Master's thesis

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Exploring difficult morning awakening in Delayed Sleep Phase Syndrome using polysomnography

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Abstract

Objective: Difficult morning awakening is an important symptom of delayed sleep phase syndrome (DSPS), but there is little knowledge about this phenomenon. The first aim of this study was to test the hypothesis that DSPS patients have more slow wave sleep (SWS) during the latter half of the night than what is normally observed. The second aim of the study was to examine whether the difficult awakening had any relation to a specific sleep stage upon awakening and sleep inertia. The third aim was to test if cognitive performance was reduced upon awakening.

Methods: Six patients diagnosed with DSPS and four healthy controls were recruited. The subjects kept a sleep diary for 14 days and underwent actigraphy recording for seven days prior to an experimental night at the sleep laboratory where a polysomnography (PSG) recording took place. In addition, saliva samples were collected for dim light melatonin onset (DLMO) testing.

Results: The PSG analysis showed that the DSPS patients do not have more SWS during the latter half of the night. In fact, the controls were observed to have more SWS than the patients during the latter half of the night. All subjects woke up from either stage N1, N2 or REM. Two of the patients were difficult to wake up from REM sleep. No significant differences were observed between the groups regarding sleep inertia. Likewise, no significant difference was found in the patients' cognitive performance when tested in the afternoon compared to immediately after awakening.

Conclusions: The most interesting finding is the difficult awakening from REM sleep in two of the patients. This yields for further investigation. As no differences were observed regarding cognitive performance, it is possible to conclude that the patients in this study did not experience any major temporary cognitive impairment upon awakening.

1. Introduction

Delayed sleep phase syndrome (DSPS) is one in the line of several circadian rhythm disorders characterized by a desynchronization between the endogenous body clock and the external cues (zeitgeibers) [1]. DSPS was first reported by Weitzman et. al in 1981 [2]. The circadian rhythm is controlled by a small nucleus in the hypothalamus known as the suprachiasmatic nucleus (SCN). It runs approximately on a 24 h cycle, compatible with the external cues of the nature [3]. Sometimes though, this 24 h cycle is disturbed so that it does not completely align with nature's signals. Patients who have a delay in their circadian phase find it difficult to fall asleep and wake up at what is considered socially (and biologically) desirable times [2, 4]. This phase delay of the circadian rhythm causes undesirable social and occupational effects. Many patients struggle with getting to school or work on time. Many reported cases of DSPS are adolescents [5]. Prevalence of DSPS based on a large sample of Norwegian high school students was reported to be 8.4% [6], while a study based on a large sample of Norwegian adult individuals (18-67 years) reported a prevalence of 0.17% [7]. If young patients are experiencing difficulty at school because of the daily struggle of not being able to wake up, then an uncertain future might lay ahead. Even for older patients there are many reports of difficulty with holding a job, at least within normal working hours. This is a practical issue that might cause a great deal of social insecurity.

While the sleep phase is delayed, most authors report no other abnormalities recorded regarding the quality of sleep. It is believed that DSPS patients sleep as well as any healthy person once they enter sleep mode [8, 9]. In contrast, Watanabe et al. found in their study that DSPS patients have a disturbed sleep structure [10].There is also some uncertainty regarding whether sleep duration is altered in DSPS. Whereas some studies report DSPS patients having a longer duration of sleep than what is considered normal [11], others have failed to find support for this concept [12]. So one may say that there is still some dispute regarding sleep quality in DSPS. In addition, several studies report a pattern of sleep onset insomnia once patients are enforced to a normal rhythm [2, 11]. This points towards a problem with the entrainment of the endogenous body clock.

The study of biological aspects of DSPS might give important insights about the nature of the disorder. One of the most investigated factors is melatonin [13]. Melatonin, known as the 'molecule of darkness' is a hormone secreted by the epiphyseal gland that sends signals to the SCN about the absence of light. In other words melatonin-producing cells are highly

active during night, but quite silent during day time. The secretion of melatonin starts a few hours before habitual bed-time. For most people that would be around 2000 h. During the night melatonin level reaches its maximum around 0200 h after which it gradually decreases during the day. [14]

In DSPS patients it has been observed that melatonin secretion is delayed by approximately two hours [12]. This could provide one answer as to how falling asleep at a normal time might be difficult, although one should always keep other factors in mind as well. Those other factors might be either of neurophysiological nature, possibly involving dysfunction in arousal or the transitions from sleep to wakefulness (sleep inertia) or they might be of social and motivational nature and showing symptoms characteristic of DSPS, but without the biological disturbances [11]. In a clinical setting it may be important to be able to distinguish these two (potentially causal) mechanisms in order to initiate a proper treatment.

What is most likely though, is that DSPS is influenced by a combination of both biological and psychological factors. A comorbidity that has been documented in a recent study is depression [15]. An evening chronotype, which is common amongst DSPS patients, is more likely to show depressive symptoms than a morning-chronotype. Furthermore, cognition also seems to be temporarily impaired in the morning. This is probably most obvious right after a difficult awakening where patients either wake up very slowly or fall asleep right after awakening, sometimes not even remembering the attempts to wake them up.

The temporarily impaired memory and feelings of disorientation and confusion upon awakening are characteristics of sleep inertia (SI) [16]. It most probably occurs following a transit from one form of consciousness to another, namely from sleep to wakefulness. SI is not unusual in a healthy population but the degree of it varies strongly [16]. A study by Tassi et al. suggests that waking up from deep sleep results in greater SI, while waking up from stages N1 or N2 results in only minor or none cognitive decline [17]. Yet, a study done by Jewett et. al some years before had reported no such difference with respect to stage upon awakening [18].

However, whereas difficult morning awakening is a key symptom in DSPS, research on this symptom remains limited [19]. In a review on the literature on awakening from sleep, Åkerstedt reported only one study on this in DSPS [20]. This study tested the difficult morning awakening using the multiple sleep latency test (MSLT) and it was found that the

sleep onset latencies in DSPS were shorter in the morning compared to the afternoon [20]. However, the author argues that the MSLT may not be optimal to study this phenomenon and suggests that other measures should be used to study difficult morning awakenings.

Research on hypersomnia, where difficult morning awakenings may also be found, have utilized PSG recordings prior to awakening and cognitive evoked potentials just after awakening to determine if sleep stages are altered during sleep and if cognitive functioning is altered immediately after awakening [19, 21]. Hence, a similar approach with PSG recording with a particular focus on the sleep structure immediately before awakening and testing of cognitive function after awakening could also provide important information about the nature of biological dysfunctions in DSPS.

