis of the data once the test had been completed and produced values of latency, average **smooth pursuit gain** and **smooth pursuit accuracy** for the smooth pursuit eye movements. Prior to the analysis of the smooth pursuit data, the recorded ENG data was low-pass filtered at a cut-off frequency of 15 Hz. Thereafter, the data was deemed to obtain the velocity of the eye movements for each target movement. The recorded **smooth pursuit latency** was measured as the time taken from the start of target movement until the velocity of recoded eye movement exceeded 5 º/s. As illustrated in figure 1, the most common response to the start of the smooth pursuit target movement was an initial short delay followed by a brief period of faster than target smooth pursuit to catch up with the visual target, but sometimes catch up saccades also occurred. However, the analysis method used is designed to handle both these kinds of responses. The calculated latency time was rejected if the latency was below 0.1 seconds or above 0.6 seconds.

To calculate the average **smooth pursuit gain**, the analysis procedures identified and removed time periods where the recorded eye movements were presumed to be saccades. This was achieved by removing all data where the eye movement velocity exceeded the velocity of the visual target by 40 º/s. Following this filtration, the average eye movement velocity for each remaining time periods were calculated using linear regression. If the calculated average eye movement velocity within a time period was below 5 º/s, the time period was deemed to contain no smooth pursuit eye movements and was rejected. The smooth pursuit gain value was calculated by dividing the average eye movement velocity by the target velocity value. The **smooth pursuit accuracy** for each target movement was calculated as the percentage of time the smooth pursuit eye movement velocity was within the target velocity boundaries of less than 20% absolute error from the visual target velocity.

For all parameters, the final values presented are the average values from all smooth pursuit movements assessed during the same target velocity in both movement directions.

2.4.2. Saccadic data analysis

Vestcon™ was also used to automatically analyze the data and produce values of latency, saccade velocity and saccade accuracy. Prior to the analysis of the saccadic data, the recorded ENG data was low-pass filtered at a cut-off frequency of 70 Hz. Thereafter, the data was deemed to obtain the velocity of the eye movements during each individual target movement.

The recorded **saccade latency** was measured as the time taken from the start of target movement for the recoded eye movement velocity to exceed 80 º/s. The calculated latency was rejected if the latency was below 0.1 seconds or above 0.6 seconds. The saccade was also rejected if the duration of the saccade was shorter than 25 ms, as it was regarded a measurement artifact. **Saccade velocity** was calculated by identifying and removing time periods where the recorded eye movements were slower than 80 º/s and where saccades were shorter than 25ms. Thereafter, in the remaining time periods where saccades were found, the 4 ms period (e.g., 5 samples) where the saccade velocity was highest during the saccade was determined and the average saccade velocity during this 4-ms period was calculated. If the subject made several saccades to achieve the target movement, the saccade with the highest saccade velocity and with the largest movement distance (in degrees, usually defined as saccade amplitude) was selected. The **saccade accuracy** for each target movement was calculated as a quotient value in percent between the movement distance of the largest eye movement saccade (if several saccades were made), divided by the movement distance of the visual target reference.

To determine whether the was any saccade ratio decrease between saccade velocity and saccade amplitude, individual quotients between saccade velocity divided by saccade amplitude were calculated using data from all saccade target amplitudes and statistically analyzed.

For all parameters, the final values presented are the average values from all saccadic eye movements assessed during the same movement amplitude in both movement directions.

2.4.3. EEG data Analysis

The EEG data was analyzed for evidence of alertness using the alpha wave activity. Scoring of wakefulness/sleep was carried out according to Rechtschaffen & Kales (Rechtschaffen and Kales 1968). Uninterrupted sleep stage II for more than 2 minutes was considered sleep. All recordings were investigated manually for large decreases in alertness suggesting stage 1 and stage 2 sleeps by an expert (S Berg).

2.5. Statistical Analysis

The Wilcoxon matched-pairs signed-rank test (Exact sig. 2-tailed) [1] was used for the statistical comparison between tests. Oculomotor performance was based on the analysis of rightward and leftward smooth pursuit and saccadic eye movements.

A statistical evaluation of sleep deprivation and the target movement velocity (smooth pursuit eye movement) or target movement amplitude (saccadic eye movements) and the interaction effects was performed with a GLM univariate ANOVA (General Linear Model univariate Analysis of Variance) test [1]. The GLM model accuracy was evaluated by testing the model residual for normal distribution. Normality of the distribution was tested with the Shapiro-Wilk test.

Correlation analysis was performed between subjective VAS scores of sleepiness and recorded eye movement values using Spearman correlation test [1].

Non-parametric statistics were used in the statistical evaluation since all obtained analysis values were not normally distributed before or after logarithmic transformation. The statistical analysis was carried out with Bonferroni correction for multiple comparisons and in the analysis, p-values <0.01 were considered statistical significant [1]. However, we present the p-values <0.05 in the figures (in red) and tables for reasons of consistency. The statistical analysis was performed with SPSS version 14.0 (SPSS Inc.) and mathematically analyzed using LabView version 6.1 (National Instruments Inc.).

