Delayed Sleep Phase Disorder

Prevalence, sleep, circadian rhythm and treatment

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Scientific environment

Working with the theses, I have been employed as a PhD student at the Department of Global Public Health and Primary Health Care, University of Bergen, Norway (2007-2012). The PhD grant was provided by the University of Bergen. I have also had a position at the Norwegian Competence Center for Sleep Disorders, Haukeland University Hospital, Bergen Norway (2011-2012).

The theses include results from two studies. The first study was conducted in 2004 by Helge Molde and Ståle Pallesen at the Faculty of Psychology, University of Bergen, Norway, and I was later given the opportunity to report from the data set. The second study was a clinical trial conducted between 2008 and 2012. Several researchers at the University of Bergen, Norway were involved in the clinical trial, including my main supervisor Bjørn Bjorvatn at the Department of Global Public Health and Primary Health Care, co-supervisors Ståle Pallesen and Inger Hilde Nordhus at the Faculty of Psychology, my colleague Ane Wilhelmsen-Langeland at the Department of Global Public Health and Primary Health Care and psychology student Øystein Vedaa. Eli Sørensen at the Department of Child and Adolescent Psychiatry, Haukeland University Hospital, Bergen, Norway was also involved in parts of the project. Data collection was performed by Ane Wilhelmsen-Langeland and me, helped by psychology student Øystein Vedaa. Facilities for the study (sleep laboratory) were provided by Ståle Pallesen at the Faculty of Psychology. Melatonin analyses were performed by Nina Harkestad, staff engineer at the Research Group on Experimental and Clinical Stress and Sleep (RECSS), University of Bergen, Norway. The clinical trial received a 75.000 NOK grant from the Meltzer foundation, Bergen, Norway.

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ABBREVIATIONS

ANOVA	Analysis of variance
AUDIT	Alcohol use disorders identification test
BMI	Body mass index
BIS	Bergen insomnia scale
CTmin	Core body temperature minimum
DLMO	Dim light melatonin onset
DSP	Delayed sleep phase
DSPD	Delayed sleep phase disorder (delayed sleep phase syndrome)
DSPS	Delayed sleep phase syndrome (delayed sleep phase disorder)
EEG	Electroencephalogram
ELISA	Enzyme-linked immunosorbent assay
EMG	Electromyogram
EOG	Electrooculogram
GCP	Good clinical practice
HADS	Hospital anxiety and depression scale
ICSD-1	The international classification of sleep disorders, 1st edition
ICSD-2	The international classification of sleep disorders, 2nd edition

NREM	Non-rapid eye movement
NSD	Norwegian social science data service
PRC	Phase response curve
PSG	Polysomnography
PSQI	Pittsburgh sleep quality index
REK	The regional ethics committee
REM	Rapid eye movement
RCT	Randomized controlled trial
SCID-I	Structured clinical interview for DSM-IV axis I disorders
SCN	The suprachiasmatic nucleus
SOL	Sleep onset latency
SWS	Slow wave sleep
TIB	Time in bed
TST	Total sleep time
τ	Tau, the spontaneous length of the endogenous period

Abstract

Adolescence is often characterized by delayed and irregular sleep patterns, with potential negative consequences in terms of school performance and daytime functioning. At the most extreme, a stably delayed sleep phase may reflect a circadian rhythm sleep disorder of the delayed sleep phase type (delayed sleep phase disorder, DSPD). DSPD is assumed to be common amongst adolescents and young adults, but little is known about its prevalence and aetiology, and no guidelines exist with respect to treatment.

The aims of the theses were 1) to address the prevalence and correlates of a delayed sleep phase (DSP) in a large and representative sample of Norwegian high school students, 2) to investigate objective measures of sleep, circadian rhythm and phase angle relationship between the underlying circadian rhythm and sleep timing in adolescents and young adults with DSPD when allowed to sleep on a self-chosen schedule and 3) to investigate short- and long-term effects on sleep of bright light and melatonin administered alongside gradual advancement of rise time in adolescents and young adults with DSPD. The aims were addressed using two different study designs.

Study 1 was an internet-based, cross sectional survey conducted on 1285 Norwegian high school students. The survey included items on background, demography, sleep and daytime functioning and the validated questionnaires the Hospital Anxiety and Depression Scale (HADS) and the Alcohol Use Disorder Identification Test (AUDIT). DSP was operationalized as difficulties falling asleep before 2 a.m. at least 3 nights per week together with much/very much difficulties awakening in the morning. The results showed a prevalence of DSP of 8.4% (paper 1). Students with DSP slept less during weekdays and had more often weekend rebound sleep than students without DSP. DSP was associated with negative outcomes in terms of poorer school grades, more smoking, more use of alcohol and increased symptoms of anxiety and depression.

Study 2 combined a case control study and a clinical trial in a comprehensive design into which 40 adolescents and young adults with DSPD and 21 healthy controls were recruited to participate. In the case control study, sleep on a self-chosen schedule was assessed in patients with DSPD and controls by means of polysomnography (PSG). Circadian rhythm was assessed by measuring salivary dim light melatonin onset (DLMO). Results showed delayed timing of sleep and delayed DLMO in the patients with DSPD compared to the healthy controls (paper 2). Sleep, however, appeared to occur at a similar phase angle, and once sleep was initiated no differences in sleep duration or sleep architecture were observed between the groups. In the clinical trial, the DSPD patients were randomized to receive treatment for two weeks in one of 4 treatment conditions: dim light and placebo capsules, bright light and placebo capsules, dim light and melatonin capsules and bright light and melatonin capsules. In a follow-up study, participants were re-randomized to either receive treatment with the combination of bright light and melatonin or no treatment in an open label trial for approximately three months. Light and melatonin were always administered alongside gradual advancement of rise times. Sleep was assessed by sleep diaries, actigraphy recordings, the Pittsburgh Sleep Quality Index (PSQI) and the Bergen Insomnia Scale (BIS). Circadian rhythm was assessed by measuring DLMO. Results showed that the timing of sleep and DLMO were advanced in all groups after short-term treatment, with no additional effect of bright light and melatonin (paper 3). Termination of treatment produced a relapse to baseline levels whereas continued treatment using bright light and melatonin together with adjunct behavioural instructions allowed maintenance of the sleep rhythm.