Patients, and their parents, report that they require very rigorous awakening methods and once out of bed the patients act confused, are very slow and seem almost unable to be awake and focused. This might suggest abnormal brain activity upon awakening which could be assessed by PSG. Another method to document and quantify the reported impaired cognitive functioning is a battery of cognitive tests that assess the patients' performance and ability to focus their attention. The Continuous Performance Test (CPT) may be an useful method in this respect as it assesses the ability of sustained attention, has well established norms, and does not have a learning-effect [22]. Thus, patients can take the test in the afternoon when they feel refreshed and again just after awakening. The working hypothesis is that the discrepancy in performance is larger in a DSPS group compared to healthy controls.

In the light of sleep inertia, looking into the importance of last sleep stage before awakening might also be interesting. In an overview article it is reported that most awakenings follow a REM stage, or rather that the last REM episode is interrupted once the brain is ready to make the transition to wakefulness [23]. In the same overview, it is mentioned how awakening from SWS most often results in confusion and low arousal, which is not surprising as waking up from deep sleep is difficult.

The aims of this study were to investigate the physiological nature of the difficult awakening process and possible cognitive impairment related to a difficult awakening by means of polysomnography (PSG), including electroencephalographic (EEG) activity, and the use of neuropsychological test to investigate the cognitive effects of early awakening (i.e. 0700 am) in DSPS patients compared to healthy controls. One working hypothesis was that DSPS

patients have more SWS in the latter part of the night and immediately before a difficult awakening. Another working hypothesis was that cognitive function will be reduced and related to the sleep stage upon awakening in DSPS patients compare to healthy controls. In addition, because psychological and sleep-related behavioral factors may also be involved, these factors are also assessed.

2. Methods

2.1 Participants

Based on a previous assessment by a psychologist or a psychiatrist at Department of Psychiatry, St. Olav's Hospital, six patients diagnosed with delayed sleep phase syndrome were included in the study, four males and two females. A brief case history for each patient follows bellow. Four healthy controls were included as well, two males and two females. Healthy controls were recruited by posting an announcement on the university's homepage. All subjects ranged between 18- 33 years. All participants were screened and checked up against a list of exclusion factors as to minimize comorbidity. Table 1 contains all the exclusion factors. The study protocol was approved by the Regional Committees for Medical and Health Research Ethics and all subjects signed an informed consent form prior to their participation.

Table 1. Exclusion factors
Migraine
Other sleep related problems (insomnia, sleep apnea, RLS)
High blood pressure
Infectious disease
Metabolic, endocrine or neuromuscular disease
Connective tissue diseases
Other acute or chronic illness
Newly acquired injuries that might impair function
Heart disease or use of heart medication
Lung disease
Cerebrovascular disease
Neurological disease with major functional impairment (Parkinson's disease, epilepsy, MS)
Psychological disorders (schizophrenia, bipolar disorder, antisocial personality disorder)
Neoplastic disease
Use of any medicine that might influence sleep, neurological or muscular function
Pregnancy or nursing
Undergone neurosurgery with craniotomy or cervical spine surgery
Abuse of alcohol or narcotics
Impaired hearing

2.2 Procedure

2.2.1 Pre-experimental weeks

In the first phase, participants were asked to fill in a sleep diary for 14 days. After the first week, each person was in addition provided with an *actigraph watch* that was to be used for seven days. Actigraphy is a simple, non-invasive way of recording activity, and in this scenario it was used to record each person's sleep pattern for the seven day period.

For the second phase of the study, participants underwent polysomnography (PSG) for two nights. The first night was an ambulatory polysomnography where patients slept at home with the equipment. This was done in order to minimize the "first-night effect" [24]. Brain activity (EEG), eye movement (EOG), muscle activity (EMG), as well as breathing was monitored.

On the first day of the PSG recording, participants were asked to come to the sleep laboratory at 1400 h where a technician mounted the equipment they were to wear for the night.

Each person was to answer a set of short questions regarding their medicine use and intake of nicotine or caffeine for the past 24 hours, followed by a semi-structured interview regarding sleep behavior and quality. The last questionnaire each person was provided with was to be filled out at home. The schema contained some demographic questions as well as Epworth sleepiness scale (ESS) [25].

After the equipment was placed and the questionnaires were completed, participants were allowed to go home. They were free to decide when to go to bed and when to get up. In other words, they were to follow their natural rhythm. The only condition was to be back at the sleep laboratory by 1300 h at the latest so that the equipment could be demounted and prepared in time for the next person coming for the examination.

2.2.2 The experimental night

The experimental night was two nights after the initial ambulatory PSG. Participants came back to the sleep laboratory at 1400 h. At 1500 h participants were subjected to a neuropsychological test to assess their ability for sustained attention as a measure of vigilance during the day. From 1800 h the light was dimmed to below 200 LUX to ensure a dim-light environment. A Hagner Digital Luxmeter Model EC1 was used to measure light intensity. As most participants had brought their own laptops, both light from the ceiling and light from the laptop were measured to ensure that the limit was upheld. Subjects were then asked to complete psychiatric and sleep-related questionnaires during the evening. To measure circadian phase each participant provided saliva samples for melatonin testing every hour from 1900 h. Participants were to go to bed at midnight, right after the last saliva sample was given. They were reminded to shut off their computers and mobile phones or anything else that might cause distraction. At exactly 0700 h the following morning, an alarm clock was activated. A custom made software was developed using Test tone generator 3.8. Sound pressure level measured in decibel (dB) was used. The alarm clock was programmed to be active for 3 minutes. It started at 72 dB and the sound was increased with 2 dB at equal intervals until 104 dB which was the maximum sound intensity. A standard set of Sony speakers (SONY, Active speaker system, SRS- Z510) was placed on each side of the bed, approximately 50 cm from the subjects head. The exact time and decibel value at which each person reacted were noted. The few subjects that did not react at all even at 104 dB had to be wakened manually. Six minutes after awakening the participants were again administered the same neuropsychological test as the prior day. The instructions were read up in the same

manner to every participant for each time they took the test. After a short practice-test, the main test was started at which point the subject was left alone in the room with no distractions.

With the test completed, the examination was complete and the subject was allowed to get a 30 minute sleep if desired. At 0800 h the sensors were taken off and the subject was free to go home.