3. Results

The EEG assessment of alpha wave activity confirmed that no subject had fallen asleep according to Rechtschaffen & Kales (Rechtschaffen and Kales 1968) criteria for effective sleep during the entire sleep deprivation period of 36 hours. The VAS scores increased from 5.2 after 24SDep to a level of 6.8 after 36SDep (p<0.001).

3.1. Smooth Pursuit Eye Movements

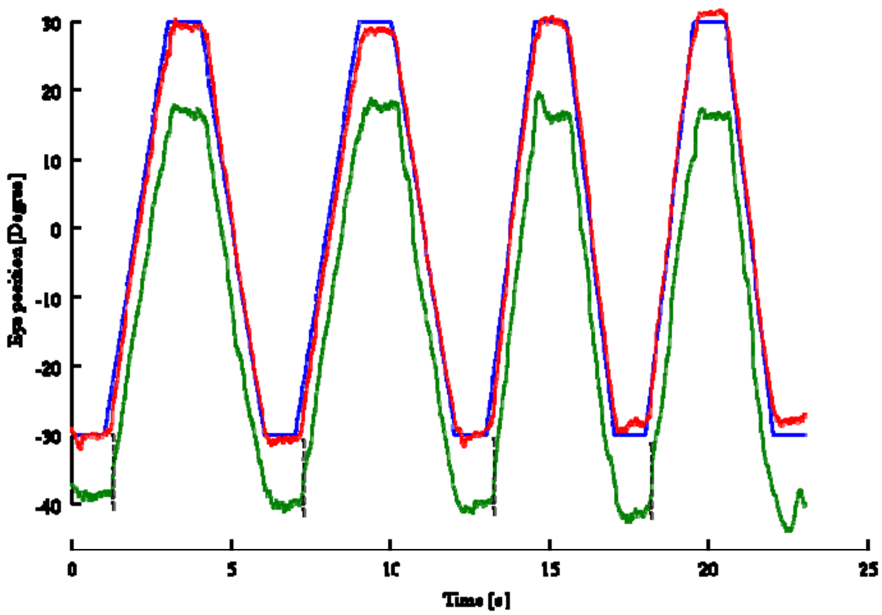

Figure 1: Recordings of a subject performing the 40 degree/s smooth pursuits in the smooth pursuit test sequence while rested (red) and after 24 hours of sleep deprivation (green). The smooth pursuit eye target is presented in blue, and as illustrated in the figure, the subjects performed the smooth pursuit between 30 degrees to the left (denoted –30 on the axis) and 30 degrees to the right (denoted +30 on the axis). The recorded smooth pursuit latency was measured as the time taken from the start of target movement until the velocity of recoded eye movement exceeded 5 °/s. For illustration, smooth pursuit starts based on this criterion are marked in the eye position figure above by vertical dashed lines for the left-to-right smooth pursuits at 24SDep. Note the increased difficulty to maintain a steady and accurate smooth pursuit while sleep deprived. Additionally, note that some recordings have been moved arbitrarily somewhat in the vertical direction for presentational reasons.

3.1.1. Smooth Pursuit Gain

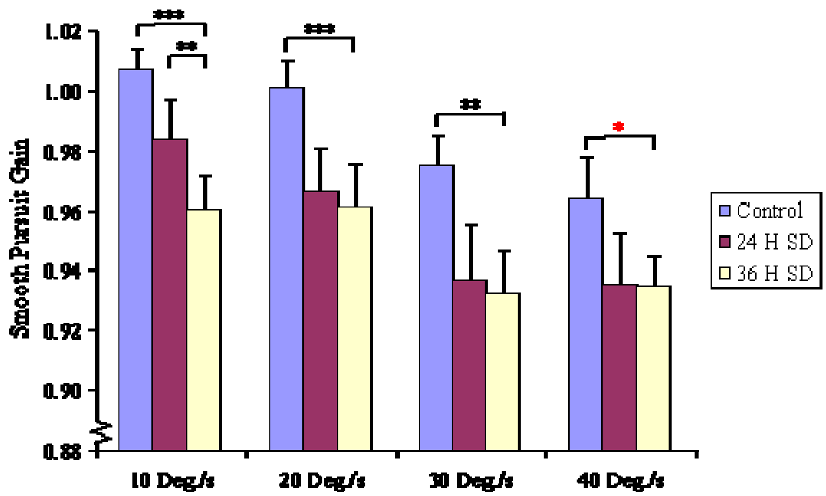

Figure 2: Gain values between average smooth pursuit eye movement velocity and target movement velocity at increasing target velocities (mean and standard error of mean (SEM)) during different stages of sleep deprivation (24 H SD and 36 H SD). A value of 1.00 represents perfect average smooth pursuit gain and a value below 1 represent that the average smooth pursuit velocity was below the target velocity (*denotes P<0.05, ** denotes P<0.01 and *** denotes P<0.001). Results show greater differences between the measurements at the slower velocities.

