Sleep structure and awakening threshold in delayed sleep-wake phase disorder patients compared to healthy sleepers

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List of abbreviations:

DSWPD: Delayed sleep-wake phase disorder

AT: Awakening threshold

SOI: Sleep onset insomnia

PSG: Polysomnography

DLMO: Dim light melatonin onset

SOL: Sleep onset latency

TST: Total sleep time

SE: Sleep efficiency

ESS: Epworth sleepiness scale

FFS: Flinders fatigue scale

SHI: Sleep hygiene index

MEQ: Morningness - eveningness questionnaire

BDI: Beck depression inventory

BAI: Beck anxiety inventory

Abstract

Study Objectives:

Difficult early morning awakening is a primary symptom of delayed sleep-wake phase disorder (DSWPD), however, it remains poorly investigated. Our main objective was to quantify the awakening threshold in DSWPD-patients and healthy controls and investigate a possible relationship with sleep stage. A secondary objective was to compare habitual sleep measured by polysomnography and actigraphy between patients and controls.

Methods:

Twenty DSWPD patients and 16 controls had two polysomnographic recordings at a sleep laboratory. Participants followed their habitual sleep-wake schedule on the first night and a forced sleep-wake schedule (0000 - 0700 h) on the second night. A custom-made alarm clock was used for forced-night awakening, starting from 72 dB sound intensity and increasing up to 104 dB.

Results:

Mean awakening threshold in dB was higher in patients compared to controls; 75.5 vs. 72.6, $p = 0.01$, and the difference could not be explained statistically by sleep-time. Patients who were in REM sleep upon attempted awakening had a higher awakening threshold compared to patients who were in NREM sleep; 80.0 vs 74.7, $F = 6.4$, $p = 0.02$. Patients had increased sleep onset latency both at home with actigraphy and by PSG during the first laboratory night (20.6 vs 12.1 min, $p=0.004$), but no further differences between the groups were observed regarding sleep structure.

Conclusions:

High early-morning forced awakening threshold in DSWPD was related to REM sleep. Sleep onset problems, even with habitual bedtimes, may also be an integral feature of DSWPD.

Keywords

Delayed sleep wake phase disorder, difficult early morning awakening, awakening threshold, REM sleep, polysomnography.

1.1 Introduction

Delayed sleep-wake phase disorder (DSWPD) is a circadian rhythm disorder characterized by a misalignment of the circadian phase with later than optimal sleep onset time and difficult early morning awakening [1-3]. Difficult morning awakening is a primary symptom included in the diagnostic criteria for DSWPD in the third edition of the International Classification of Sleep Disorders (ICSD -3) [4]. This symptom often causes severe occupational and educational impairment, and it is described as an essential feature of DSWPD in ICSD-3. Still, research regarding the specific pathophysiological nature of difficult awakening is scarce.

We recently performed a controlled pilot study quantifying the awakening threshold (AT) in DSWPD-patients using an alarm clock with increasing sound intensity from 72 to 104 dB [5]. We observed a high AT in several patients, but a small sample size limited the generalizability of the results. We also had hypothesized, based on results by Saxvig et al., [6] that high AT would be related to slow wave sleep (N3). However, we did not observe any awakening from N3, and all patients who did not respond to the alarm clock at the highest sound-intensity were in REM sleep prior to awakening [5]. Such an unexpected finding should be retested in a different group of DSWPD patients. In addition, it is not yet clear if the high objective AT can be explained by the expected short sleep-time in DSWPD-patients who are awakened at 0700 h or by other mechanisms related to sleep structure or stage-specific arousability in DSWPD.

According to ICSD-3 [4], DSWPD patients sleep normally once sleep is initiated. Equivalent sleep structure and quality between DSWPD patients and normal sleepers is found in several studies [1, 6, 7]. However, at least one study has reported that quality and structure of sleep is compromised in this group of patients [8]. In addition, sleep onset insomnia (SOI) is a clinical problem as patients struggle to initiate sleep at a conventional time, [4] and SOI has also been

reported in patients going to bed at their preferred bed time [6, 9, 10]. To better understand sleep in DSWPD it is therefore important to measure sleep both during a habitual sleep-wake schedule and during a forced sleep-wake schedule similar to conventional bed- and rise times.

The first aim of the present study was to quantify difficult early morning awakening and the relationship with sleep stage in a larger DSWPD patient group. We hypothesized that patients would have a clearly higher auditory morning AT compared to healthy age-matched controls. We further hypothesized that difficult forced morning awakening would be related to REM sleep based on our previous study. Our second aim was to compare polysomnographic sleep structure between patients and healthy sleepers on a night with a habitual sleep-wake schedule and on a night with a forced sleep-wake schedule. In addition, we aimed to perform an exploratory correlation analysis between AT and sleep duration, sleep efficiency, insomnia symptoms, fatigue, sleepiness, and morningness-eveningness.

2.1 Methods

2.1.1 Participants

Patients were recruited from the Østmarka Sleep Clinic's waiting-list and by general advertisement on the University web page, local campuses and a local newspaper. To be included the patients had to meet the Diagnostic and Statistical Manual for Mental Disorders, 4th edition (DSM-IV 307.45) diagnostic criteria for circadian rhythm sleep disorder [11] and the ICSD-3 criteria for DSWPD [4]. All patients completed a 14-day sleep diary prior to a diagnostic interview at the Østmarka Sleep Clinic at St. Olavs Hospital. An experienced psychiatrist (twenty years of general clinical experience and thirteen years of clinical experience specifically with DSWPD) performed the diagnostic interview and the diagnostic assessments. If there was uncertainty about the diagnosis, the sleep diaries and patient sleep history were discussed with a clinical psychologist at the Sleep Clinic before final inclusion.