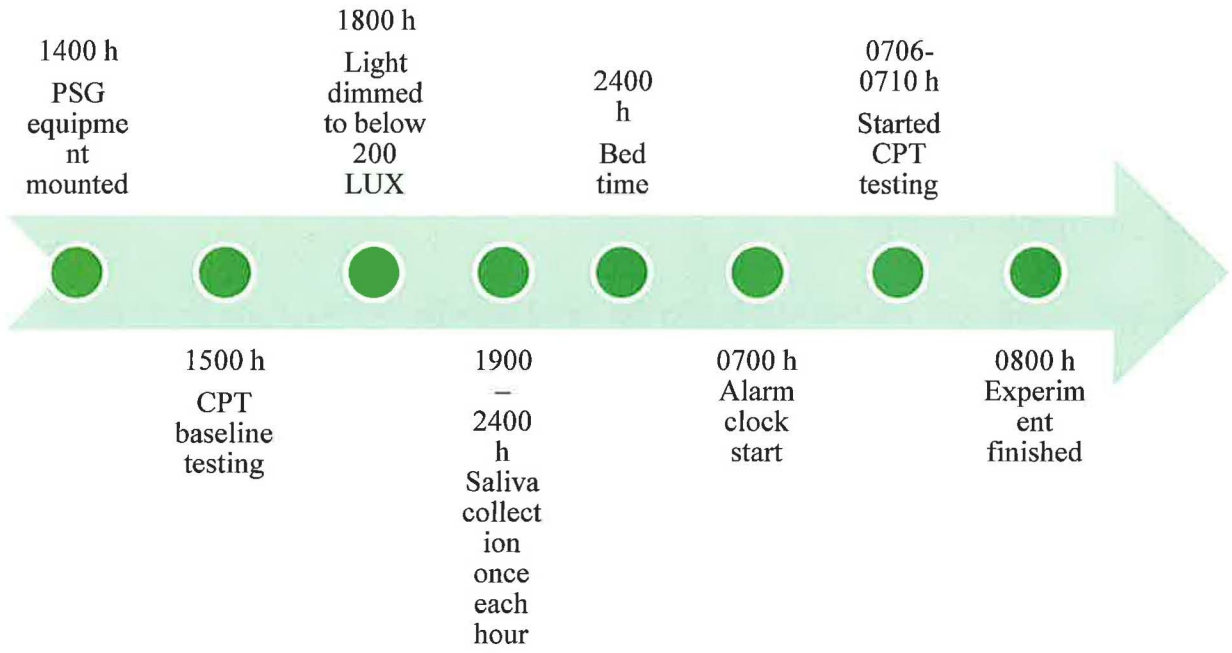

Fig. 1 A timeline showing the course of events during the experimental night

2.3 Assessments

2.3.1 Diagnosis

Patients were interviewed by psychiatrist with six years of clinical experience with sleep disorders or a medical doctor with one year of clinical experience with sleep disorders using a semi-structured interview. The six included patients met the Diagnostic and Statistic Manual for mental disorders – IV (DSM-IV 307.45) diagnostic criteria for Circadian Rhythm Sleep Disorder [8].

2.3.2 Physiologic sleep measures

Actigraphy: Participants used an *actigraph* unit AW4 from Cambridge Nanotech. The Actigraph recorded 30-second epochs for seven days prior to the experimental night with medium sensitivity.

Polysomnography: A standard Somnomedics montage, which consists of several electrodes attached to the head according to the international 10-20 system, i.e. F3, F4, C3, C4, O1, O2, A1, A2 with a Cz reference electrode for measuring EEG (analyzed with 0.2-35 Hz filter) and EOG electrode (placed 1 cm over/under the left/right lateral canthus), plus two electrodes placed on the chin to monitor muscle activity (EMG) with a 10 Hz high pass filter for analysis, was used. A nasal airflow sensor and an oronasal thermistor were used to monitor nasal flow, pressure and oronasal airflow (calculated from the temperature of the expired air). Participants were given instructions on how to place the sensor below the nostrils right before bed time. Also, an infrared oxygen sensor was placed on top of the index finger. On the experimental night a stationary video camera was placed by the bed as an additional source of behavioral recording.

Melatonin: Each person was to provide saliva samples for melatonin testing. From 1900 h until midnight, with 60 minute intervals, participants were asked to provide a minimum 2 ml of saliva. This was done non-invasively by placing their saliva directly into a tube. 30 minutes prior to each sample taking, no food or drink was to be consumed. Five minutes before each time a sample was to be given, the person had to rinse their mouth with cold water. Thereafter, they were given a few minutes to provide two samples of minimum 2ml each. A piece of Parafilm® was chewed as to enhance saliva production. As soon as both samples were delivered, they were placed in the refrigerator at 4 °C. For each sample taken, no more than 10 minutes passed from when the collection started until the samples were in the refrigerator. The procedure was repeated once an hour until midnight when last the sample was taken. A total of 12 samples, two samples of 2 ml for each hour, were immediately after placed in the freezer at <-20 °C.

The samples were analyzed using the Non-Extraction Melatonin Saliva ELISA kit, provided by IBL International GmbH. The procedure was followed as instructed in the manual.

Due to unexpected large values in the first sample from 1900h in some subjects, only samples from 2000 h to 2400 h were included in the analysis. A melatonin baseline was calculated as the mean value for samples taken at 20, 21 and 22 h (see Wyatt 2006 Fig 1) [26]. Normal (early) melatonin secretion onset was defined when a concentration equal to twice the baseline value was measured either at 2300h or at 2400h. An alternative cutoff equal to individual baseline + 3 standard deviations was used as well. In DSPS patients a delayed onset was presumed if concentrations had not reached the cutoff value in the last midnight sample.

2.3.3 Self-reported sleep measures

Sleep diary: Participants kept a sleep diary of their subjective experience of sleep timing and duration for 14 days prior to the experimental night.

Sleep Hygiene Index (SHI). The Sleep Hygiene Index is a 13-item questionnaire assessing the frequency of the individual engages in sleep inhibitory behaviors [27].

Flinders Fatigue Scale (FFS). The Flinders Fatigue Scale is a 7-item assessing daytime fatigue related to poor sleep [28].

Horne – Östberg Morningness Eveningness Questionnaire (MEQ). The MEQ is a 19-item questionnaire assessing level of diurnal preference [29]. A score of 16 to 30 is defined as "definitely evening type", 31 to 41 as "moderately evening type", 42 to 58 as "neither type", 59 to 69 as "moderately morning type" and 70 or above as "definitely morning type".

Sleep-related behaviors questionnaire (SRBQ). A 32-item questionnaire designed to assess the use of safety behaviors employed to promote sleep and cope with tiredness [30].

2.3.4 Psychiatric assessments

Beck Depression Inventory (BDI). The BDI is a 21-item self-report measure of behavioral, cognitive, and affective symptoms associated with clinical depression. Higher scores indicate greater depression. The BDI has been shown to be highly valid and reliable [31].

Beck Anxiety Inventory (BAI). The BAI is a 21-item state anxiety scale measuring the intensity of cognitive, affective, and somatic anxiety symptoms the last 7 days on a 4-point Likert scale. The BAI has shown to possess good reliability and acceptable validity [32].

2.3.5 Continuous performance test

The continuous performance test (CPT) is used for measuring several variables, attention being one of them [22]. Participants are required to respond to the stimuli on a computer screen by pressing a space bar for every letter except the letter "X". Multiple dependent measures exist, including Omissions, Commissions, Response Time, Variability or Standard Error and Detectability.