Sleep deprivation decreased average smooth pursuit gain with increasing target velocity, see figure 1, figure 2 and table 1. There was no statistical difference between the Control test and 24SDep at any velocity, whereas statistical difference was found at most target velocities between the Control test and 36SDep; for 10 and 20 °/s target velocities at p<0.001; for 30 °/s target velocity at p<0.01. The smooth pursuit gain deceased by about 4% on average for all target velocities after sleep deprivation. Statistical values also suggested that smooth pursuit gain decreased significantly between 24SDep and 36SDep by about 2.4% for 10 °/s smooth pursuits (p<0.01).

3.1.2. Smooth Pursuit Accuracy

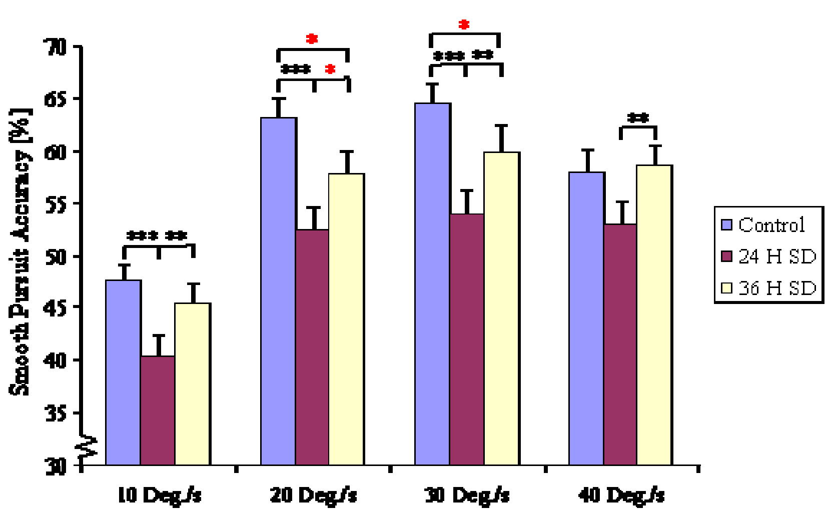

Figure 3: Average smooth pursuit accuracy values, representing the percentage of time the smooth pursuit velocity were within the target velocity boundaries of less than 20% absolute velocity error compared with the

visual target velocity, during different stages of sleep deprivation (mean and standard error of mean (SEM)). Of note, since the acceptable velocity error is given in percent the acceptable absolute velocity marginal in degrees/s is smaller during slower smooth pursuit tests than in faster smooth pursuit tests. A value of 100 represents that the smooth pursuit eye movement velocity always were within the boundaries of less than 20% velocity error.

The smooth pursuit accuracy levels peeked between target velocities of 20 and 30 °/s as shown by figure 3. The greatest smooth pursuit accuracy was found in the Control test, and the least after 24SDep. The smooth pursuit accuracy was about 16% higher in the Control test compared with 24SDep for 10, 20 and 30 °/s target velocities ($p<0.001$). Moreover, the smooth pursuit accuracy recovered between 24SDep and 36SDep. The smooth pursuit accuracy was on average about 11% higher at most target velocities after 36SDep compared with 24SDep; for 10, 30 and 40 °/s target velocities at statistical level $p<0.01$, and for 20 °/s target velocity at statistical level $p<0.05$. Values also suggested that the smooth pursuit accuracy was lower after 36SDep compared with the Control test at 20 and 30 °/s target velocities, though this was only verified at statistical level $p<0.05$.

3.2. Saccadic Eye Movements

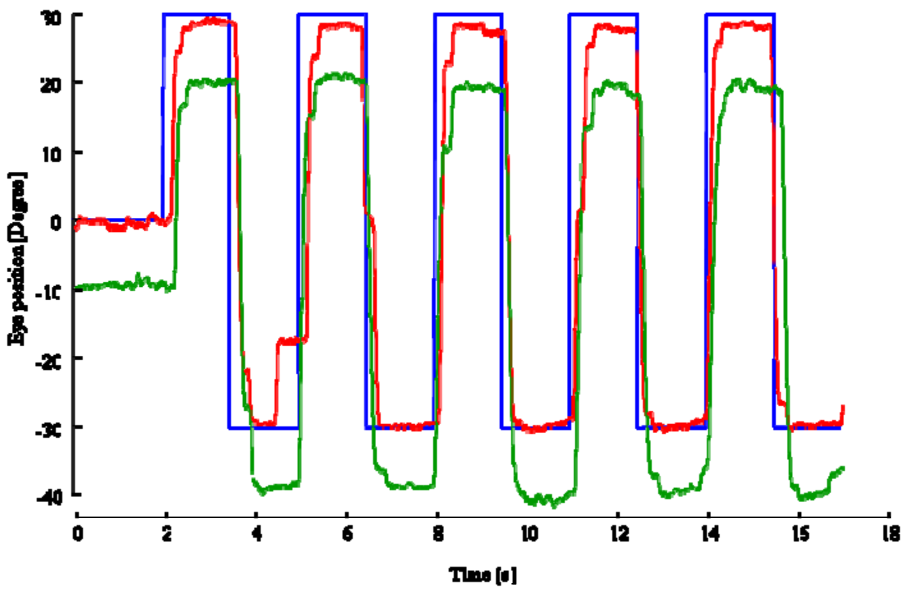

Figure 4: Recordings of a subject performing the 60 degree/s saccades in the saccade test sequence while rested (red) and after 24 hours of sleep deprivation (green). The saccade eye target is presented in blue, and the subjects performed the saccades between 30 degrees to the left and 30 degrees to the right. Note that the subject while rested often made one large saccade followed by a smaller corrective saccade whereas while sleep deprived the subject sometimes gradually decreased the saccade velocity thereby making a gradual adjustment of the final position, a response which could explain the increased saccade accuracy values presented in figure 5. Additionally, note that some recordings have been moved arbitrarily somewhat in the vertical direction for presentational reasons.