Twenty patients and sixteen healthy age-matched controls were included in the study. All participants in the control group had a normal circadian rhythm (defined as bedtime between 2300 - 2400 h and wake up time between 0700 - 0800 h) and were otherwise healthy (applying the same exclusion criteria as for patients).

Exclusion criteria were: Other sleep disorders, migraine, infections, cancer, neurological disorders, schizophrenia, other psychotic disorders, bipolar disorder, substance-related disorders, obsessive compulsive disorder, attention-deficit hyperactivity disorder, heart and lung disease, brain or cochlear implants, pregnancy/breastfeeding and daily use of sleep-disturbing medications the last four weeks. Only selective serotonin reuptake inhibitors were allowed (prescribed for one patient).

The study protocol was approved by the Regional Committees for Medical and Health Research Ethics (2011/1406). All participants signed a consent form upon inclusion.

2.1.2 Procedure

Data were collected from March 2014 to June 2016. One week prior to the experimental week, all participants kept a sleep-diary and wore a wrist-actigraph.

In order to familiarize subjects with the equipment and to minimize a possible ‘first-night effect’ [12] an ambulatory run-in polysomnography (PSG) was scheduled first. A fixed test-order was used for the two experimental nights, starting with habitual sleep (Table 1), in order to prevent sleep deprivation by forced sleep from interfering with habitual sleep.

Table 1. An overview of the experimental week.	
Monday - Tuesday	Ambulatory PSG at home.
Tuesday - Wednesday	Sleep laboratory night 1: Self-report questionnaires, DLMO, and PSG recording with free sleep opportunity between 2300 and 1200 h.
Wednesday - Thursday	Recovery night at home
Thursday - Friday	Sleep laboratory night 2: PSG recording with forced bed time (0000-0700 h). Awakening threshold test at 0700 h.
DLMO: Dim light melatonin onset. PSG: Polysomnography	

On Mondays participants came to St. Olavs Hospitals sleep laboratory at 1400 h for run-in equipment mounting by an experienced technician. They slept at home and followed their preferred sleep-wake schedule. The equipment was dismounted Tuesday morning, no later than 1200 h.

Tuesday was the first day and night spent at the sleep laboratory. The participants were asked to come at 1400 h to be prepared for a PSG. They completed several questionnaires during the evening (see below). At 1800 h the lights in the room were dimmed slightly below 100 lux. Lights on any electronic devices such as PCs or tablets were dimmed to slightly below 30 lux. Light intensity was measured by placing a lux meter just above the head facing the ceiling and just in front of the head facing the computer/tablet screen. Saliva for dim light melatonin onset (DLMO) was sampled hourly from 1900 h until self-determined bedtime. Earliest bedtime was set to 2300 h to ensure at least a five-hour span for saliva collection. Latest bedtime was set to 0400 h to ensure that at least eight hours of sleep was possible. Participants who did not wake up by 1200 h were woken up manually. Wednesday night the participants slept at home with no PSG assessments.

Thursday (the second night spent at the sleep laboratory), participants were allowed to leave the laboratory for few hours if requested after the PSG mounting at 1400 h, all returned to the

laboratory at 2300 h at the latest. Bedtime was scheduled for 0000 h. The PC-based alarm clock was activated at 0700 h on Friday morning. A set of speakers (SONY, Active Speaker System, SRS - Z510) was placed on each side of the bed, approximately 50 cm from the head. A custom-made tone generator was programmed to start at 72 dB sound pressure level, increasing by 2 dB with equal intervals (4.4 seconds sound, 5 seconds pause) up to 104 dB. The maximum sound intensity of 104 dB was reached after approximately 3 minutes. Activation threshold (AT) was scored in real time by one observer (BS) based on direct observation of behavior supplemented by online PSG recording. Subjects that woke up by themselves before alarm clock activation should have AT imputed to 70 dB. Subjects who did not react to the alarm clock and were to be awakened manually, should have AT imputed to 105 dB.

2.1.3 Actigraphy

A Philips Actiwatch Spectrum (Respironics Inc., PA, USA) was used to record 30-second epochs for seven days prior to the experimental week. Data were automatically scored using Philips Actiware 6.0.7 software with default ("medium") sensitivity. In addition, all data were manually inspected. Sleep start was cross-checked and adjusted according to the sleep diary. Sleep start, sleep end, sleep onset latency (SOL), total sleep time (TST), awakenings and sleep efficiency (SE) were averaged and used for analyses.

2.1.4 Polysomnography

We used a standard PSG-montage in accordance with the international 10-20 system and AASM technical recommendations [13]. Electroencephalography (EEG) included electrodes F3, F4, C3, C4, O1, O2, A1, A2 with Cz as reference. Electrooculography (EOG) included one electrode placed 1 cm over and 1 cm lateral to the right lateral canthus and one electrode placed 1 cm under and 1 cm lateral to the left lateral canthus. Electromyography (EMG)

electrodes were placed on the chin to monitor muscle activity. A nasal airflow sensor and an oronasal thermistor monitored nasal flow, pressure and airflow. An infrared O_2-sensor was placed on the index finger. During the two sleep laboratory nights, video and sound were recorded (VID65A, HD resolution 2048x1536, SOMNOmedics). Due to technical failure, we missed PSG recordings of the last 15 minutes of sleep for three patients who were awakened manually at 1200 h during the first laboratory night.

We scored sleep and respiration according to the AASM manual version 2.0. For respiration analyses the alternative ≥ 4% oxygen desaturation criteria were applied [14].