In conclusion, we found in study 1 a high prevalence of DSP in our sample of Norwegian high school students (paper 1). DSP was associated with lower school grades, more smoking, more alcohol use and higher scores on anxiety and depression. In study 2 we found that patients with DSPD had delayed timing of sleep and DLMO, whereas sleep architecture and phase angle relationship appeared to be normal when patients were allowed to sleep according to a self-chosen sleep schedule (paper 2). Short-term treatment of patients with DSPD involving bright light and melatonin

alongside gradual advancement of rise time produced a phase advance irrespective of treatment condition (paper 3). Long-term treatment with bright light and melatonin alongside gradual advancement of rise time allowed maintenance of the sleep rhythm whereas termination of treatment caused relapse into delayed sleep times.

Sammendrag på norsk

Ungdom har ofte et forsinket og uregelmessig søvnmønster, med potensielt negative effekter på skoleprestasjoner og dagtidsfungering. I ekstreme tilfeller kan et stabilt forsinket søvnmønster reflektere døgnrytmeforstyrrelsen forsinket søvnfasesyndom (delayed sleep phase disorder, DSPD). Pasienter med DSPD har innsovningsproblemer og problemer med å våkne til ønsket tid om morgenen, og de kan derfor ha store vansker med å tilpasse seg samfunnsrytmen. Kunnskapen vi har om utbredelse og etiologi av DSPD er imidlertid mangelfull, og det eksisterer i dag ingen retningslinjer for behandling.

Målsetningene for denne avhandling var derfor 1) å estimere forekomst og korrelater til forsinket søvnfase (delayed sleep phase, DSP) i et representativt utvalg av elever ved norske videregående skoler, 2) å sammenligne objektive mål på søvn, døgnrytme og fasevinkelen mellom den endogene døgnrytmen og søvnperioden hos ungdom og unge voksne med DSPD og friske kontroller når de selv valgte tidspunkt for søvn og 3) å undersøke kort- og langtidseffekter på søvn av behandling med lys og melatonin sammen med instruksjoner om å stå opp gradvis tidligere hos ungdom og unge voksne med DSPD. Disse målsetningene ble adressert gjennom to studiedesign.

Studie 1 var en tverrsnittstudie gjennomført blant 1285 elever i norsk videregående skole. Elevene besvarte et internettbasert spørreskjema med spørsmål relatert til bakgrunn, demografi, søvn og dagtidsfungering, i tillegg til validerte spørreskjema på symptomer på angst og depresjon (Hospital Anxiety and Depression Scale, HADS) og bruk av alkohol (Alcohol Use and Disuse Identification Test, AUDIT). DSP ble operasjonalisert som vansker med å sovne før klokken 02:00 minst 3 netter per uke sammen med store/svært store vansker med å våkne om morgenen. Resultatene viste en forekomst av DSP på 8,4 % (artikkel 1). Studenter med DSP sov mindre på ukedager og tok oftere igjen søvn i helgene enn studenter uten DSP. DSP var assosiert med negative utfall i form av dårligere skolekarakterer, mer røyking, mer bruk av alkohol og økte symptomer på angst og depresjon.

Studie 2 kombinerte en sammenlikningsstudie og en klinisk studie i et helhetlig forsøksdesign. Tilsammen 40 ungdommer og unge voksne med DSPD og 21 friske kontroller ble rekruttert til å delta. I sammenlikningsstudien undersøkte vi polysomnografiske (PSG) mål på søvn hos pasienter med DSPD og kontroller når de selv fikk velge tidspunkt for søvn. Vi estimerte døgnrytme ved å finne tidspunkt for økt melatoninkonsentrasjon i spytt ved fravær av lys (dim light melatonin onset, DLMO). Resultatene viste at tidspunktet for søvn og DLMO var forsinket hos pasienter med DSPD (artikkel 2). Imidlertid fant søvnperioden hos deltakere i begge grupper sted på samme fasevinkel, og etter innsovning observerte vi ingen forskjeller mellom gruppene med tanke på søvnlengde og søvnarkitektur. I den kliniske studien ble DSPD-pasientene randomisert til behandling i to uker i en av fire behandlingsbetingelser: svakt lys og placebo kapsler, sterkt lys og placebo kapsler, svakt lys og melatonin kapsler og sterkt lys og melatonin kapsler. I en oppfølgingsstudie ble deltakerne re-randomisert til enten å få behandling med kombinasjonen sterkt lys og melatonin eller ingen behandling i 3 måneder. Lys og melatonin ble alltid kombinert med instrukser om å stå opp gradvis tidligere. Søvn ble målt ved hjelp av søvndagbøker, aktigrafer og de validerte spørreskjemaene Pittsburgh Sleep Quality Index (PSQI) og Bergen Insomnia Scale (BIS). Døgnrytmen ble estimert ved å måle DLMO. Resultatene viste at tidspunkt for søvn og DLMO ble framskyndet i alle grupper under korttidsbehandling, uten at sterkt lys og melatonin ga forsterket effekt (artikkel 3). Avsluttet behandling ga et tilbakefall til forsinket søvnmønster etter tre måneder, mens fortsatt behandling med lys og melatonin opprettholdt et fremskyndet søvnmønster.

Oppsummert, fant vi en høy forekomst av DSP hos elever i norsk videregående skole (artikkel 1). DSP var assosiert med dårligere skolekarakterer, mer røyking, mer alkoholbruk og økte symptomer på angst og depresjon. Pasienter med DSPD hadde forsinket tidspunkt for søvn og DLMO, men søvnarkitektur og fasevinkel syntes å være normal når pasientene selv fikk velge tidspunkt for søvn (artikkel 2). Korttidsbehandling av pasienter med DSPD med lys og melatonin og instrukser om å stå opp gradvis tidligere fremskyndet tidspunktet for søvn og DLMO uavhengig av

behandlingsbetingelse. Langtidsbehandling av DSPD med lys og melatonin opprettholdt et framskyndet søvnmønster mens avslutning av behandling forårsaket tilbakefall til forsinket søvnmønster.

List of publications

Saxvig IW, Pallesen S, Wilhelmsen-Langeland A, Molde H, Bjorvatn B. (2012). Prevalence and correlates of delayed sleep phase in high school students. Sleep Medicine 13, 193-199.