2.4 Data analysis and statistics

Considering that the present study is a pilot study there is a relatively low sample size and our focus is for this reason mainly on qualitative and descriptive analysis of the cases. In addition, exploratory statistical data analysis was done in order to compare the patients and healthy controls. With a low sample size it is hard to find significant difference, but the numbers can nonetheless be of interest. The p- value cutoff was set between 0.05-0.10 where $p<0.05$ was considered significant while $p<0.10$ was reported as a trend or tendency. Non-parametric measures were mainly used, such as Mann-Whitney U test and Wilcoxon signed-rank test while differences in proportions were tested with a standardized normal deviate.

The polysomnograms were analyzed by a single blinded scorer (a certified clinical neurophysiologist) according to standardized criteria [33]. The recordings were analyzed using SomnoPanel software version 2.5.0. The sleep parameters included Sleep Onset Latency (SOL), amount of Slow Wave Sleep (SWS), amount of light sleep (N1, N2), amount of REM sleep, total sleep time (TST), sleep efficiency (SE), stage in last epoch before alarm clock start and stage in last epoch before awakening.

A statistical analysis of alarm clock was done to compare the sound intensity measured in dB. In order to avoid missing data, the value of 105 dB was entered for cases that did not wake up to 104 dB, which was the maximum sound intensity. In cases where the subjects had awakened before the alarm clock, a value of 72 dB was entered as it was the lowest sound intensity of the alarm clock.

2.5 Case history

Patient 1

The patient is a male, 22 years old who lives alone. He finished one year of high school, but lost his trainee position in 2008 due to sleep problems. For the past 2-3 years he has been receiving psychiatric treatment for anxiety and depression. Otherwise the patient is physically healthy.

Sleep related problems had been reported for the past 15 years, starting when the patient was 7 years old. The condition was stable throughout the years. Main problem is not being able to fall asleep until late in the night and difficulty with awakening. The patient is dependent on help for waking up as he does not react to an alarm clock. Sleep pattern: goes to bed after 2400 h, falls asleep between 0200-0400 h and wakes up between 1100-1300 h. Due to sleep problems, he has neither graduated nor gotten a job.

According to the Diagnostic and Statistical Manual for Mental Disorders (DSM-IV 307.45) criteria, the patient satisfies all five conditions and has been diagnosed with delayed sleep phase syndrome.

Patient 2

The patient is a male, 24 years old who lives with his parents. He has finished elementary school, but terminated his high school education and lost his job due to sleep problems. For the past year he has managed to hold a trainee position. The patient is otherwise physically and mentally healthy.

The patient has had sleep related problems since elementary school, approximately for the past 15 years. The condition has been stable throughout the years. The main problem is difficulty with falling asleep until late in the night, followed by a difficult awakening. The patient feels unwell when forced to get up at a 'normal' time. Usually he goes to bed around 0200 h and gets up between 0730- 1400 h. He sleeps well into the afternoon on weekends and feels well after awakening when allowed to follow his own rhythm. Due to sleep problems, he has not graduated and has had difficulties finding a full time job. The patient has not received any previous treatment for his sleep problems.

According to the DSM-IV 307.45 criteria, the patient satisfies all five conditions and has been diagnosed with delayed sleep phase syndrome, with a suspect of the condition being socially related.

Patient 3

The patient is a female, 24 years old who lives with her boyfriend and attends high school. She is experiencing academic difficulties due to her sleep problems. The patient had formally received treatment for psychosis, but has recovered and no treatment has been needed for the past two years. The patient had undergone a knee surgery as well as hip surgery and reports occasional back pain.

The patient has had sleep problems since the age of 12 years. A stable pattern of falling asleep late in the night and difficulty with waking up in the morning has prevailed. For the past few years the patient has reported a lighter sleep as well as one or two short waking episodes during the night. Usually the patient falls asleep around 0100-0200 h and wakes up between 0700- 1200 h. Due to sleep problems the patient has not graduated nor gotten a job. The patient had formally used hypnotic medications as well as melatonin, but without any effect. Anti-psychotic medication had also been previously used which had a soporific effect.

According to the DSM-IV 307.45 criteria, the patient satisfies all five conditions and has been diagnosed with delayed sleep phase syndrome and insomnia.

Patient 4

The patient is a male, 33 years old who lives alone but has had other people live with him in order to help him get up in the morning. He has held a stable employment for the past seven years. The patient has recovered from poliomyelitis with some remaining symptoms in both legs.

The patient reports a preference towards going to sleep late since childhood years. In his early years he was dependant on his parents to wake him up. He still occasionally needs help from others to wake up in the morning. Due to few hours of sleep each night, the patient feels tired and exhausted during the day. Usually he goes to bed around 2400 h and falls asleep between

0200-0330 h. During the week days he gets up at 0730 h and during the weekends between 1000-1200 h. The patient has tried hypnotic medications and bright light therapy for a short period of time with no effect.

According to the DSM-IV 307.45 criteria, the patient satisfies all five conditions and has been diagnosed with delayed sleep phase syndrome.

Patient 5

The patient is a male, 18 years old and lives with his parents. The patient attends his second year of high school, but due to his sleep problems he is in danger of not fulfilling the school year. The patient has reported feeling in despair of the situation and having concerns about his future if the same pattern is to continue. The patient is otherwise physically healthy.

Since childhood years the patient has preferred to fall asleep late and sleep longer in the mornings. For the past two years there has been a gradual worsening. The longest the patient can hold a normal rhythm is three consecutive days. The patient prefers to fall asleep around 0800-0900 h and wake up around 1700-1800 h. Once asleep the patient seems impossible to wake up. This pattern is affecting his school performance and overall mood. Hypnotic medications and melatonin had been used sporadically with little effect.

According to the DSM-IV 307.45 criteria, the patient satisfies conditions A, B, C, D, but not condition E. He has been diagnosed with delayed sleep phase syndrome, but longer sleep diary registration shows a free running circadian rhythm. Mild depressive symptoms had been reported which had required no treatment.

Patient 6

The patient is a female, 25 years old and lives alone. The patient has not graduated from high school. For the past six months the patient has been going to a psychologist due to anxiety and depression problems. Otherwise the patient is physically healthy.

The patient has had sleep problems since she was 13 years old. Falling asleep late in the night and difficulty with waking up is a pattern that has been stable throughout many years. Usually the patient falls asleep between 0100-0430 h and wakes up between 0700-1600 h, most often around 1200 h. The situation is reported to be somewhat worse during the winter months. Due

to the sleep problems the patient has not graduated from high school and has not gotten a job. The patient had attempted bright light therapy seven years ago.

According to the DSM-IV 307.45 criteria, the patient satisfies all five conditions and has been diagnosed with delayed sleep phase syndrome. Anxious and elusive personality as well as some depressive symptoms which need no further treatment other than the follow up the patient is getting.