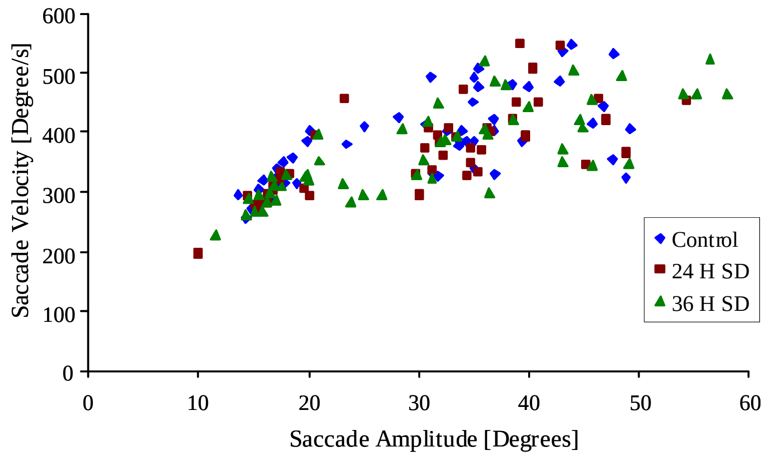

Figure 5: Illustration of individual saccade responses as plotted in an XY-diagram with saccade velocity as y-value and saccade amplitude as x-axis value for each subject and for each of the 20, 40 and 60 degree tests during Control trial, at 24 SDep and at 36 SDep.

Figure 4 shows the recordings of a subject performing the 60 degree/s saccades in the saccade test sequence while rested and after 24 hours of sleep deprivation. Figure 5 shows an illustration of individual saccade responses as plotted in an XY-diagram with saccade velocity as y-value and saccade amplitude as x-axis value for each subject and for each of the 20, 40 and 60 degree tests during Control trial, at 24 SDep and at 36 SDep. Note that several subjects have larger saccade amplitudes at 36 SDep with 60 degree target amplitudes than during the Control and 24 SDep test occasions, though the saccade velocity is similar to the other test occasions.

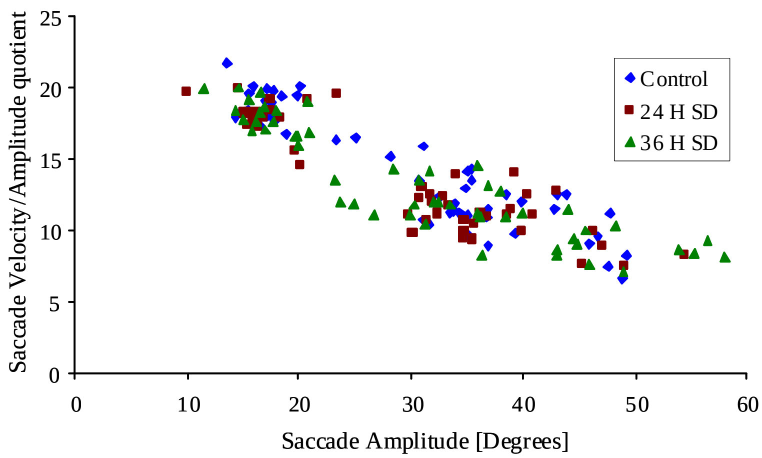

Figure 6: Illustration of individual ratio between saccade velocity divided by saccade amplitude at different saccade amplitudes as plotted in an XY-diagram with the ratio value as y-value and saccade amplitude as x-axis value for each subject and for each of the 20, 40 and 60 degree tests during Control trial, at 24 SDep and at 36 SDep.

Figure 6 shows an illustration of individual ratio between saccade velocity divided by saccade amplitude at different saccade amplitudes as plotted in an XY-diagram with the quotient values as y-values and saccade amplitudes as x-axis values for each subject and for each of the 20, 40 and 60 degree tests during Control trial, at 24 SDep and at 36 SDep. The ratio between saccade velocity and saccade amplitude were on average 14.2 during the Control trial, 13.5 at 24 SDep and 13.3 at 36 SDep. The statistical difference between Control trial and 24 SDep ration values was p=0.001, and the statistical difference between Control trial and 36 SDep ration values was p<0.001. The ratio values were not significantly different between 24 SDep and 36 SDep trials.