Saxvig IW, Wilhelmsen-Langeland A, Pallesen S, Vedaa Ø, Nordhus IH, Sørensen E, Bjorvatn B. (2013). Objective measures of sleep and dim light melatonin onset in adolescents and young adults with delayed sleep phase disorder compared to healthy controls. Journal of Sleep Research (in press).

Saxvig IW, Wilhelmsen-Langeland A, Pallesen S, Vedaa Ø, Nordhus IH, Bjorvatn B. (submitted). A randomized controlled trial with bright light and melatonin for delayed sleep phase disorder. Effects on subjective and objective sleep.

Contents

1. INTRODUCTION

All animal species alternate between periods of activity and rest, in accordance with fluctuations of the surrounding environment such as variations in access to food, ambient temperature and daylight. Biological rhythms that are aligned with the 24 hour light/dark cycle are referred to as circadian, from the Latin circa diem (approximately one day). The circadian oscillations are innate to the organisms and are present also in temporal isolation, although usually slightly longer than 24 hours (Aschoff, 1965; Czeisler *et al.*, 1999). These rhythms entrain to match the length of a day through external cues (zeitgebers) of which daylight is believed to be the most important (Czeisler *et al.*, 1989). Circadian rhythms may promote adaptive behaviour, for example by ensuring inactivity when access to food is low and/or predator risk is high. Some animals are active during the dark phase (nocturnal) whereas others, like humans, are mostly active during the light phase of the day (diurnal). The inactivity phase is usually accompanied by sleep, defined as a reversible behavioural state of perceptual disengagement from and unresponsiveness to the environment (Carskadon and Dement, 2011).

Circadian rhythm sleep disorders are characterized by a misalignment between the internal sleep/wake cycle and the surrounding environment (World Health Organization, 1992; American Psychiatric Association, 2000; American Academy of Sleep Medicine, 2005) and result in complaints of insomnia, excessive daytime sleepiness or both (American Academy of Sleep Medicine, 2005). Whereas secondary circadian rhythm sleep disorders are inflicted by external factors such as traveling across time zones (jet lag type) or shift work (shift work type), less is known about the aetiology of primary types (e.g. delayed and advanced sleep phase disorders) (American Academy of Sleep Medicine, 2005). In the delayed sleep phase disorder (DSPD) the timing of the sleep period is delayed in relation to normal or desired sleep times. DSPD has potentially large impact on the lives of afflicted individuals, still little is known about DSPD in terms of prevalence, pathophysiology and treatment.

The present thesis aims to address some of these aspects, hopefully contributing to increased understanding and ultimately to better clinical management of patients with DSPD.

1.1 Sleep

Sleep in mammals is characterized by two distinct stages, rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep. These sleep stages can be identified by polysomnography (PSG) recordings which involves electrophysiological recordings of brain activity (electroencephalogram, EEG), eye movements (electrooculogram, EOG) and muscular activity (electromyogram, EMG) (Rechtschaffen and Kales, 1968; Iber *et al.*, 2007; Carskadon and Dement, 2011). Wakefulness is defined by high frequency (beta range, >17 Hz), low voltage oscillations in the EEG. The NREM stages N1, N2 and N3 are characterized by progressively slower EEG activity. Background activity during the stages N1 and N2 is in the theta frequency range (5-7 Hz) whereas N3 is defined by the presence of high amplitude EEG waves in the low delta frequency range (<2 Hz) (Iber *et al.*, 2007; Carskadon and Dement, 2011). Accordingly, stage N3 is often referred to as slow wave sleep (SWS) or delta sleep. Due to a high wake up threshold in N3, the term deep sleep is sometimes used. In contrast, REM sleep displays high frequency, low amplitude EEG together with rapid eye movements and muscular atonia (Iber *et al.*, 2007; Carskadon and Dement, 2011). REM sleep normally constitutes approximately 20-25% of the sleep period, whereas 5%, 50% and 20-25% are spent in the NREM stages N1, N2 and N3, respectively (Carskadon and Dement, 2011). The sleep stages are temporally organized within the major sleep period into 4-6 NREM-REM cycles of approximately 90 minutes each. N3 is abundant during the first sleep cycles of the sleep period, REM sleep during the latter (Carskadon and Dement, 2011) .

Sleep regulation

According to the two-process model for sleep regulation, sleep is regulated in an interplay between circadian and homeostatic processes (Borbely, 1982). The circadian factor promotes sleep during certain periods of the day, to a large degree determining the duration of the sleep period (Czeisler *et al.*, 1980; Dijk and Czeisler, 1995) (Fig.1). The homeostatic factor represents a sleep propensity that accumulates during time spent awake and is reflected by the amount and intensity of N3 (Borbely *et al.*, 1981; Banks and Dinges, 2007). Extended periods of wakefulness are followed by increased amounts of N3, and after sleep deprivation the rebound of deep sleep occurs at the cost of other sleep stages. Deep sleep is believed to be crucial in order to feel awake and alert. In humans, habits and behaviour may override and influence the biological drives (e.g. voluntary wake during the normal sleep period, drinking coffee, etc.) (Carskadon *et al.*, 2004; Bjorvatn and Pallesen, 2009).

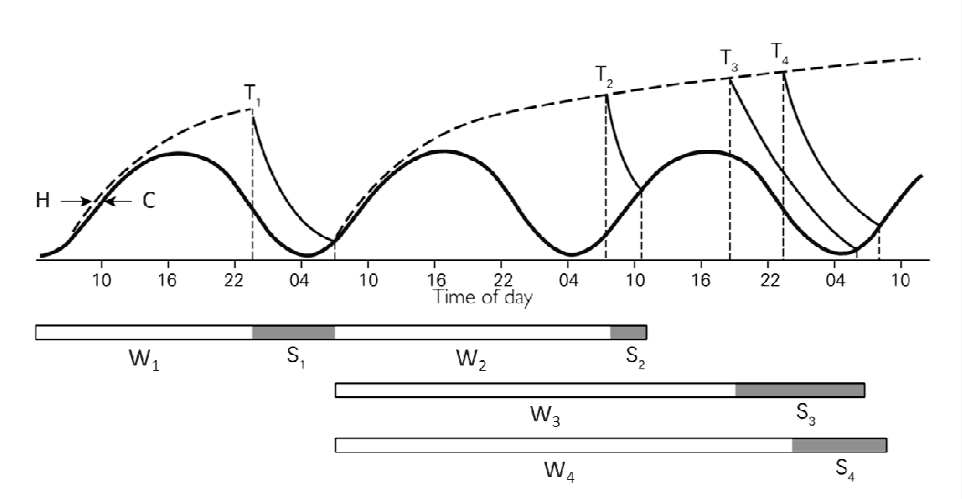

Figure 1. Sleep duration is controlled by the interplay between the homeostatic factor (H) and the circadian factor (C). H builds up during wakefulness (W) and is released during sleep (S). Wake up occurs when the H curve crosses the C curve. Habits and behaviour determine the time for sleep onset (T) (Ursin, 2008).