3. Results

Based on the self reported sleep-diary data there is an obvious trend in the difference of sleep-wake pattern between patients and healthy controls. Table 2 shows mean values based on the fourteen day period. One can observe that on average, patients go to bed and wake up later than the controls.

Table 2. Sleep diary. Mean values for sleep-start, sleep-end, amount of sleep and latency for patients (Pas) and controls (Co)

	Sleep-start	Sleep-end	Hours-of-sleep	Latency
Pas1	00:46	10:48	8,7	30-90 min
Pas2	02:16	08:38	6,4	0 min
Pas3	02:59	10:07	7,1	0 min
Pas4	02:50	08:25	5,5	0 min
Pas5	02:41	11:08	7,4	<30 min
Pas6	02:28	11:45	7,7	<30 min
Co1	00:06	07:37	6,8	<30 min
Co2	01:06	08:45	6,9	<30 min
Co3	23:30	07:19	6,9	30-90 min
Co4	23:59	07:54	6,7	30-90 min
p-value	0.019**	0.038**	0.476	0.171
Mean (SD) Pas	02:20 (00:48)	10:08 (01:21)	7.1 (1.1)	30 min (30 min)
Mean (SD) Co	00:10 (00:40)	07:53 (00:37)	6.8 (0.01)	30-90 min (15 min)

P-values < 0.05 are indicated by a double asterix. P-values between 0.05 and 0.10 are indicated by one asterix (Mann-Whitney U test)

Data recorded from the actigraphy shows a supporting result. Based on a seven-day period, mean values show that patients fall asleep and wake up later that the controls. Table 3 presenting the exact numbers is shown below.

Table 3. Actigraphy. Mean values for sleep-start and sleep-end for patients (Pas) and controls (Co)

	Sleep-start	Sleep-end
Pas1	02:22	09:09
Pas2	-	-
Pas3	02:05	09:40
Pas4	01:22	07:54
Pas5	-	-
Pas6	01:13	08:55
Co1	23:40	07:07
Co2	00:53	08:37
Co3	00:01	07:14
Co4	00:41	08:10
p-value	0.029**	0.114
Mean (SD) Pas	01:45 (00:33)	08:54 (00:44)
Mean (SD) Co	00:18 (00:34)	07:47 (00:43)

P-values <0.05 are indicated by a double asterix. P-values between 0.05 and 0.10 are indicated by one asterix (Mann-Whitney U test)

The Dim light melatonin onset (DLMO) results are presented in Table 4. Three of four controls had the expected early melatonin secretion while 5 (or 6 depending on the method) of DSPS patients had indications of a delayed response. Absolute levels are quite variable.

Table 4. Melatonin concentration in saliva (pg/ml)

	Basal evening level[1]	2300h pm	0000h am	DLMO 2x basal	+3SDbasal
Pas1	6.7	**11.0**	13.2	delay	**Early**
Pas2	17.9	19.5	20.8	delay	Delay
Pas3	17.5	19.5	25.9	delay	Delay
Pas4	10.0	16.3	9.4	delay	Delay
Pas5	16.4	26.3	28.6	delay	Delay
Pas6	5.9	7.6	4.4	delay	Delay
Co1	9.9	10.1	**20.7**	**early**	**Early**
Co2	6.6	7.0	9.2	delay	Delay
Co3	11.0	**46.2**	50.0	**early**	**Early**
Co4	7.1	8.6	**14.2**	**early**	**Early**

DLMO: Delayed melatonin onset categorization with two different cutoffs (2x baseline and $3SD_{(baseline)}$. (individual baseline SDs are not tabulated) [1] Basal evening level is the mean of measurements at 20, 21 and 22h. Normal (early) onsets are shown in bold

The polysomnography recordings from the experimental night show that patients have a greater sleep onset latency (SOL) compared to the controls. Values for SOL for stages N1, N2 and N3 deep sleep are noted in Table 5 below. The numbers show that patients have a greater SOL through all stages except for REM compared to the controls.

Table 5. PSG recorded SOL values for patients (Pat) and controls (Co)

	SOL N1	SOL N2	SOL N3	SOL REM
Pas1	01:52	01:55	02:21	01:03
Pas2	00:09	00:29	00:39	02:14
Pas3	01:09	01:20	01:33	01:27
Pas4	00:40	00:59	01:05	01:31
Pas5	00:21	00:29	02:53	01:37
Pas6	00:48	00:59	01:13	01:34
Co1	00:02	00:03	00:14	01:04
Co2	00:12	00:20	00:34	01:02
Co3	00:01	00:02	00:10	02:04
Co4	00:22	00:26	00:33	03:33
p-value	0.067*	0.010**	0.010**	1.000
Mean (SD) Pas	00:50 (00:36)	01:02 (00:32)	01:38 (00:50)	01:34 (00:22)
Mean (SD) Co	00:09 (00:10)	00:13 (00:11)	00:23 (00:12)	01:55 (01:10)

P-values < 0.05 are indicated by a double asterix. P-values between 0.05 and 0.10 are indicated by one asterix (Mann-Whitney U test)

In Table 6 the values for total sleep time (TST), sleep efficiency (SE) and amount of time spent in each stage N1, N2, N3, including the amount of deep sleep for the last three hours before waking (0400-0700 h) and the amount of REM sleep throughout the night are summed up. The numbers show a significant difference in amount of deep sleep where controls have more deep sleep than the patients. In addition, there is a significant difference in the amount of REM sleep, where controls spent more time in REM stage than the patients. Amount of sleep spent in each stage relative to total sleep time is shown in Figure 2.

Table 6. PSG recorded TST, SE, amount of time spent in stages N1, N2, N3, N3 (0400-0700 h) and REM

	Total-sleep-time	Sleep-efficiency	N1	N2	N3	N3 04-07 h	REM
Pas1	04:21	68.9 %	00:05	02:21	01:08	00:22	00:46
Pas2	06:04	91.1 %	00:32	03:28	01:11	00:16	00:51
Pas3	04:26	67.7 %	00:47	02:32	00:51	00:01	00:13
Pas4	05:30	83.0 %	00:23	02:48	01:27	00:21	00:50
Pas5	04:10	62.7 %	01:51	00:58	00:46	00:12	00:34
Pas6	05:40	85.3 %	00:19	02:54	01:19	00:06	01:07
Co1	06:33	97.4 %	00:11	02:47	02:05	00:43	01:27
Co2	05:29	80.8 %	00:42	02:00	01:42	00:56	01:03
Co3	06:26	96.0 %	00:16	03:00	01:29	00:02	01:40
Co4	05:57	89.3 %	00:34	03:07	01:18	00:20	00:56
p-value	0.114	0.114	0.762	0.762	0.038**	0.352	0.038**
Mean (SD) Pas	05:02 (00:48)	76.5 % (11.5 %)	00:40 (00:37)	02:30 (00:50)	01:07 (00:15)	00:13 (00:08)	00:43 (00:18)
Mean (SD) Co	06:06 (00:48)	90.8 % (7.6 %)	00:26 (00:14)	02:44 (00:30)	01:39 (00:20)	00:30 (00:23)	01:17 (00:20)