2.1.5 Melatonin

Food or beverage consumption was not allowed 30 minutes prior to each saliva sample taking. Samples were taken hourly between 1900 h and 2300-0300 h. Saliva was collected using the Bühlmann Salivette ® collection kit. Instructions provided by the manufacturer were followed. Caffeinated drinks were not consumed on the evening of saliva collection. Certain foods were avoided, such as bananas, chocolate, milk and almonds. 15 minutes prior to each sample taking participants were asked to rinse their mouth with cold water. Saliva was collected by chewing on the provided swamp for a minimum of 1-2 minutes. Samples were immediately stored in a refrigerator at 4 °C. The samples were analyzed using the Bühlmann Direct Saliva Melatonin ELISA kit. Dim light melatonin onset (DLMO) was defined as the time at which melatonin concentration reached a 4 pg/ml threshold [15, 16].

2.1.6 Self- reported measures

Daytime tiredness: Assessed by a single item targeting the frequency of daytime tiredness on a scale from 0 – 5 where 1 = no problem with tiredness, 2 = less than 7 days per month, 3 = 7-14 days per month, 4 = more than 14 days per month, 5 = daily.

Insomnia symptoms: Scores for early, middle and late insomnia symptoms were obtained from the following questions from Karolinska sleep questionnaire: i) difficulty falling asleep, ii) nocturnal awakenings, iii) early awakenings and difficulty falling back to sleep, where 0 = never, 1 = a few times per year, 2 = a few times a month, 3 = several times per week and 4 = daily [17].

Epworth Sleepiness Scale (ESS): The ESS is an 8-item questionnaire used to assess daytime sleepiness. The questionnaire measures the probability of dozing off or falling asleep during different daily situations. Scores range from 0-24 [18].

Flinders Fatigue Scale (FFS): The FFS is a 7-item questionnaire used to assess daytime fatigue [19].

Sleep Hygiene Index (SHI): SHI is a 13-item questionnaire assessing the frequency of sleep inhibitory behaviors [20].

Horne – Östberg Morningness Eveningness Questionnaire (MEQ): MEQ is a 19-item questionnaire assessing level of diurnal preference. The cut-off values are ≤ 41 = evening type; 42-58 = intermediate; ≥ 59 morning type [21].

Beck Depression Inventory (BDI): BDI is a 21-item self-report measure of behavioral, cognitive and affective symptoms associated with clinical depression. The cut-off values are: 0-9 = normal; 10-18 = mild; 19-29 = moderate; and ≥ 30 = severe depression [22].

Beck Anxiety Inventory (BAI): BAI is a 21-item self-report measuring the intensity of cognitive, affective and somatic anxiety symptoms on a 4-point Likert scale. The cut-off values are defined as 0-21 = low anxiety; 22-35 = moderate anxiety; and ≥ 36 = potentially concerning levels of anxiety [23].

Sleep diary: All participants completed a sleep diary for a total of seven days prior to the experimental week. Participants used the Consensus Sleep Diary [24] and sleep data from seven days were averaged and used for analysis.

Subjective level of sleepiness: Tuesday and Thursday afternoon, as well as Wednesday and Friday morning immediately upon awakening, patients were asked to report their subjective level of sleepiness on a scale from 0 to 100 where 0 = not sleepy at all and 100 = could fall asleep within the next minute.

2.1.7 Data analysis:

IBM SPSS Statistics 22.0 (Armonk, NY: IBM Corp.) software was used to perform the statistical analyses. We used a non-parametric Mann-Whitney U test for all group-comparisons. We applied ANCOVA to adjust the between-group AT-difference for TST, and a two-way ANOVA to investigate how sleep stage affected AT in the two groups. In addition, we calculated exploratory multiple regression models with backward selection to predict AT based on PSG variables and self-reported measures. AT was LN-transformed for parametric testing. Non-parametric exploratory Spearman analysis was also applied for the correlation between AT and sleep duration, sleep efficiency, insomnia symptoms, fatigue, sleepiness, and morningness-eveningness. We considered p-values below 0.05 as significant. The power to detect an effect = 0.96 SD, based on a two-sample Student's t-test, was 80 %. In order to preserve statistical power in the exploratory part of the study, we did not apply Bonferroni-type adjustments [25]. For partially missing night-1 PSG-data for about 15 minutes before awakening in two patients, we also performed a sensitivity analysis by imputing missing epochs with "extreme" sleep stage values, first by N3 and then with REM.

3.1 Results

3.1.1 Descriptive data

We present demographic and health data regarding sleep, chronotype, depression and anxiety as well as timing of DLMO in Table 2. Daytime tiredness and fatigue affected patients significantly more than healthy controls but there was no statistical difference for daytime sleepiness. Patients scored significantly higher for early insomnia-symptoms, however, we observed no differences between the groups regarding middle or late insomnia-symptoms. Further, patients reported slightly poorer sleep hygiene compared to healthy sleepers. All patients scored as evening chronotypes on the MEQ, compared to controls who scored as either intermediate or morning chronotypes. DLMO occurred more than two hours later in patients compared to controls, although with a noticeably large variance within the patient group. In addition, as illustrated in Figure 1, the concentration of melatonin was significantly lower in patients and eight patients never reached the 4 pg/ml threshold during the saliva collection period. Patients with DSWPD reported significantly higher levels of depression and anxiety (Table 2).

Actigraphy data confirmed the patients' delayed phase with average sleep onset delayed by almost three hours compared to healthy sleepers (Table 2). Sleep-offset was also delayed by approximately 2.5 hours. Patients had longer sleep onset latency (SOL) and lower sleep efficiency (SE) than controls, but we observed no significant differences regarding total sleep time (TST). Data from sleep diaries confirmed the later sleep onset and offset times in the patient group compared to healthy controls. Sleep diary SOL was also greater in the patient group, although the difference was not significant due to the large variance within patients. Four patients and zero controls overestimated SOL by more than 50 minutes ($p = 0.06$ for difference in proportions), but the overall diary-actigraphy difference was not different between groups (Mann-Whitney p-value = 0.95).

Table 2. Descriptive, clinical, actigraphy and sleep diary data for DSWPD-patients and controls (mean and SD).