1.2 Circadian rhythms

Circadian rhythms are seen not only as oscillations in behaviour (sleep/wake, activity/inactivity), but also as daily variations in physiological processes such as hormone secretion (e.g. melatonin, cortisol), body temperature, renal activity and gene transcription (Kryger *et al.*, 2011). Peripheral organs and cells in vitro display circadian rhythmicity in the absence of external cues, with period lengths (τ) ranging from 20 – 30 hours (Gachon *et al.*, 2004; Yoo *et al.*, 2004). These circadian oscillations are of genetic origin, and rely on transcriptional feedback loops involving the clock genes Clock, Bmal1, Per1, Per2, Cry1 and Cry 2, recently reviewed by Mohawk *et al.* (2012).

Circadian regulation

The mechanisms through which the peripheral circadian rhythms are synchronized and aligned with the 24 hour light/dark cycle are known to mainly rely on the action of a central pacemaker, the suprachiasmatic nucleus (SCN) (Kryger *et al.*, 2011; Mohawk *et al.*, 2012). SCN resides in the hypothalamus just above the optic chiasma. The τ of the SCN is normally slightly longer than 24 hours (Aschoff, 1965; Czeisler *et al.*, 1999; Herman, 2011) but entrains to the length of a day through external stimuli (zeitgebers). Light appears to be the most important zeitgeber in humans (Czeisler *et al.*, 1989; Duffy *et al.*, 1996), and works by activating retinal cells such as the melanopsin expressing photoreceptive ganglion cells which project directly to the SCN through the retino-hypothalamic tract (Do and Yau, 2010). The SCN coordinates the peripheral rhythms through effects on hormone secretion and body temperature, in addition to more complex pathways and feedback loops involving autonomic control and local signals (Gachon *et al.*, 2004; Mohawk *et al.*, 2012). Through indirect neuronal pathways, the SCN regulates the pineal secretion of melatonin (Pandi-Perumal *et al.*, 2008). Melatonin is suppressed by light and released in the dark, and is involved in the regulation of several physiological processes with a reciprocal influence on the circadian phase of the SCN.

Measuring circadian rhythms

The phase, length (τ) and amplitude of the endogenous period can be identified by measuring circadian oscillations in physiological processes, often core body temperature or melatonin secretion, under conditions that minimize the influences of external temporal cues (unmasking). Unmasking is often done by imposing days of abnormal length, outside the range of entrainment by the internal clock (forced desynchrony) or by using a constant routine protocol (Mills *et al.*, 1978; Minors and Waterhouse, 1984; Brown and Czeisler, 1992). In a constant routine protocol, unmasking is achieved by keeping potential temporal cues such as sleep/wake, lightning, heating, humidity, posture, activity, mealtimes etc. constant over 24 hours or more (Minors and Waterhouse, 1984). On a constant routine protocol the core body temperature, usually measured using a rectal temperature probe, is higher during the subjective day and declines in the evening to reach a minimum (core body temperature minimum, CTmin) about 2 hours before spontaneous/habitual wake up time (Bjorvatn and Pallesen, 2009). Melatonin (N-acetyl-5-methoxytryptamine) is measured in saliva or plasma, or through its metabolite 6-sulphatoxymelatonin in urine (Benloucif *et al.*, 2008). Melatonin levels during the day are low, but increase in the evening in the absence of light, about 7 hours before CTmin (Brown *et al.*, 1997), to reach an acrophase in the middle of the sleep period. The time of CTmin and the time at which melatonin secretion increase in the evening in the absence of light (dim light melatonin onset, DLMO) are considered reliable markers of circadian phase (Lewy *et al.*, 1985; Herman, 2011). In recent years, partial protocols for salivary DLMO assessment have been validated, allowing at home based assessment of circadian phase (Pandi-Perumal *et al.*, 2007; Pullman *et al.*, 2012).

Entrainment by light

Although behaviour and other non-photic zeitgebers (e.g. sleep/wake cycle, activity levels, food intake, ambient temperature) may contribute to entrainment, it has been established that light is the most important zeitgeber in humans (Czeisler *et al.*, 1989; Duffy *et al.*, 1996). Its effects (direction and size) largely depend on the time of

exposure, as described by the phase response curves (PRC) for light (Minors *et al.*, 1991; Khalsa *et al.*, 2003) (Fig.2). Morning bright light, shortly after the core body temperature minimum (CTmin), produces a phase advance whereas light prior to CTmin produces a phase delay (Minors *et al.*, 1991; Khalsa *et al.*, 2003; Bjorvatn and Pallesen, 2009). Light in close proximity to CTmin produces a larger phase advance/delay, and in a non-linear manner, light with higher intensity (measured in lux) has a stronger phase shifting capacity (Zeitzer *et al.*, 2000).

When administered at adequate times, intensities and durations, bright light can be used to manipulate the circadian rhythm, for example in the treatment of circadian rhythm sleep disorders or affective disorders with associated circadian dysregulation (Wirz-Justice, 2003). In clinical practice, patients may be advised to ensure exposure to outdoor light at optimal times. Light therapy lamps for indoor use are also commercially available and may be a good alternative, in particular at high or low latitudes during the dark season. Normally, these lamps emit high intensity light (5000 – 10 000 lux) in the visible part of the spectrum (380-760 nm). Using full spectrum light with 10 000 lux intensity, common exposure time is 30 minutes (Terman and Terman, 2011). The side-effects of bright light therapy are usually minor and of short-term duration (Wyatt, 2004). A line of research has indicated that the SCN may be particularly sensitive to high energy light (short wavelength light, blue spectrum ~480 nm) (Wright and Lack, 2001; Lockley *et al.*, 2003), and lamps emitting blue enriched lights are also available for purchase. However, PRC's for lights of the different wavelengths (Revell *et al.*, 2012; Ruger *et al.*, 2013) as well as safety of use should be established before clinical application can be recommended.