3.2.1. Saccade Velocity

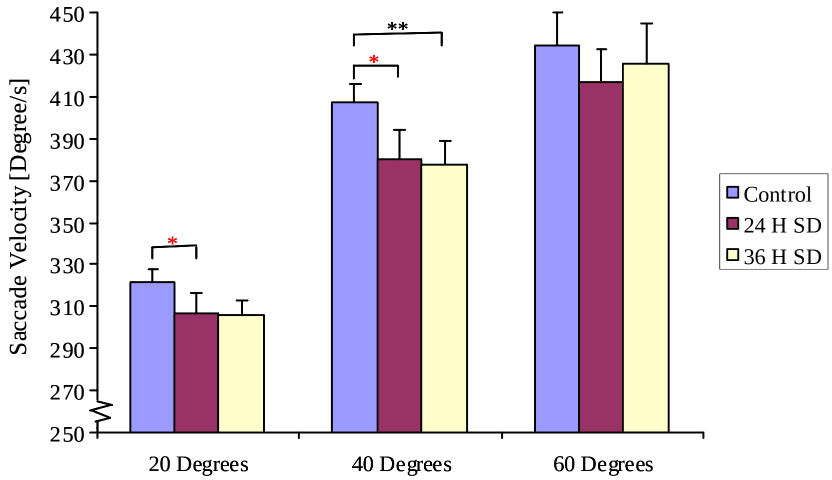

Figure 7: Saccadic eye movement velocity with increasing amplitudes of the target saccades (mean and standard error of mean (SEM)) during different stages of sleep deprivation. Testing started with the target stationary straight forward (0º). Thereafter, the visual target moved horizontally according to the following sequence of amplitudes: ±10, ±20, ±30º, yielding saccades of a total range of respectively 20, 40 and 60º amplitude. Hence, the visual target moved at maximum 30º right (+) and 30º to the left (-).

There was some evidence that saccade velocity decreased after sleep deprivation, see figure 6 and table 1. The saccade velocity was about 7% lower after 36SDep compared with the Control test for 40 degree target movements (p<0.01) and about 6% lower after 24SDep compared with the Control test for 20 and 40 degree target movements, though this was only verified at statistical level p<0.05.

3.2.2. Saccade accuracy

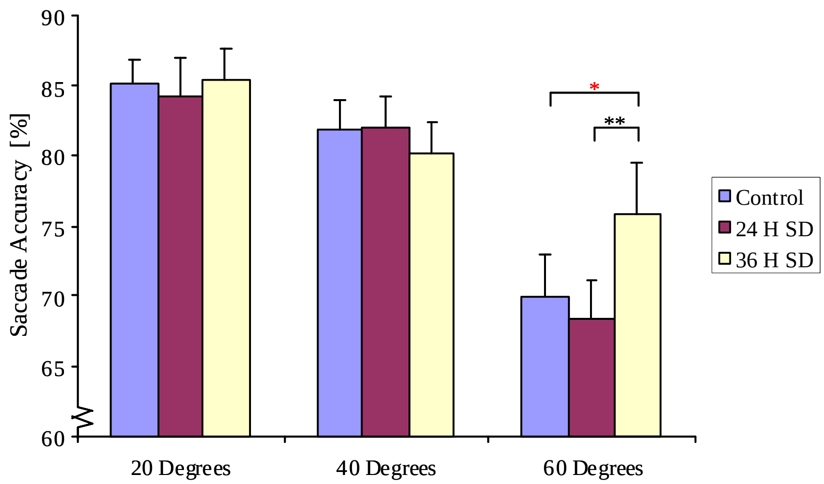

Figure 8: Average saccade accuracy in percentage (mean and standard error of mean (SEM)) during different stages of sleep deprivation. A value of 100 represents perfect saccade accuracy whereas values below 100 represent short saccades (hypometric).

The pro-saccade accuracy was only affected by sleep deprivation for saccades of 60 degrees amplitude, see figure 4 and figure 7. Saccade accuracy was about 11% higher after 36SDep than after 24SDep (p<0.01), and on average about 12 % higher after 36SDep compared with the Control test (p<0.05).

3.3. Smooth pursuit and saccade latencies

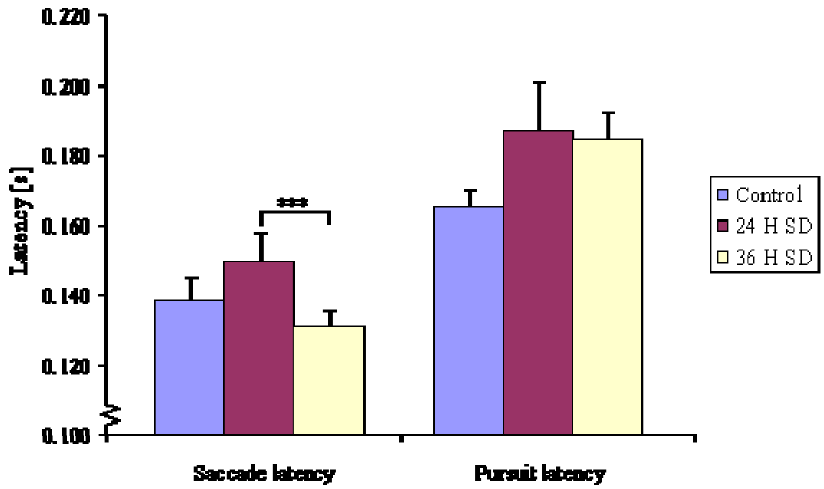

Figure 9: Latency values for smooth pursuit eye movements and saccadic eye movements in all tests (mean and standard error of mean (SEM)) during different stages of sleep deprivation.