	DSWPD	Controls	p-value[e]
Number	20	16	ns
Age	24.8 (3.0)	24.4 (3.4)	ns
Male/Female (N)	9/11	4/12	ns
Student/Employed/Neither (N)	16/1/3	11/5/0	ns
Daytime tiredness (1-5)[a]	3.3 (1.3)	1.4 (0.6)	< 0.0005
Early insomnia symptoms (0-4)[b]	2.9 (1.0)	0.9 (0.7)	< 0.0005
Middle insomnia symptoms (0-4)[b]	1.1 (1.0)	1.7 (1.2)	ns
Late insomnia symptoms (0-4)[b]	0.8 (0.9)	0.9 (1.0)	ns
Epworth sleepiness scale	6.5 (4.4)	4.2 (3.0)	ns
Flinders fatigue scale	12.0 (7.2)	5.4 (5.5)	0.007
Sleep hygiene index	41.1 (4.5)	44.8 (6.1)	0.049
Morningness-Eveningness	29.3 (4.9)	54.7 (6.2)	< 0.0005
Beck depression inventory	10.8 (9.4)	3.4 (3.1)	0.006
Beck anxiety inventory	9.5 (7.1)	4.9 (8.2)	0.007
DLMO[c] (h)	00:45 (01:24)	22:22 (00:37)	< 0.0005
Actigraphy [d]			
Bed time	02:53 (01:36)	00:10 (00:36)	< 0.0005
Get up time	11:11 (01:47)	08:32 (00:40)	< 0.0005
Sleep onset latency (min)	19.3 (12.0)	11.3 (6.9)	0.034
Total sleep time (min)	422.0 (38.0)	446.0 (30.0)	ns
Sleep efficacy (%)	84.4 (3.6)	88.6 (1.9)	< 0.0005
Sleep diary [d]			
Lights off (h)	02:38 (01:50)	00:25 (01:10)	< 0.0005
Lights on (h)	10:58 (01:50)	08:51 (01:13)	< 0.0005
Sleep onset latency (min)	43.5 (55.6)	17.3 (11.2)	ns
Total sleep time (min)	462.0 (42.0)	480.0 (42.0)	ns

h: hours, [a] (1: never, 2: less than 7 days a month, 3: 7-14 days a month, 4: more than 14 days a month, 5: daily). [b] Scores for early, middle and late insomnia symptoms (scored as 0=never to 4= daily in the Karolinska sleep questionnaire). [c] Dim light melatonin onset (4 pg/ml threshold). Eight patients did not reach the 4 pg/ml

threshold, and their missing DLMO-values were imputed to the nearest whole hour after self-chosen bed-time (time for the next planned saliva test) for statistical analysis. Patients who reached the threshold (n=12) had mean DLMO = 00:20 (SD = 01:44). [d] Average from 7 days. [e] Mann-Whitney U test. ns: Not significant.

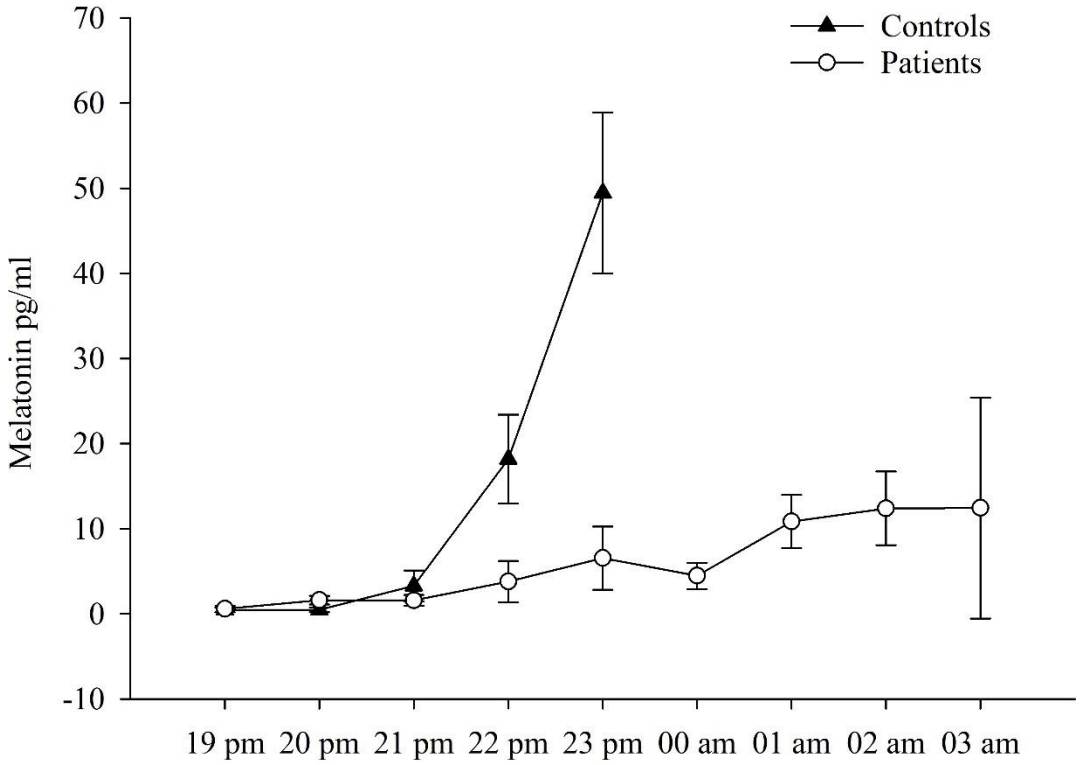

Figure 1. Hourly melatonin concentration (means and SD) in DSWPD-patients and healthy controls. Samples taken from 1900 h until self-chosen bed-time. We calculated melatonin concentration from nine patients at 0100 h, from four patients at 0200 h, and from two patients at 0300 h.