Entrainment by melatonin

Chronobiotics are agents with the ability to shift the endogenous circadian phase (Dawson and Armstrong, 1996). Melatonin is a potent chronobiotic that has been extensively studied, likely exerting its effects through its reciprocal interaction with the SCN (Pandi-Perumal *et al.*, 2008). The PRC for melatonin is about 12 hours out of phase with light (Lewy *et al.*, 1992; Lewy *et al.*, 1998) (Fig.2).

In clinical practice exogenous melatonin (0.3-5 mg) may be administered in the evening to achieve a phase advance (Fuardiola-Lemaître and Quera-Salva, 2011). The phase delaying effects of melatonin administered in the morning have been more questioned (Wirz-Justice *et al.*, 2002; Fuardiola-Lemaître and Quera-Salva, 2011). When melatonin and light therapy are employed together, melatonin may be administered about 12 hours prior to bright light exposure (Bjorvatn and Pallesen, 2009). No classical dose response relationship for exogenous melatonin has been found, but different doses appear to have different time windows for optimal effects (PRCs), probably due to wash-over effects (Burgess *et al.*, 2010). There has been some controversy regarding use of melatonin in children and adolescents since there is little knowledge of teratogenicity, interaction effects, long-term effects and effects on the reproductive system of melatonin (Arendt, 1997).

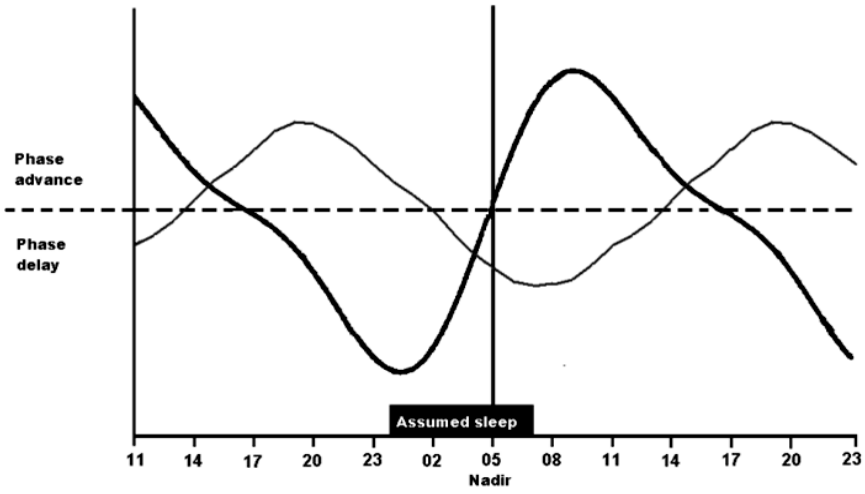

Figure 2. Phase response curves for light (thick black line) and melatonin (thin grey line) based on studies by Khalsa *et al.* (2003) and Lewy *et al.* (1998). Light/melatonin at times when the curves are above the dotted line will produce a phase advance, when curves are below the dotted line they produce a phase delay. Nadir is the core body temperature minimum (CTmin).

Diurnal preference

Some people feel more alert and prepared for mental and physical activity during the early part of the day, others in the later. Such diurnal preference is closely connected to the sleep/wake pattern, and evening types (night owls) tend to go to bed and rise at later times than morning types (morning larks) and they often have more irregular sleep schedules (Giannotti *et al.*, 2002). The diurnal preference is believed to be an innate trait reflecting differences in the underlying circadian phase, possibly related to different alleles of several clock genes (yielding chronotypes along a continuum from morningness to eveningness) (Katzenberg *et al.*, 1998). Eveningness appears to be a risk factor for poor mental and physical health (Randler, 2011), as well as low school attendance, reduced school performance, risk-taking and bad health behaviour (e.g., use of tobacco and alcohol as well as inadequate dietary habits, see Cavallera *et al.* (2011) for review).

1.3 Sleep in adolescents

Adolescence is often characterized by irregular weekday/weekend sleep patterns as recently reviewed by Gradisar *et al.* (2011b), as well as increased daytime sleepiness and increased evening preference (Carskadon *et al.*, 1980; Carskadon *et al.*, 1993; Crowley *et al.*, 2007). Late bed times together with early imposed rise times due to school obligations produce sleep curtailment during weekdays. In contrast, sleep duration during weekends is often prolonged, in part due to rebound or recovery sleep, and the late bed times are accompanied by late rise times. However, sleeping in on weekends may further contribute to the sleep/wake rhythm delay, making it difficult to fall asleep at appropriate times on following evenings. By this, some adolescents conceive themselves trapped in a vicious cycle/positive feedback loop.

A biological basis for the adolescent sleep phase delay is suggested by the facts that it is not confined to western cultures or even the human species (Hagenauer *et al.*, 2009), and that it appears to correlate with pubertal development rather than age

(Carskadon *et al.*, 1993). According to sleep regulatory principles (Borbely, 1982) alterations in the timing of sleep may originate from homeostatic or circadian alterations, and there is evidence that both are involved in the adolescent sleep phase delay as reviewed by Hagenauer *et al.* (2009). Still, adolescence is accompanied by reduced parental control, and the 24 hour society (services open and available around the clock), television, computers, internet, cellular phones and increased norm pressure from peers produce great enticements for being awake at night (Ferrara and De Gennaro, 2001), potentially causing a delay of sleep onset.