The latency was generally shorter in the Control test compared with the sleep deprivation tests for the smooth pursuit eye movements, see figure 8. However, the only statistical change was a decrease in saccade latency between 24SDep and 36SDep by about 14% (p<0.001).

3.4. GLM analysis of oculomotor parameters.

A, Smooth pursuit parameters		Values			p-values		
	Target velocities	Control	24SDep	36SDep	SDep	Target velocity	SDep × Target velocity
Gain#	10	1.01 (0.01)	0.98 (0.01)	0.96 (0.01)	<0.001	<0.001	ns
	20	1.00 (0.01)	0.97 (0.01)	0.96 (0.01)			
	30	0.97 (0.01)	0.94 (0.02)	0.93 (0.01)			
	40	0.96 (0.01)	0.94 (0.02)	0.93 (0.01)			
Accuracy#	10	47.6 (1.4)	40.3 (2.0)	45.3 (2.0)	<0.001	<0.001	ns
	20	63.1 (1.9)	52.4 (2.1)	57.6 (2.4)			
	30	64.4 (2.1)	54.0 (2.3)	59.9 (2.5)			
	40	57.8 (2.4)	52.9 (2.2)	58.6 (1.9)			

B, Saccade parameters		Values			p-values		
	Target amplitudes	Control	24SDep	36SDep	SDep	Target amplitude	SDep × Target amplitude
Velocity#	20	321 (6)	307 (10)	305 (7)	ns	<0.001	ns
	40	407 (9)	380 (14)	378 (10)			
	60	422 (20)	416 (15)	425 (19)			
Accuracy#	20	85.1 (1.7)	84.2 (2.7)	85.4 (2.2)	ns	ns	ns
	40	81.9 (2.0)	81.9 (3.1)	80.1 (2.3)			
	60	67.5 (3.8)	68.3 (3.3)	75.9 (4.4)			

Table 1: Statistical evaluation of the smooth pursuit (A) and saccade (B) values using the GLM univariate ANOVA method for the smooth pursuit and saccade parameters (mean and standard error of mean (SEM)). #The GLM model residual was not normally distributed. These statistical values may therefore be somewhat less accurate.

Sleep deprivation significantly decreased smooth pursuit gain and smooth pursuit accuracy (p<0.001), see table 1A. Moreover, the average smooth pursuit gain was more accurate during the slow pursuit movements, though it was easier to maintain correct smooth pursuit velocity within the boundaries during faster smooth pursuit movements. GLM analysis also showed no interaction effect of sleep deprivation and the smooth pursuit target velocity.

The saccadic eye movements were not significantly affected by sleep deprivation, see table 1B. However, the saccade velocity was significantly faster for larger saccade amplitudes (p<0.001). In addition, there was no interaction effect of sleep deprivation and saccade target amplitude.

3.5. Correlation between the VAS scores and the oculomotor parameters.

Smooth pursuit parameter		Statistics	
Sleep Deprivation	Parameter	R-value	p-value
24 hours	Gain 10 º/s	ns	ns
	Gain 20 º/s	0.339	0.043
	Gain 30 º/s	0.365	0.029
	Gain 40 º/s	ns	ns
	Accuracy 10 º/s	ns	ns
	Accuracy 20 º/s	ns	ns
	Accuracy 30 º/s	ns	ns
	Accuracy 40 º/s	ns	ns
	Latency	ns	ns
36 hours	Gain 10 º/s	ns	ns
	Gain 20 º/s	ns	ns
	Gain 30 º/s	ns	ns
	Gain 40 º/s	ns	ns
	Accuracy 10 º/s	ns	ns
	Accuracy 20 º/s	ns	ns
	Accuracy 30 º/s	ns	ns
	Accuracy 40 º/s	ns	ns
	Latency	ns	ns

Table 2: Correlation analysis between the subjective individual scores of sleepiness (VAS) after 24SDep and 36SDep and smooth pursuit oculomotor parameters: Gain of the smooth pursuit movement, accuracy of the smooth pursuit movements [%] and latency [s], for visual target movements of velocities 10, 20, 30 and 40 º/s. The average values are presented in table 1A and figure 6. The average VAS scores were 5.2 at 24SDep and 6.8 at 36SDep.

Smooth pursuit eye movements were only partially correlated to subjective VAS scores, see table 2. However, after 24SDep, subjects with high VAS scores had higher average smooth pursuit gain during 20 and 30 º/s target velocities, though this was only determined at $p<0.05$.