3.1.2 Hypothesis 1: Difficult awakening

Patients were more difficult to wake up than healthy controls, as indicated by higher mean dB threshold of the alarm clock, 75.3 (SD = 4.3) vs. 72.6 (SD = 1.0), p = 0.01, Cohen's d = 0.63 (Figure 2).

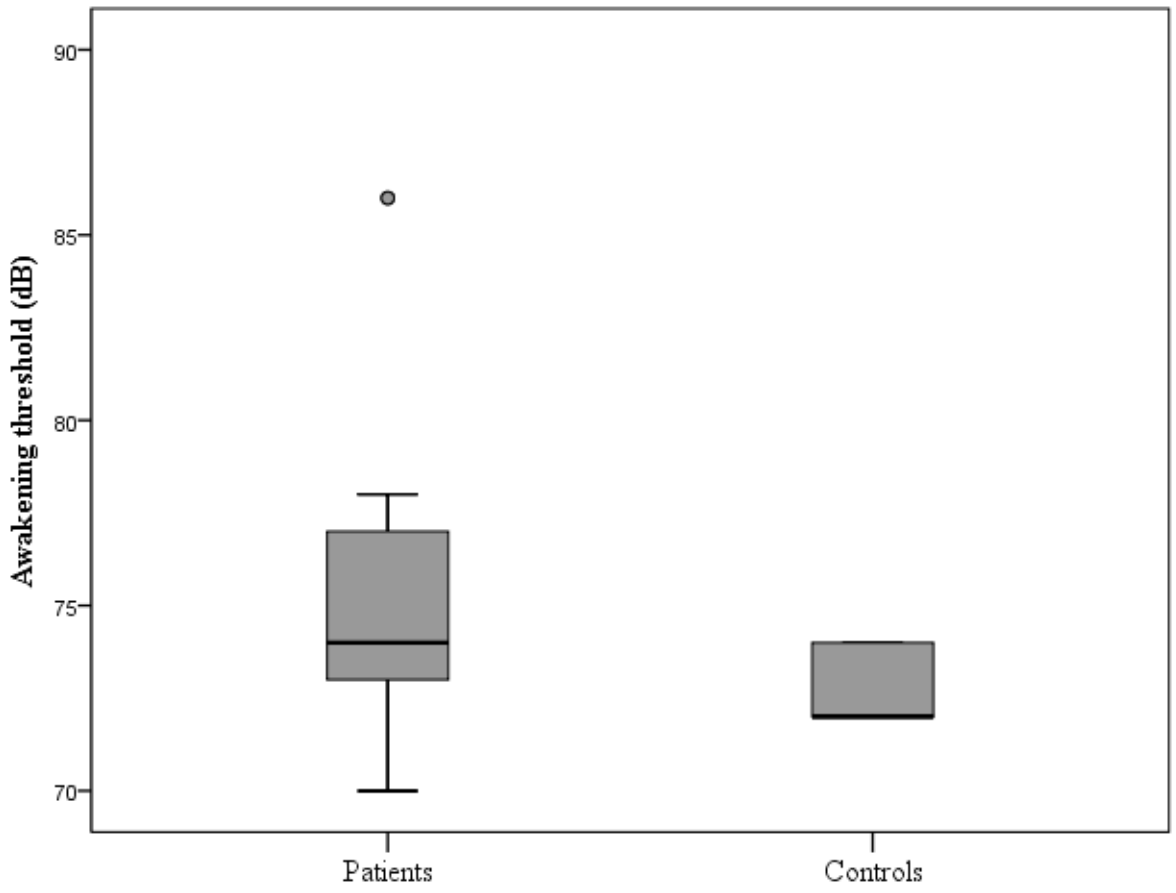

Figure 2. A box plot illustrating the differences in awakening threshold during the second laboratory night between DSWPD-patients and controls.

Analysis of covariance confirmed that the significant group difference remained, F (1,33) = 5.3, p = 0.02 and could not be explained by TST, F (1,33) = 0.50, p = 0.48.

Two patients woke up spontaneously before the alarm; one patient directly from N3 two minutes before the alarm clock and one patient from N1 one hour and twenty minutes before the alarm clock.

Only one patient did seemingly not react to the highest intensity of 104 dB, and the investigator had to awaken him manually. Upon subsequent analysis of the sleep profile, it was determined that the patient had an arousal from stage N2 sleep a few seconds before the alarm clock was activated, and he was in light sleep stage N1 upon attempted awakening. EEG analyses disclosed that the patient had another arousal to the auditory stimuli at 72 dB followed by a typical occipital alpha rhythm throughout the increasing sound intensity from the alarm clock. In spite of the seemingly "awake" EEG, he remained motionless in bed, and afterwards he had no memory of any alarm-clock sound. His social hearing did not seem to be otherwise impaired.

3.1.3 Exploratory correlation analysis of awakening threshold

Table 3 shows significant correlations between awakening threshold (AT) and descriptive variables, PSG variables and actigraphy variables in the merged patients and controls group (n=36). A positive correlation was observed between AT and SOL, daytime fatigue, degree of tiredness after forced awakening, bed time and get up time. AT correlated negatively with TST, SE and MEQ (evening chronotypes have a higher AT). We did not observe any significant correlation between AT and DLMO for the merged groups, or when the groups were analyzed separately.

Table 3. Significant correlations between awakening threshold and sleep-quality measured by polysomnography (PSG), fatigue score, chronotype, subjective feeling of sleepiness and actigraphy in patients and controls (n=36).