The adolescent sleep phase delay may have severe consequences. Rising at times when the biological clocks are set for sleep, adolescents with a delayed sleep phase may experience problems performing optimally during school hours due to the circadian fluctuations in subjective sleepiness, cognition, attention and physical performance (Carrier and Monk, 2000). Moreover, the need for sleep does not decline during puberty (Carskadon *et al.*, 1980), and since school normally starts early in the morning many adolescents are forced to rise before they have received adequate sleep. Chronic sleep curtailment causes sleepiness and may have widespread implications on both physiological and neurobehavioral functioning (Banks and Dinges, 2007; Akerstedt *et al.*, 2012), the neurobiological effects of sleep debt accumulating over time (Van Dongen *et al.*, 2003). In line with this, several studies have showed an association between poor sleep habits and reduced academic performance in adolescents (Wolfson and Carskadon, 1998; Wolfson and Carskadon, 2003; Dewald *et al.*, 2010). Poor sleep habits also appear to be linked to overweight, depressive mood, smoking and alcohol usage (Carskadon *et al.*, 1998; Meijer *et al.*, 2010; Owens *et al.*, 2010; Pallesen *et al.*, 2010; Pasch *et al.*, 2010; Garaulet *et al.*, 2011). Oversleeping is another common consequence of a delayed sleep phase, affecting school attendance and consequently contributing to poor school performance.

1.4 Delayed sleep phase disorder

Circadian rhythm sleep disorders are characterised by a misalignment between the sleep/wake rhythm and the external environment (American Academy of Sleep Medicine, 2005). Delayed sleep phase disorder (DSPD, previously termed delayed sleep phase syndrome, DSPS) (American Academy of Sleep Medicine, 1990; American Academy of Sleep Medicine, 2005) was first described by Weitzman *et al.* (1981) as a chronobiological disorder with sleep onset insomnia. In the second edition of the International Classification of Sleep Disorders (ICSD-2), DSPD is defined as "*a delay in the phase of the major sleep period in relation to the desired sleep time and wake-up time, as evidenced by a chronic or recurrent complaint of inability to fall asleep at a desired conventional clock time together with the inability to awaken at a desired and socially acceptable time*" (American Academy of Sleep Medicine, 2005). According to the ICSD-2, sleep quality and duration are normal when patients are allowed to sleep on a self-preferred sleep schedule, and sleep diary or actigraphy for at least a week show a delayed but stable habitual sleep period (American Academy of Sleep Medicine, 2005).

1.4.1 Prevalence

DSPD is assumed to be the most frequent circadian rhythm sleep disorder (Dagan and Eisenstein, 1999), but few studies have been published addressing the prevalence of DSPD as diagnosed according to ICSD-2 (American Academy of Sleep Medicine, 2005) (Table 1). A large Norwegian study yielded a prevalence of 0.17% (Schrader *et al.*, 1993) in an adult sample whereas a prevalence rate of 0.14% was reported from a corresponding Japanese study (Yazaki *et al.*, 1999). Although the prevalence in adolescent samples is believed to be higher, 7-16% according to the ICSD-2 (American Academy of Sleep Medicine, 2005), only data from survey studies are available. Without the use of sleep logs to confirm the diagnosis it is difficult to differentiate between DSPD, eveningness and a normal adolescent sleep phase delay, and different sets of criteria have been used to identify DSPD-like symptoms.

Accordingly, results from survey studies have been ambiguous, ranging from 0.48 to almost 17 % (Table 1). Nevertheless, it appears that at least a mild form of DSPD/a severe sleep phase delay/extreme eveningness is common amongst adolescents and young adults, potentially causing problems for the youngsters.

Table 1. Prevalence studies of delayed sleep phase disorder/a severe sleep phase delay

Publication	N	Age	Methods	Prevalence (%)
(Yazaki *et al.*, 1999)	1525	15-59	Confirmed diagnosis	0.13
(Schrader *et al.*, 1993)	9918	18-67	Confirmed diagnosis	0.17
(Hazama *et al.*, 2008)	4971	Students	Survey	0.48
(Ohayon *et al.*, 2000)	3294	15-24	Telephone interview	<0.5 CRSD[2]
(Ando *et al.*, 1995) [1]	417	40-64	Survey	0.7
(LeBlanc *et al.*, 1999) [1]	1743	12-20	Confirmed diagnosis	1.3
(Pelayo *et al.*, 1988) [1]	109	12-19	Survey	7.3
(Brown *et al.*, 2001)	191	17-55 (average 19)	Survey	11.5
(Lack, 1986)	211	16-50 (average 23)	Survey	16.6

[1]Abstract, [2]Circadian rhythm sleep disorder

1.4.2 Correlates and comorbidity

Patients with DSPD experience challenges commonly associated with delayed and irregular sleep patterns in terms of school adherence and daytime functioning

(Crowley *et al.*, 2007). DSPD has also been shown to have high comorbidity with other conditions such as depression (Thorpy *et al.*, 1988; Regestein and Monk, 1995; Regestein and Pavlova, 1995), learning disabilities (Dagan and Eisenstein, 1999) and personality disorders (Dagan *et al.*, 1998a).

1.4.3 Aetiology

Although genetic markers related to DSPD have been described (Archer *et al.*, 2003; Hohjoh *et al.*, 2003) the pathophysiology of DSPD is still largely unknown (American Academy of Sleep Medicine, 2005). The sleep phase delay is assumed to reflect an underlying delay in the endogenous rhythm, and accordingly several studies have shown delayed measures of circadian rhythms in terms of DLMO (Shibui *et al.*, 1999; Wyatt *et al.*, 2006; Chang *et al.*, 2009) and CTmin (Watanabe *et al.*, 2003; Chang *et al.*, 2009) in patients with DSPD. Czeisler *et al.* (1981) suggested that patients with DSPD have long τ, making it hard for them to adequately entrain to the light/dark cycle. Campbell and Murphy (2007) recorded τ in a patient with DSPD in temporal isolation. This patient had an average τ of 25.38 hours, compared to 24.44 in the healthy controls. Reduced phase advancing capacity may also be caused by abnormalities in light sensitivity, and it has been suggested that patients with DSPD have reduced sensitivity to light. In contrast, one study has found a more pronounced suppression of nocturnal melatonin secretion by light in these patients (Aoki *et al.*, 2001). Thus, it is possible that patients suffering from DSPD are hypersensitive to light, allowing evening light to cause a phase delay. Another source of pathophysiology is suggested by several reports of longer phase angle relationships (time interval) between measures of circadian rhythmicity and sleep in patients with DSPD (Ozaki *et al.*, 1996; Uchiyama *et al.*, 2000a; Uchiyama *et al.*, 2000b; Watanabe *et al.*, 2003; Campbell and Murphy, 2007). The prolonged phase angle in patients with DSPD may be explained by the finding by Uchiyama *et al.* (1999) of reduced sleep propensity in patients with DSPD after sleep deprivation, indicating that homeostatic processes may play a role in DSPD either in terms of reduced accumulation of homeostatic factor during wakefulness or reduced ability to release