Saccadic parameter		Statistics	
Sleep Deprivation	Parameter	R-value	p-value
24 hours	Velocity 20º	ns	ns
	Velocity 40º	ns	ns
	Velocity 60º	ns	ns
	Accuracy 20º	ns	ns
	Accuracy 40º	ns	ns
	Accuracy 60º	ns	ns
	Latency	0.582	<0.001
36 hours	Velocity 20º	0.484	0.003
	Velocity 40º	0.480	0.003
	Velocity 60º	0.460	0.021
	Accuracy 20º	0.424	0.010
	Accuracy 40º	0.363	0.029
	Accuracy 60º	ns	ns
	Latency	ns	ns

Table 3: Correlation analysis between the individual subjective scores of sleepiness (VAS) after 24 hours and 36 hours of sleep deprivation and saccadic oculomotor parameters: Velocity of the saccade [º/s], saccade accuracy [%] and latency [s], for visual target movements of 20, 40 and 60º amplitude. The average values are presented in table 1B and figure 6. The average VAS scores were 5.2 at 24SDep and 6.8 at 36SDep.

Saccadic measurements correlated better to VAS scores than smooth pursuit measurements, particularly after 36SDep, see table 3. Correlations show that after 24SDep, subjects with high VAS scores had significantly longer latency time ($p<0.001$) and after 36SDep, subjects with high VAS scores had significantly higher saccade velocity (20, 40º target movement amplitude, $p<0.01$; 60º target movement amplitude, $p<0.05$). Correlation values also suggest that subjects with high VAS scores had higher saccade accuracy for 20º and 40º target movement amplitudes, though this was only determined at $p<0.05$.

4. Discussion

There is a need to find practical, non-invasive, objective methods to measure the effects of sleepiness, especially when reaching levels involving higher risks for accidents. Several studies have elected to use the deterioration of smooth pursuit and saccadic eye movements as an estimator of sleepiness, and have shown that eye movement performance deteriorate after a period of sleep deprivation. However, when reviewing the literature, the findings vary considerably between studies, as some show deterioration of smooth pursuit eye movements and saccadic performance, whereas others found no significant changes. For example, De Gennaro et al. and Porcu et al. [7, 18], both found that smooth pursuit gain was negatively affected by sleep deprivation. Similarly, we found that smooth pursuit gain was markedly decreased by sleep deprivation, though it should be noted that both of these studies measured smooth pursuit gain using a sinusoidal target movement pattern whereas we used a movement pattern with fixed target velocity. However, whereas Porcu et al. found that smooth pursuit accuracy was not affected by sleep deprivation [18], De Gennaro et al. did [7]. In our study, the smooth pursuit accuracy was found to decrease after sleep deprivation, though it should be noted that the parameter used in the present study is not directly compatible to the parameter used in other reports.

Similar to some reports [8, 18], we found some deterioration of maximum saccade velocity after sleep deprivation, though other studies did not [6]. Whereas some studies found that saccadic accuracy was affected sleep deprivation [5, 18], we did not. Hence, although several eye movement properties are clearly deteriorated by sleep deprivation, the inconsistent findings in the performed studies raise questions about whether investigation of oculomotor performance offers sufficient reliability to provide an objective measure of sleepiness.

From previous reports, the largest effects of sleep deprivation were found by De Gennaro et al. in a study repeatedly measuring performance in 2 hour intervals [7]. One possible reason for this could be that if the number of tests is restricted, motivation and attention might be temporarily increased while these tests are carried out, thus suppressing any sleep deprivation effects. However, if a number of similar tests are performed during a period of sleep deprivation, the act of repeatedly performing the tests might no longer raise attention and motivation, subsequently allowing a larger influence of sleep deprivation.

4.1 Sleep deprivation and eye movements

Saccadic and smooth pursuit eye movements are important for our visual orientation and are also involved when capturing images of interest on the fovea, and for preservation of focus on images independently of head movements. In line with some previous studies, we found that the oculomotor function can be affected by sleep deprivation, though our findings showed that smooth pursuits were more affected than saccades. Previous reports have shown that the effects of sleep deprivation are primarily marked when a task is not allocated sufficient attention [20]. However, another explanation might be that a slow movement in the visual field might be considered somewhat less important, and therefore given less attention than a rapid movement. Consistent with this explanation, we found that the smooth pursuit eye movements were more affected at slower velocities of the target and saccadic velocity at smaller saccade amplitudes than at faster target velocities and larger saccade target amplitudes. Another explanation could be that the subjects found it generally more monotonous to trace a slowly moving target, whereas tracing a target at higher velocities was more exciting and sufficient attention was allocated. This observation is in line with the proposal by Horne that interesting and stimulating activities can mask sleepiness, whereas monotonous task and boredom can make sleepiness worse [10].

4.2 Circadian rhythm and oculomotor function

An unexpected finding was that several oculomotor functions did not continue to decrease uniformly from 24SDep to 36SDep. Instead, smooth pursuit gain and saccade velocity maintained fairly the same between 24SDep and 36SDep, whereas smooth pursuit accuracy and saccadic accuracy even recovered to near Control test values. These findings are in line with the reports by De Gennaro supporting the notion that the circadian rhythm can affect oculomotor performance [7, 8]. However, in contrast to De Gennaro, we found no clear improvements of saccade velocity but only of smooth pursuits. The effects of sleep deprivation have also been studied when performing other kinds of motor tasks, and similar to our findings various reports have found that some motor performances follow a circadian cycle, rather than gradually decrease with the length of sleep deprivation [2, 9, 13, 14].