	Correlation coefficient[a]	p-value[a]
PSG from the second forced-schedule night		
Sleep onset latency	0.515	0.001
Total sleep time	-0.339	0.043
Sleep efficiency	-0.360	0.031
Questionnaires		
Flinders fatigue scale score	0.452	0.006
Morning-Eveningness questionnaire score	-0.409	0.013
Subjective sleepiness upon awakening[b]	0.351	0.036
Actigraphy[c]		
Sleep onset latency	0.580	< 0.0005
Lights off	0.340	0.046
Lights on	0.448	0.007

[a]Spearman's rho. [b] Subjective level of sleepiness measured by VAS upon awakening from the second laboratory night. [c] Average from 7 days.

3.1.4 Multiple regression:

A backward multiple regression with AT as dependent variable and TST, SOL, FSS, ESS and insomnia-symptom score as initially included independent variables showed that only SOL during forced-schedule laboratory night ($p = 0.009$) and FFS ($p = 0.007$) significantly predicted AT upon awakening ($F (2, 33) = 8.97$, $p = 0.001$, adjusted $R^2 = 0.31$).

3.1.5 Hypothesis 2: Sleep stage upon awakening

Table 4 shows similar sleep stage distributions upon awakening between groups and between the two sleep schedules. We performed a two-way ANOVA to investigate how sleep stage (REM vs NREM) and group (18 DSWPD vs 16 controls) affected AT, and a significant

interaction was observed between group and stage upon alarm clock activation F (1, 30) = 5.93, p = 0.02. Contrast analyses, by considering the main-effects in separate one-way ANOVAs per group, showed that patients had a significantly higher AT when in REM sleep (mean = 80.0 dB (SD = 7.1), median = 86 dB), as opposed to NREM sleep (mean = 74.7 dB (SD = 2.0), median = 72 dB), F (1, 16) = 6.41, p = 0.02. No such difference was observed within the control group (REM sleep: mean = 72.7 dB (SD = 1.0), median = 72 dB, NREM sleep: mean = 72.6 dB (SD = 1.0), median = 72 dB, F (1, 14) = 0.02, p = 0.90).

Table 4. Stage upon awakening during first and second night at the laboratory.

	Habitual sleep schedule (Night 1)		Forced 00-07 h sleep schedule (Night 2)	
	DSWPD[a]	Controls	DSWPD[b]	Controls
N1	3	2	5	1
N2	8	6	9	9
N3	1	1	2	0
REM	5	7	4	6

[a] Missing data for 3 patients on night 1. [b] One patient woke up spontaneously from N3 while another patient woke up spontaneously from N1.

3.1.6 Sleep structure

Habitual sleep onset and offset was significantly delayed in patients compared to healthy controls, as expected (Table 5). SOL was also longer in patients than controls. Both groups had similar TST, SE, as well as percent of sleep spent in each sleep stage.

Table 5. Polysomnographic data from the first laboratory night (free sleep opportunity between 23:00 h and 12:00 h).

	DSWPD[b]	Controls	p-value[c]
Lights off (h)	01:10 (01:18)	23:29 (00:20)	< 0.0005
Lights on (h)	10:53 (01:13)	08:26 (00:50)	< 0.0005
SOL N1 (min)	20.6 (8.8)	12.1 (6.6)	0.004
SOL N2 (min)	30.0 (14.7)	21.2 (7.5)	0.041
SOL N3 (min)	44.9 (15.4)	35.0 (7.2)	0.017
SOL REM (min)	82.2 (30.6)	95.3 (35.5)	ns
SE (%)	91.7 (4.2)	92.7 (3.2)	ns
Sustained SE (%)	95.3 (3.5)	95.0 (3.1)	ns
TST (min)	527.1 (101.8)	498.9 (52.4)	ns
N1 (min)	43.3 (26.5)	42.2 (20.0)	ns
N2 (min)	270.9 (71.0)	255.1 (43.6)	ns
N3 (min)	80.7 (31.6)	87.9 (27.8)	ns
N3 3h before awakening[a] (min)	9.1 (12.2)	9.8 (10.5)	ns
REM (min)	132.2 (52.2)	113.7 (27.7)	ns
N1 percent of TST	8.3 (5.3)	8.6 (4.3)	ns
N2 percent of TST	51.1 (6.4)	51.0 (5.2)	ns
N3 percent of TST	16.3 (7.9)	17.7 (5.7)	ns
REM percent of TST	24.3 (7.0)	22.7 (4.8)	ns
WASO (min)	24.4 (14.1)	26.4 (15.6)	ns
AHI (h^{-1})	0.5 (1.0)	0.1 (0.2)	ns
Awakening index (h^{-1})	1.7 (0.7)	1.9 (0.6)	ns

Values are presented as mean (SD). [a] A study by Saxvig et al.[6] found that DSWPD patients had significantly more deep sleep in the early morning hours. [b] Missing last 15 minutes of PSG recording for two patients. [c] Mann-Whitney U test. ns: Not significant. SOL: sleep onset latency; SE: sleep efficiency; TST: total sleep time; WASO: Wake after sleep onset; AHI: Apnea-hypopnea index.

On the forced-sleep night (Table 6), we observed larger SOL in patients compared to healthy controls as expected. SE was accordingly also significantly lower in the patient group,

however, there was no significant difference in ”sustained SE” (that is SE after sleep onset). N2, N3, REM-times and TST were significantly shorter in the patient group, however, there were no significant differences regarding awakening index or percentage of sleep in any particular sleep stage. The sensitivity analysis also showed that the statistical conclusions would have been unchanged with complete data for two patients in question.

Table 6. Polysomnographic data from the second laboratory night (forced bedtime 0000-0700 h).