the homeostatic factor when allowed to sleep. A longer phase angle may promote sleep during a larger part of the most potent phase advance period, thus impairing the ability to phase adjust. Research is, however, not univocal, and neither Wyatt *et al.* (2006) nor Chang *et al.* (2009) found differences in phase angles, whereas Shibui *et al.* (1999) found a prolonged phase angle relationship between melatonin and sleep offset but not sleep onset, a result that may be explained by longer total sleep time. It appears that the correlation between DLMO and sleep onset is lower in patients with DSPD than in healthy individuals (Keijzer *et al.*, 2011). Adding further complexity, evening types have been found to actually have shorter phase angle than morning types (Baehr *et al.*, 2000; Liu *et al.*, 2005). It has been debated whether patients with DSPD display extreme eveningness or if the two conditions are caused by different aetiologies. Mongrain *et al.* (2004) have suggested that evening types often follow an enforced rhythm, hence shortening the phase angle whereas patients with DSPD more often sleep according to their internal rhythm, the late sleep offset producing a longer phase angle relationship. Hence, the inconsistencies in phase angle can be explained in terms of external constraints acting differentially on individuals along the morningness – eveningness continuum. It is, however, possible that the two conditions are caused by qualitatively different aetiologies. According to the view of Dagan and Eisenstein (1999), DSPD reflects a distinct clinical entity characterized by rigidity in the circadian rhythm. Also social factors and habits are likely to play important roles in the aetiology of DSPS. Bad habits such as staying up late and sleeping in will phase delay the circadian rhythm, and a vulnerable subject may enter a vicious circle facilitating development of the syndrome (Whyte and Schaefer, 1995).

1.4.4 Sleep

Since DSPD is commonly believed to rely on abnormalities in the circadian regulation system, sleep quality and duration have been assumed to be normal when patients are allowed to sleep on a self-preferred sleep schedule (American Academy of Sleep Medicine, 2005). It is possible, however, that other sleep regulatory mechanisms responsible for the sleep phase delay may influence duration and/or architecture of

sleep. Disturbances in homeostatic processes may affect amount and distribution of SWS. Furthermore, sleeping at a later endogenous time (longer phase angle) may have implications for both sleep duration and architecture (Dijk, 1999). The only controlled study where polysomnographic measures of sleep were reported for patients with DSPD (Watanabe *et al.*, 2003) on non-imposed sleep schedules revealed prolonged sleep onset latency, more wake after sleep onset, reduced sleep efficiency and reduced SWS. The reduced sleep quality was attributed by the authors to a longer phase angle in which sleep offset was delayed in relation to the core body temperature nadir (Watanabe *et al.*, 2003).

1.4.5 Treatment

The most common treatment approaches for DSPD are based on behavioural interventions, administration of bright light and/or exogenous melatonin or combinations of these (Barion and Zee, 2007; Morgenthaler *et al.*, 2007; Bjorvatn and Pallesen, 2009). The use of hypnotics is generally not an option in these patients, since effective treatment should aim to correct the underlying circadian delay. Although much is known about the chronobiological effects of light and melatonin, no standardized guidelines exist regarding treatment for DSPD, and treatment is often available only at specialized sleep clinics.

Bright light therapy

The use of bright light exposure in treatment of DSPD was first suggested by Lewy *et al*. (1985), but its effect has been assessed in few controlled studies (Rosenthal *et al.*, 1990; Cole *et al.*, 2002; Lack *et al.*, 2007; Gradisar *et al.*, 2011a). In a cross-over study by Rosenthal *et al.* (1990), CTmin was advanced with almost one and a half hour in patients with DSPD when receiving 2500 lux for two hours in the morning over two weeks but not in the dim light condition. Similarly, Lack *et al.* (2007) reported a two and a half hour advance of DLMO in DSPD patients receiving morning blue light for one week with no phase advance in the dim light control group. Restriction of evening light facilitates sleep phase advancement (Rosenthal *et al.*,

1990; Cole *et al.*, 2002). Hence, in line with current evidence and chronobiological principles, light after CTmin should produce an advance of the endogenous circadian rhythm in patients with DSPD.

Behavioural interventions

The first treatment proposed for DSPD was chronotherapy; a gradual delay of the sleep period (usually 3 hours per day) until the desired timing of sleep was achieved (Czeisler *et al.*, 1981). This treatment regime was based on the assumption that patients with DSPD had reduced capacity to phase advance, and in the original report (Czeisler *et al.*, 1981) chronotherapy successfully shifted the timing of sleep in 5 patients who also were able to maintain the newly achieved sleep phase over longer periods by adhering to strict stable sleep schedules. Chronotherapy is, however, time consuming, and several cases of hypernyctohermal syndrome/free running disorder (American Academy of Sleep Medicine, 2005), have been reported after chronotherapy (Oren and Wehr, 1992). Hence, chronoterapy is generally not a recommended treatment for DSPD.

Bright light is usually administered together with behavioural instructions such as gradual advancement of rise time (Cole *et al.*, 2002; Lack *et al.*, 2007; Gradisar *et al.*, 2011a) or fixed advanced sleep/wake schedules (Sharkey *et al.*, 2011). Interestingly, some of these studies have shown phase advancement also in dim light control groups (Cole *et al.*, 2002; Sharkey *et al.*, 2011). In the study by Sharkey *et al.* (2011), participants with "subclinical DSPD" advanced DLMO with approximately one and a half hour in both a blue light and a dim light condition by adherence to fixed advanced sleep/wake schedules. Hence, it appears that sleep schedules may act as a determinant for circadian phase in patients with DSPD. It is, however, possible that behavioural instructions are sufficient to produce a phase advance only in the patients with less severe phase delay. Accordingly, Cole *et al.* (2002), reported superior effects of the bright light condition only in the participants whose initial circadian delay was most severe. Behavioural interventions addressing possible

associated conditioned sleep onset insomnia have also been suggested as part of DSPD treatment protocols (Lack and Wright, 2007; Gradisar *et al.*, 2011a).