Our observations also show that increased saccade amplitude does not necessarily coincide with an increase saccadic velocity under sleep deprivation, as is the case in normal conditions [4]. The saccade properties are described by 2 factors, the saccade velocity and saccade amplitude. Importantly, both of these factors might be influenced by sleep deprivation and the saccade velocities might not necessarily be changed in the same manner as the saccade amplitudes. This fact is clearly illustrated in the responses to the 20, 40 and 60 degree target movements. In the saccade responses to 20 and 40 degree target movements, it was found that sleep deprivation primarily decreased the average saccade velocities but the average saccade amplitudes were unchanged. However, when studying the saccade responses to 60 degree target movements it was found that the average saccade amplitudes were significantly larger at 36 SDep but the average saccade velocities were not significantly changed. Hence, as illustrated by the ratio values presented in figure 6, the relationship between saccade velocity and saccade amplitude is significantly changed by sleep deprivation in that the subjects can still initiate large saccades if necessary but not necessarily with the same high velocity. Possibly, the predictable target position in the pro-saccade tests used may have influenced the changes found under sleep deprivation. However, our findings are well in line with other reports [7, 8].

4.3 Subjective sleepiness and oculomotor function

VAS scores showed that subjective sleepiness increased between 24SDep and 36SDep. However, smooth pursuit gain, smooth pursuit accuracy and saccade velocity did not show the same gradual deterioration. Subsequently, we found poor correlation between subjective sleepiness and smooth pursuit eye movements. Therefore, ones own estimation of sleepiness might not be a reliable indictor of actual performance for some oculomotor functions. This further stresses the need to find a reliable, objective measurement of tiredness. Moreover, we also found that when subjects believed they were tired, they were able to perform better in saccadic tests. This response could imply that awareness of sleepiness encourages subjects to increase their levels of attention, and concentrate harder on a certain task. However, we also found that the subjectively sleepiest subjects had a more delayed saccadic response, which suggest that these subjects were slower to identify and track a moving target. No such evidence was found with smooth pursuit latency, which suggests that subjective sleepiness may have a different effect on smooth pursuit and saccade functions. In contrast to our results, De Gennaro et al found a significant correlation between subjective sleepiness and decreased oculomotor performance [7]. However, our findings may differ as subjective scores are influenced by motivation, personal factors, experience, training etc [2].

4.4 Methodological Considerations

In most studies of smooth pursuits, sinusoidal stimulations at various frequencies have been used and the properties of the smooth pursuit movements have been evaluated from gain and phase characteristics to measure smooth pursuit accuracy [7, 18]. A sinusoidal movement is well-defined with respect to spectral contents. However, with sinusoidal movements, the target velocity is never constant but changes non-linearly over time and the target velocity is always lowest at the maximum amplitude positions. In the present study, we have instead used a stimulus with a constant target velocity over an amplitude of 60º. Such movement enhances the possibility to determine whether a subject or patient is able to perform a pursuit movement of a specific velocity. A constant target velocity over the entire movement range can also be used to determine whether a detected smooth pursuit movement deficit is influenced by eye position amplitude. The velocity accuracy parameter introduced in this study, therefore allows additional information to be investigated and was found to be the most sensitive measure of sleep deprivation. In addition, with smooth pursuit accuracy measurements, the standard procedure is to remove all artifacts and analyze whether the remaining data fulfils the gain and phase requirements. However, as illustrated in the present study, sleep deprivation or oculomotor deficits might influence the ability to maintain an accurate smooth pursuit within acceptable boundaries over longer periods of time. The velocity accuracy parameter solves this problem, as it quantifies the extent a subject or patient is able to maintain an accurate smooth pursuit movement during a test. Therefore, the velocity accuracy parameter might be sensitive to a number of disorders where other measurements are not.

In this study, the saccade latencies were generally shorter than the smooth pursuit latencies, which are not common. One possible reason why our study results are different could be the difference in smooth pursuit test sequences used in the trials. In most prior studies of smooth pursuit, sinusoidal stimulation has been used. In this study, smooth pursuit movements with fixed velocities were used, where each smooth pursuit target movement was preceded by a 1 second period where the visual target was stationary. These 1-second periods where the target was stationary might have made the subjects more uncertain about when the next pursuit movement might start than other smooth pursuit tests used. Another possible reason for the longer smooth pursuit latencies could be that it might be easier to detect the start of smooth pursuit movements with a clear initial target velocity change than at the start of a smooth pursuit movement with a slow fixed velocity. However, the effect of both these factors needs to be investigated further in future studies.

Acknowledgements

The authors' wishes to acknowledge the financial supported from the Swedish Medical Research Council (grant nr. 17x-05693) and the Medical Faculty, Lund University, Sweden. We also acknowledge Janet Lindblad for her invaluable help in the study and Lars Beijer and

Fredrik Alvik, ResMed Sweden AB, for providing the Embletta™ EEG measurement equipment for the study.

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