	DSWPD	Controls	p-value[a]
SOL N1 (min)	47.9 (35.7)	11.5 (5.8)	< 0.0005
SOL N2 (min)	67.0 (50.4)	24.0 (11.4)	< 0.0005
SOL N3 (min)	87.8 (50.0)	40.7 (12.9)	< 0.0005
SOL REM (min)	108.5 (79.9)	84.0 (24.8)	ns
SE (%)	80.0 (14.8)	94.2 (1.8)	< 0.0005
Sustained SE (%)	90.8 (15.2)	97.0 (1.2)	ns
TST (min)	334.9 (62.5)	395.8 (7.5)	< 0.0005
N1 (min)	39.9 (33.6)	31.3 (16.4)	ns
N2 (min)	171.5 (45.0)	202.0 (22.8)	0.015
N3 (min)	61.8 (19.1)	84.0 (23.5)	0.004
N3 3 h before awakening[b] (min)	16.2 (13.5)	9.5 (11.0)	ns
REM (min)	61.8 (25.9)	78.5 (14.1)	0.042
N1 percent of TST	11.9 (9.3)	7.9 (4.1)	ns
N2 percent of TST	50.7 (8.1)	51.1 (6.0)	ns
N3 percent of TST	19.5 (8.4)	21.2 (5.8)	ns
REM percent of TST	18.0 (6.8)	19.8 (3.6)	ns
WASO (min)	35.0 (58.4)	12.1 (5.0)	ns
AHI (h^{-1})	0.4 (0.7)	0.0 (0.1)	ns
Awakening index (h^{-1})	2.5 (1.7)	1.9 (0.6)	ns

Values are presented as mean (SD). [a]Mann-Whitney U test. ns: Not significant. SOL: sleep onset latency; SE: sleep efficiency; TST: total sleep time; WASO: Wake after sleep onset; AHI: Apnea-hypopnea index. [b] A study by Saxvig et al.[6] found that DSWPD patients had significantly more deep sleep in the early morning hours.

4.1 Discussion

We report three main findings in the present study: 1) DSWPD-patients are objectively more difficult to wake up than controls with a normal circadian rhythm. Although the variability was large among patients and the measured mean difference in dB was rather small, Cohen's effect difference was moderately sized [26]. 2) Although *severely* difficult awakening from REM-sleep was not a prevalent feature, the present study confirmed that DSWPD-patients, as opposed to controls, were significantly more difficult to wake up from REM sleep than from NREM sleep. 3) Increased SOL was the only habitual sleep-variable difference between DSWPD-patients and controls. This confirms previous observations of increased SOL even with self-chosen habitual bedtime routines in DSWPD-patients [6, 9, 10]. Although the observed objective habitual SOL in the present patient sample was not alarmingly high, it was double the time of healthy controls, and a similar difference was seen both in actigraphy and the sleep diary.

We confirmed our hypothesis that patients would have a higher AT than healthy sleepers when forced to an early awakening schedule. A possible explanation is that patients due to prolonged sleep onset latency had less total sleep time, thus not being adequately rested upon forced awakening. This idea is further strengthened by the observed correlation between shorter sleep time and higher sound intensity required for arousal. This is highly representative of patients' everyday life as they struggle to wake up upon early morning no matter how early they try to go to sleep. However, ANCOVA-analysis showed that TST could not explain this difference statistically. In addition, the positive significant correlations between AT and both lights-off and lights-on times on actigraphy suggest that the "trait DSWPD-severity", i.e. the magnitude of circadian delay, and not only the state "low TST on forced awakening night" is related to and reflected by objective awakening difficulty measured by AT in the present study.

Since all patients were either clinically or electroencephalographically awake before the maximum sound intensity of the alarm clock was reached, we could not directly replicate the findings from our previous study where three out of nine patients did not react to the highest intensity of the alarm clock while in REM sleep [5]. However, we did observe that patients who were in REM sleep upon alarm clock activation had a significantly higher AT, meaning that they required higher auditory stimulation in order to wake up than patients who were in NREM sleep. Older studies suggest in general that awakening thresholds are elevated in SWS compared to REM and stage-2 sleep [27], and Busby et al. [28] confirmed this result for adults. However, elevated auditory thresholds in REM have been described [29], and awakening thresholds in REM were much higher in adolescents and children than in adults [28]. Pilon et al. [30] also reported that higher auditory stimulation is required for awakening from REM than NREM in sleepwalkers compared to healthy controls, possibly caused by fragmented NREM sleep and 'overflow' of slow wave activity into REM. These findings seem rather interesting considering that REM sleep has been referred to as an easy transition from sleep to wakefulness [31] and most healthy subjects have a preference towards awakening from REM sleep [32, 33]. Another study has taken a step further and investigated auditory AT in tonic REM sleep vs. phasic REM sleep [34]. The latter study concludes that arousal threshold in tonic REM was indifferent from that in stage N2, while arousal threshold in phasic REM was larger and similar to that of deep sleep N3. Hence, both the present and previous studies clearly suggest that processing of external stimuli is reduced during REM sleep, possibly related to the well-known phenomenon called "stimuli incorporation": External stimuli from the alarm clock can be incorporated into a dream during REM sleep, preventing arousal and subsequent awakening [35]. However, we could neither compare tonic and phasic REM nor compare N3 to N2 with the present design and sample size.

During the night of self-chosen sleep-wake schedule, patients went to sleep almost two hours earlier than their diary-reported (and actigraphy-confirmed) habitual lights-off time. One of the factors contributing to their unusual 'early' bed time could be the restricted, light-deprived, unfamiliar, and probably a bit boring, laboratory environment. This interpretation is in line with a recent study by Joo et al. [36]. These observations suggest that DSWPD patients might fall asleep earlier than their habitual reported bedtime if the environment is kept really optimal for sleep-induction.

However, it is also important to notice that patients had longer sleep latency than controls both on the night of the self-chosen schedule and measured with actigraphy at home [6, 9, 37]. They used on average about 20 minutes to fall asleep, almost double the time of healthy sleepers, but slightly less than 36 minutes reported by Saxvig et al. [6]. Hence, DSWPD-patients seem to fall in the "high-normal range" for sleep latency, suggesting a tendency towards early insomnia. This was also detected by the early insomnia question in the Karolinska questionnaire in the present study (Table 2). In addition, diary-reported and actigraphy-calculated TST was shorter in patients than controls, suggesting that increased SOL cannot be explained by a difference in accumulated sleep pressure. Also, SOL (both from PSG and actigraphy) correlated positively with high AT, strengthening the notion that this trend towards early insomnia may be an integral feature of DSWPD, possibly a conditioned problem with sleep onset based on previous experiences [38].