P-values < 0.05 are indicated by a double asterix. P-values between 0.05 and 0.10 are indicated by one asterix (Mann-Whitney U test)

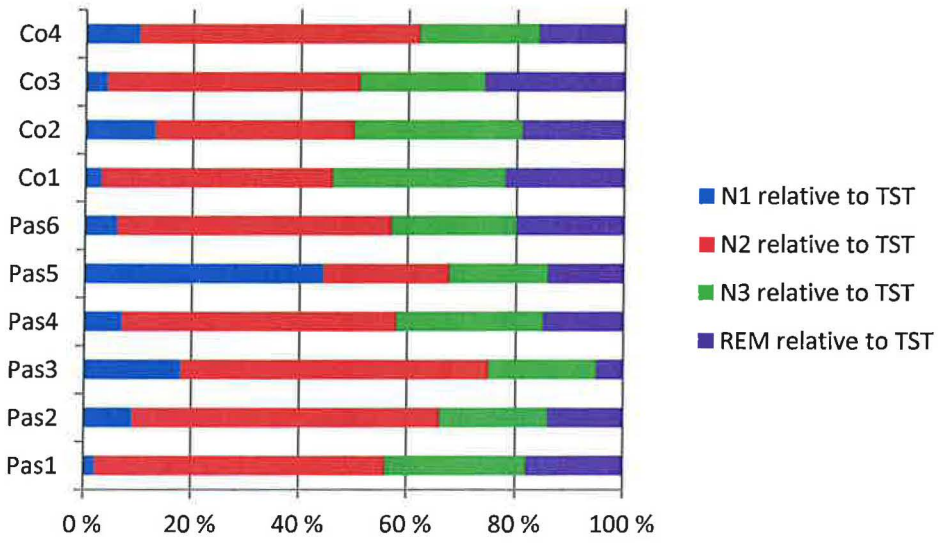

Fig.2 Amount of sleep in each stage relative to total sleep time

Upon awakening, it was noted whether the subject was woken up by the alarm clock or by the experimenter. The decibel (dB) value at which the subject reacted is also presented in Table 7 bellow. In addition, the stage in last epoch before alarm clock onset and the stage in last epoch before awakening are shown in the table. There is a significant difference in the sound intensity of the alarm clock at which the subjects react. Controls have demonstrated to be easier to wake up than the patients. Patient 1 and patient 6 did not react at all and had to be woken up by the experimenter. The PSG recording shows that they were both in REM stage of sleep. Patient 5 was awake before the alarm clock was activated. The patient had woken up on his own from REM stage. Most subjects woke up from either S2 or REM.

Table 7. Waking and dB values; stage in last epoch before alarm clock onset and stage in last epoch before awakening

	Wake up manually	**dB wake**	**Stage in last epoch before alarm clock onset**	**Stage in last epoch before awakening**
Pas1	yes	105	REM	REM
Pas2	no	78	REM	REM
Pas3	no	74	S2	S1
Pas4	no	80	S2	S2
Pas5	no	72	WAKE	REM
Pas6	yes	105	REM	REM
Co1	no	74	S2	S2
Co2	no	74	REM	REM
Co3	no	72	WAKE	S2
Co4	no	72	WAKE	S2
p-value		0.032**	0.2	0.43
Mean (SD) Pas		88.4(15.3)		
Mean (SD) Co		73.0 (1.2)		

P-values <0.05 are indicated by a double asterix. P-values between 0.05 and 0.10 are indicated by one asterix (Mann-Whitney U test and Difference between two proportions test (categorized as REM vs NREM))

Results from the continuous performance test (CPT) are summed up in Table 8 for baseline and Table 9 for the test. No significant differences were observed between the patient group and the control group on the baseline performance apart from a $p<0.067$ on hit response time. No significant differences were observed between the patient group and the control group on the test performance in the morning apart from a $p<0.067$ on standard error by block change.

Table 8. CPT values baseline

	Omissions	Commissions	Hit-rt	Perservations	Hit-rt-block-change	Hit-se-block-change
Pas1	good performance	within average	atypically fast	within average	good performance	within average
Pas2	within average	within average	a little fast	good performance	good performance	good performance
Pas3	good performance	within average	atypically fast	within average	good performance	good performance
Pas4	good performance	markedly atypical	atypically fast	within average	within average	within average
Pas5	within average	within average	within average	good performance	good performance	within average
Pas6	markedly atypical	markedly atypical	a little fast	mildly atypical	within average	markedly atypical
Co1	good performance	within average	a little fast	within average	good performance	within average
Co2	good performance	good performance	a little fast	within average	within average	good performance
Co3	within average	good performance	a little slow	good performance	mildly atypical	mildly atypical
Co4	within average	mildly atypical	a little fast	within average	markedly atypical	markedly atypical
p-value	0.762	0.257	0.067*	0.762	0.114	1.000

P-values < 0.05 are indicated by a double asterix. P-values between 0.05 and 0.10 are indicated by one asterix (Mann-Whitney U test)

Table 9. CPT values test in the morning

	Omissions	Commissions	Hit-rt	Perservations	Hit-rt-block-change	Hit-se-block-change
Pas1	good performance	within average	a little fast	mildly atypical	good performance	within average
Pas2	good performance	within average	a little fast	good performance	within average	within average
Pas3	within average	mildly atypical	within average	mildly atypical	within average	mildly atypical
Pas4	good performance	markedly atypical	a little fast	within average	within average	within average
Pas5	good performance	good performance	a little fast	good performance	good performance	good performance
Pas6	mildly atypical	markedly atypical	within average	markedly atypical	good performance	very good performance
Co1	within average	within average	a little fast	within average	good performance	within average
Co2	good performance	good performance	a little fast	good performance	within average	within average
Co3	good performance	good performance	a little slow	good performance	within average	mildly atypical
Co4	good performance	within average	within average	within average	mildly atypical	within average
p-value	0.914	0.171	0.476	0.352	0.257	0.067*

P-values < 0.05 are indicated by a double asterix. P-values between 0.05 and 0.10 are indicated by one asterix (Mann-Whitney U test)

The results of psychiatric measures including BDI and BAI as well as self-reported sleep measures including FFS, MEQ, SRBQ and SHI are summarized up in Table 10. No significant differences between patients and controls were found in levels of depressive symptoms. There was a trend that patients reported higher levels of anxiety on the BAI. On the FFS, patients reported significantly higher degree of sleep related daytime fatigue compared to the controls. On the MEQ, all patients scored within the "definitely evening type" category whereas three controls scored within the "neither type" and one within the "moderately evening type". There was a trend that the patients reported higher levels of maladaptive coping behaviors on the SRBQ, whereas no differences were found in sleep hygiene behaviors as assessed by the SHI.