Interestingly, a few patients over-estimated their sleep onset latencies considerably, by more than 50 minutes compared to controls. Another study also observed a similar phenomenon in misinterpretation of sleep onset in DSWPD-patients, but did not have a comparable healthy control group [37]. Sleep state misperception is also often observed in insomnia, also indicating that insomnia-like mechanisms may be relevant for DSWPD patients [39].

We observed no further differences in the polysomnographic data between the two groups on the night of the self-chosen sleep-wake schedule. Patients sustained sleep as well as healthy sleepers once sleep was initiated. They spent approximately equal amount of time asleep and sleep was equally divided in regards to respective sleep stages. These findings suggest that DSWPD patients have normal sleep structure and quality after sleep onset, as has been reported by most other groups [1, 6, 7]. The differences that were observed during the night of the forced sleep-wake schedule were mainly restricted to longer SOL and consequent shorter TST and lower SE in the patient group. Thus, the significant differences observed in amount of sleep spent in N2 and N3 stages between the groups is a mere reflection of shorter TST for patients. We found no differences regarding percentage of sleep spent in each stage, further supporting the notion that sleep structure does not differ in DSWPD-patients once sleep is initiated.

The correlation analysis clearly shows that high FFS scores and low MEQ scores (and sleepiness) are related to AT, suggesting that objective difficult awakening, as measured in the present second-night test, reflects both objective traits (phase delay severity and sleep onset latency), subjective traits (eveningness and fatigue levels), and state variables measured by the forced-scheduled PSG (high SOL, low TST and low SE). As we did not have all-night temperature measures, it is difficult to say precisely to which degree difficult awakening was related to, or could be predicted by circadian phase, although we did not observe any significant correlation between AT and DLMO.

The present study may also have clinical implications. First, the finding that patients have a higher AT, indicates that it might be necessary to focus specifically on methods to wake the patients up. Second, the finding that DSWPD patients were able to fall asleep earlier in laboratory environment, indicates that it may be beneficial to focus on stimulus reduction in treatment. Finally, the finding that some DSWPD patients displayed rather large sleep phase

misperceptions is in line with a recent review highlighting the potential importance of cognitive factors in the maintenance of DSWPD, and suggests that it may be beneficial to include techniques from Cognitive Behavioral Therapy for Insomnia when treating individuals with DSWPD [40].

Some methodological limitations in the present study have to be discussed. First, a few patients had PSG shortened by about 15 minutes for technical reasons. The sensitivity analysis showed that the statistical results were unchanged even with "extreme" imputations for missing objective sleep by REM and N3 respectively.

Second, the alarm clock was not used on the habitual morning because participants were to sleep until rested, but six patients did not wake up by themselves and they had to be manually awakened at 12 pm. The 12 pm limit was set for practical reasons. However, patients slept as long as controls and no patient went to sleep later than 03 am. Accordingly, all patients had the opportunity to sleep for at least 8 hours while the recommended minimum sleep amount for adults is 7 hours [41]. Our present DSWPD-patients were also easily awakened after habitual sleep as soon as the door was opened; only one had to be gently approached and woke up as soon as the lights were turned on. Hence, we believe that subjects in this subgroup also were generally well rested and that our PSG data are representative for habitual sleep in DSWPD of moderate magnitude. The latter observation also supports the notion that difficult awakening is not a problem for these DSWPD patients once allowed to get an adequate amount of sleep or waking up at the right time of their circadian phase. However, as discussed below, it should be noted that DSWPD of severe magnitude probably was underrepresented in the present patient cohort.

Third, our patient group consisted mainly of university students. They have coped with their problems and currently they manage their studies although many expressed concern about

their future prospects and job opportunities. This concern seemed to be the main reason for volunteering for the present study and for accepting clinical treatment afterwards. The patients from our previous study [5] had generally poorer school performance or difficulty maintaining a job and had already sought professional clinical help. These differences may also explain why AT was higher in the previous than the present study, but the REM-related awakening problem was generally consistent between our two studies, suggesting that our finding is a relevant feature in DSWPD of varying severity.

Fourth, one may consider the restricted unfamiliar laboratory environment as a limitation of all sleep-laboratory studies as it is difficult to mimic one's habitual surrounding and activities performed during an average day. As already mentioned, lack of stimulation in the laboratory might have influenced patients' bedtime which was earlier than usual. Fifth, the order of the laboratory nights was not randomized. However, a baseline PSG was added to reduce any order effects and the fixed order was deliberately chosen to reduce between-subject variation and to minimize the effect of the restricted night upon on the second habitual sleep-recording.

5.1 Conclusion

In conclusion, we have replicated findings from our previous smaller study, with a new (and probably less severely affected) population, showing that DSWPD patients have more difficult objective awakening than healthy sleepers. Also, higher AT from REM sleep seems to be a feature linked to DSWPD. Lastly, we confirmed longer objective habitual sleep latency than in controls, and we observed severe sleep onset insomnia misconception in 20% of the DSWPD patients. No other differences between patients and controls regarding sleep structure were found, standing in line with the understanding that sleep structure in DSWPD patients is generally normal after a delayed habitual sleep onset.

Acknowledgments:

We wish to thank Marit Stjern, Alexander Olsen and Ina Møller for their help and assistance.

Conflict of interest:

None.

Funding:

Supported by a grant from NTNU and St.Olavs Hospital («Felles forskningsutvalg 13/10194») and «Samarbeidsorganet».

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