Table 10. Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), Flinders Fatigue Scale (FFS), Morningness- Eveningness Questionnaire (MEQ), Sleep Related Behaviors Questionnaire (SRBQ), Sleep Hygiene Index (SHI) scores

	BDI	BAI	FFS	MEQ	SRBQ	SHI	ESS
Pas1	17	11	6	30	46	32	8
Pas2	5	12	12	17	55	38	8
Pas3	0	1	13	28	20	51	0
Pas4	15	33	12	29	74	42	6
Pas5	14	8	5	20	84	50	6
Pas6	32	26	15	21	72	39	2
Co1	1	1	4	57	31	47	3
Co2	6	6	6	34	40	37	7
Co3	3	6	3	44	9	63	4
Co4	0	4	0	48	24	40	1
p-value	0.114	0.067*	0.019**	0.01**	0.067*	0.762	0.610
Mean (SD) Pas	13.8 (11.1)	15.1 (11.9)	10.5 (4.0)	24.2 (5.5)	58,5 (23.3)	42.0 (7.3)	5 (3.3)
Mean (SD) Co	2.5 (2.6)	4.3 (2.4)	3.3 (2.5)	45.8 (9.5)	26.0 (13.1)	46.8 (11.6)	3.75 (2.5)

P-values < 0,05 are indicated by a double asterix. P-values between 0,05 and 0,10 are indicated by one asterix (Mann-Whitney U test)

4. Discussion

Qualitative and preliminary quantitative analysis of the data collected by means of polysomnography, acticraphy and self-reports indicate that in certain measures there is a difference between DSPS patients and controls while there are other areas where the groups may not differ from one another. The estimated differences between the two groups might vary but nonetheless be indicative of a real difference between them, helping us to generate hypotheses to be tested in a larger future study. It is worth mentioning that we present data from a pilot study because the planned full study could not yet be performed for technical and logistic reasons. Hence we have focused on reporting case histories and data for individual participants in some detail because this is more meaningful in a study with few participants. Nevertheless, certain differences between the groups were found despite the low sample. Caution will be taken as to generalize the results to a larger population of DSPS patients. The planned main study called for a greater amount of participants where the statistical analysis will have a greater statistical power.

Salivary melatonin data yielded more variable concentrations than expected, but when we calculated an evening baseline and looked for a define increase above this baseline in the late evening (using two different cutoffs), we were generally able to confirm that our DSPS patients had the expected lack of early secretion. Due to the PSG-protocol, we were not able to follow melatonin concentrations longer, and in this design we accordingly lack definite proof of a real delayed melatonin onset in patients. Also, our experience with this method is limited and it should be tested in a larger control group sample in order to find the best way to define the onset of the response [13].

As it is shown by the subjects' personal sleep diaries, DSPS patients go to bed later than the controls, an occurrence typical of, and indeed defining the disorder [34]. The patients also prefer to sleep longer unless they make a good effort to wake up early in order to attend school or work. Sleep diary data show that several patients do indeed start their days early if obligated to, but the same data also show that they make up for lost sleep by either taking afternoon naps or sleeping in on the weekends. The latter strategy is most frequently used. Actigraphy- recorded data support the sleep diary reports quite well. The two methods, meant to measure the same basic aspects of the circadian sleep structure, are seldom perfectly aligned, though it is neither expected nor necessary as long as subjective reports and actual recorded sleep times are similar and show identical patterns, as they do in all reported cases in this study.

None of the DSPS patients had undergone polysomnography earlier to determine the physiological basis of the diagnosis. As the PSG recordings from the experimental laboratory night show, the patient group did differ from the control group on several measurements. A significant difference in sleep onset latency for stages N2 and N3 is found, confirming the results from several other earlier studies [10, 11]. No difference was found regarding REM sleep onset latency, a finding that is in agreement with a previous study showing that latency to REM sleep in DSPS patients was not longer than normal [34]. The fact that DSPS group had a greater SOL confirms their sleep onset insomnia which is among the main characteristics of DSPS and has been reported on several other occasions [2, 11].

Although no significant differences were found, we observed that DSPS group had generally less total sleep time and lower sleep efficiency. The former result is probably a direct consequence of the longer latency. As the patients needed more time to fall asleep, they also got fewer hours of sleep as a result. The design of the study was such that they were enforced

to a certain schedule, but the schedule would be no different in real life where school and work call for an early awakening. As mentioned above, most of the patients do make an effort to get up early when they have to, but due to the few hours of sleep, most of them note tiredness and sleepiness in their self-reports. The self-reported data will be discussed further below.

One interesting hypothesis to investigate in this study was whether the patients had more SWS in the latter half of the night. The PSG recordings reveal that they do not have a greater amount of SWS compared to the controls and that the distribution is rather the opposite because mean values in controls were somewhat larger than N3 (SWS) in patients. The normal distribution of sleep stages is such that one has more SWS in the first half of the night, starting with longer periods of SWS which gradually become shorter and give way to REM dominance in the latter half of the night. Patients did also seem to have most of their SWS earlier on, making a gradual shift towards more REM sleep as seen in healthy individuals. Hence our results support previous studies reporting that sleep structure and quality in DSPS patients is not disturbed [8] although this still seems to be somewhat controversial [10]. Based on a low sample in present study it is hard to draw any definite conclusions on this subject. An important detail to mention is that much time was spent in light sleep N2 which might well be one of the explanations for sparse SWS during the last half of the night. Both patients and controls had a fair amount of light sleep, but based on the distribution of sleep relative to total sleep time, the patients had more relative light sleep than the controls. Worth mentioning is the finding that the patients had significantly less REM sleep throughout the night compared to the controls. Again, this might be understood by the fact that they had more time in the light sleep stages as well as their overall delay caused by the longer sleep latencies. A delayed and accordingly shortened (due to forced early awakening) sleep period will contain less absolute and relative REM due to the normal REM-excess in the last sleep cycles.

Another hypothesis put to the test in the present study was if the difficult awakening had a relation to a specific sleep stage. First, a significant difference in alarm clock sound intensity demonstrated that the patients indeed had a higher threshold and were more difficult to wake up. Patient 1 and patient 6 did not react to the alarm clock even at the highest sound intensity. As both patients were in REM sleep the difficult awakening might be explained by the incorporation of external stimuli into the dream, which is not unusual [23]. Another hypothetical explanation might be that the patients were in a "deeper state of REM". If this

hypothesis could be verified in future studies it would be a new discovery and possibly a characteristic biomarker for a subgroup of DSPS patients. This new hypothesis could be investigated further as frequent other observations show that awakening from REM in general is easy and possibly is linked to the presence of alpha waves in REM. Indeed, another published hypothesis states that REM sleep serves as a transition from sleep to wakefulness [23]. So if REM sleep normally is "lighter" in nature in order to make the transition into wakefulness easy, possibility of a "deep non-arousable REM state" in DSPS could be further investigated, e.g. by the more detailed analysis of arousability, EEG frequency content, saw-tooth waves and eye-movement characteristics. It should be noted however, that another DSPS patient woke up from REM at 78 dB sound intensity, only marginally higher than the control maximum of 74 dB.

As the results of the CPT did not show any significant difference on the cognitive measures between the groups, it is more interesting to look at the individual performances of all patients. Considering the low sample in the study, finding significant differences is hard, but nonetheless individual variations were observed.

Patient 1 did not differ from the average apart from a slight inattentiveness which had gotten worse in the morning compared to the baseline. The patient had otherwise a good performance. The patient did not react to the alarm clock and was difficult to wake up from REM sleep. Interesting in this case is the fact that even with the difficult REM awakening no worsening in the cognitive performance was observed. It would be interesting to take a closer look at this particular phenomenon as one may wonder how a cognitive performance might be good after such a difficult awakening.

Patient 2 did well on the test on both occasions. The patient woke up from REM sleep with no particular difficulty (to a sound level only slightly above control maximum) and his good performance in the morning is well in agreement with the hypothesis that REM serves as a smooth transition from sleep to wakefulness, thereby making it possible to function properly [23]. In addition, the study by Marzano et al. had shown that waking up from REM or N2 had no difference regarding the cognitive functional level right afterwards, meaning that level of confusion is less in both instances compared to an awakening from SWS [16].

Patient 3 had a good performance on the CPT baseline, but a slight worsening was detected when the test was taken upon awakening. More errors were made and the patient was less

consistent throughout the test. Otherwise no major attention problems were detected. The patient had woken up from light sleep N1 and the absence of major cognitive impairment is explained by the previous reports showing only slight or none cognitive impairment when woken up from light sleep [16].

Patient 4 had similar performances on both occasions where many mistakes were made due to a fast reaction time. This is indicative of an impulsive style, though the reaction time was slightly slower when tested after awakening. The patient woke up from N2 so this mild change in performance is once again supported by the fact that awakenings from N1 or N2 are followed only by a slight impairment [16]. His prior polio probably did not interfere with his cognitive abilities.

Patient 5 had an overall good performance when tested in the afternoon as well as after waking up in the morning. An important detail is that the patient was (atypically) well awake before the scheduled 0700 h awakening time. He woke up spontaneously from REM sleep and had been awake for approximately 20 minutes prior to testing. His good performance may accordingly be explained by more time awake before the test, and no confusion or other cognitive impairments were detected. It should be noted that some uncertainty about the DSPS-diagnosis was revealed in this patients as the possibility of a free-running rhythm disorder emerged.

Patient 6 had many errors compared to average, and the performance was on both instances detected to be erratic and indicative of poor attention. The patient was also difficult to wake up from REM sleep so one might question if the bad performance could partly be caused by the difficult awakening. However, the fact that no difference was found from baseline test and the test in the morning indicates that neither short sleep nor a difficult awakening contributed to her low scores.

To summarize the overall CPT-results displayed in Tables 8 and 9 it seems that the variability between patients is larger than between controls as some perform as good as controls while some have inferior performances. While some controls actually improve after sleeping, some DSPS patients show some minor deterioration.

It should also be noted that patient 4 could be deemed as an exception to the excluding criteria for the study as he had recovered from poliomyelitis, although as mentioned above this did

not seem to affect his cognitive abilities. The patient was included because the disease was no longer active. However, one should not look away from the possibility that the polio diagnosis might have an effect on sleep quality, although it has not been reported to have any specific influence on the DSPS diagnosis.

Also, after a closer look, an alternative diagnosis emerged for patient 5. The sleep diary data is indicative of a free-running circadian rhythm disorder. This will be taken in consideration in order to adapt the treatment.

All patients except patient 3 had higher scores on the BDI and BAI, measuring depression and anxiety, respectively. We also found a significant difference on the MEQ measure where the DSPS patients reported being very much evening-chronotypes, three controls reported being neither morning nor evening-chronotypes and one control reported being moderately evening type. A study investigating the relation between morningness-eveningness score and depressive symptoms among DSPS patients showed a clear positive relation between the two [15]. "Reportedly, persons with evening chronotype have more attention problems and poor school achievement and are more emotionally upset… These psychological characteristics of the evening chronotype might contribute to the linkage between depressive symptoms and evening chronotype among DSPS patients." [15]

Another struggle reported by the DSPS patients is a feeling of tiredness and fatigue during the day. We found a significant difference between patients and controls on the FFS where the patients had higher scores indicative of greater feelings of tiredness and fatigue. Interestingly, no significant differences were observed on the Epworth Sleepiness Scale (ESS), which is used to measure daytime sleepiness. When looking at the individual data, none of the subjects and most importantly none of the patients reported values greater than 10, which is considered to be the limit above which medical assessment is recommended [25]. Based on the sleep diary and actigraphy data, we were able to see how most of the patients deal with the tiredness and compensate either by short naps during the day or longer sleep periods during the weekends. This method of compensating might further worsen the condition as it makes it even more difficult to find a way back to an appropriate rhythm [6].

The findings from the present study may be helpful in further improving and adjusting clinical treatment of the DSPS patients. Knowing whether the condition is caused by physiological or

social and motivational causes (or a combination) makes it easier to focus on the appropriate aspects of the treatment.

However, the study does have its limitations. Even though it is useful to focus on each case and give them the attention that they deserve, it is hard to draw any general conclusions based on a study with a low sample. Although many studies on DSPS are done on relatively low samples, it stands as a challenge to find a balance between having a sample large enough so that it is representative of a general population, while at the same time being able to explore each case in detail. Another limitation of the study is the fact that subjects were enforced to a certain schedule while at the sleep laboratory and there was no opportunity to test the subjects upon spontaneous awakening.

5. Conclusion:

In the present study, we could not confirm that difficult awakening from SWS is related to sleep inertia. No support was found for the hypothesis that DSPS patients have more SWS during the latter half of the night. We did however observe an interesting phenomenon of difficult awakening from REM sleep. Taking into account that our findings are based on a low sample, significant difference was difficult to find mainly due to a low test power.

It is therefore desirable to conduct a larger study with an additional night at the laboratory where the subjects are to follow their own rhythm such that they can be tested under the condition of spontaneous awakening. A closer analysis of EEG activity during REM and NREM sleep in DSPS patients is also of interest.

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