Exogenous melatonin

Few controlled studies have addressed the effect of melatonin in treatment of DSPD (Dahlitz *et al.*, 1991; Nagtegaal *et al.*, 1998b; Kayumov *et al.*, 2001; Mundey *et al.*, 2005; Rahman *et al.*, 2010a). Kayumov *et al.* (2001) and Rahman *et al.* (2010a) both reported reduced sleep onset latency (SOL) on an early imposed sleep schedule after treatment with melatonin. In a study by Nagtegaal *et al.* (1998b), melatonin advanced DLMO with one and a half hour whereas CTmin remained unchanged. The authors speculated whether the results could be attributed to the soporific rather than chronobiotic actions of melatonin. In contrast, Mundey *et al.* (2005) found that melatonin advanced both DLMO and CTmin with about one and a half hour, but that sleep onset and offset remained unchanged. In a study by Dahlitz *et al.* (1991) sleep onset was advanced compared to the placebo group, but not compared to pre-treatment levels. Based on these findings and results from studies on children and adolescents with sleep onset difficulties (Smits *et al.*, 2001; Smits *et al.*, 2003; Weiss *et al.*, 2006; Van der Heijden *et al.*, 2007), a recent meta-analysis concluded that melatonin effectively produce a phase advance in patients with DSPD (van Geijlswijk *et al.*, 2010).

Timing for administration of treatment

Appropriate timing for administration of bright light/melatonin in DSPD can be ensured by measuring DLMO or CTmin (Nagtegaal *et al.*, 1998b; Lockley, 2005; Mundey *et al.*, 2005). When such biological markers of circadian phase are not available, DLMO and CTmin can be estimated from behaviour and anamnestic information, based on the facts that DLMO usually occurs approximately two hours before habitual sleep onset (Revell *et al.*, 2006) and CTmin approximately two hours before habitual wake up time (Bjorvatn and Pallesen, 2009). Still, individual timing of treatment may not be superior to fixed times as shown by Nagtegaal *et al.* (1998b). In

previous treatment studies, melatonin has often been administered at approximately the same time throughout the treatment period (Dahlitz *et al.*, 1991; Nagtegaal *et al.*, 1998b; Kayumov *et al.*, 2001; Mundey *et al.*, 2005; Rahman *et al.*, 2010a), despite the fact that a shift in circadian phase will cause a parallel phase shift of the phase-response curve for melatonin (Lewy *et al.*, 1998). Gradual advancement of time for bright light/melatonin administration alongside gradual advancement of the sleep schedule has proved effective in advancing the circadian phase in healthy populations (Burgess *et al.*, 2003; Revell *et al.*, 2006), and several researchers have suggested DSPD treatment protocols based on this principle (Cole *et al.*, 2002; Revell *et al.*, 2006; Lack *et al.*, 2007; Bjorvatn and Pallesen, 2009; Gradisar *et al.*, 2011a).

Long-term treatment

Little is known about long-term treatment of DSPD. Several clinical reports indicate that the effect of light and melatonin fades out upon termination of treatment (Alvarez *et al.*, 1992; Dagan *et al.*, 1998b; van Maanen *et al.*, 2011). Accordingly, treatment protocols should address the sleep phase delay in a two-step manner by 1) achieving and 2) maintaining a phase advance. Cole *et al.* (2002) reported maintenance of advanced sleep onset times after four weeks in a bright light condition when compared to baseline but not compared to the dim light control group. Maintenance treatment by light therapy 2-4 days per week may be useful for some patients (Wyatt, 2004). It is, however, possible that some patients are able to maintain an advanced sleep phase through strict sleep schedules. In line with this, Gradisar *et al.* (2011a) showed that DSPD-patients were able to maintain treatment effects for six months by adhering to a behavioural regime following initial short-term treatment with bright light and cognitive behavioural therapy. In a study by Czeisler *et al.* (1981), patients were able to maintain an advanced sleep schedule after chronotherapy (follow-up between 6 weeks and 2.5 years).

Treatment in children and adolescents

Treating children and adolescents with DSPD involves special challenges when compared to adult populations, both in terms of physiology and behaviour. In particular, motivational issues may have a strong impact on compliance to treatment, since most treatment protocols involve structuring the daily schedule. Hence, it seems important to address treatment effectiveness within a particular age group. Few controlled studies have, however, addressed treatment of children and young adults with DSPD. To our knowledge, the study by Gradisar *et al.* (2011a) in which a phase advance was produced through cognitive behavioural therapy in combination with morning bright light, is the only previous controlled treatment study conducted on adolescents diagnosed with DSPD. The use of melatonin in children and adolescents with sleep onset difficulties (possibly due to a delayed sleep phase) has been more extensively investigated and appears to advance sleep onset and DLMO, and possibly also increase sleep duration (Smits *et al.*, 2001; Smits *et al.*, 2003; Weiss *et al.*, 2006; Van der Heijden *et al.*, 2007; Eckerberg *et al.*, 2012).

2. Research aims

The main aims of this thesis were 1) to investigate prevalence and correlates of delayed sleep phase (DSP) in a representative adolescent sample, 2) to compare objective measures of sleep and circadian phase in patients with delayed sleep phase disorder (DSPD) to that of healthy controls, and 3) to address short- and long-term treatment effects on sleep of bright light and melatonin in patients with DSPD in a randomized controlled design. These aims were addressed in three papers:

Paper 1

The aims of paper 1 were to estimate the prevalence of DSP, operationalized as difficulties falling asleep before 2 a.m. at least 3 nights per week together with much/very much difficulties awakening in the morning, in a large sample of Norwegian high school students, and to identify correlates of DSP in terms of smoking, alcohol use, body mass index (BMI), school grades, and anxiety and depression scores.

Paper 2

The aims of paper 2 were to investigate polysomnographic measures of sleep in adolescents and young adults with DSPD on a self-chosen sleep schedule compared to healthy controls, and to estimate circadian phase (DLMO) and its phase angle in relation to the sleep period.

Paper 3

The first aim of paper 3 was to investigate and compare short-term treatment effects of bright light and melatonin when administered alongside gradual advancement of rise times in patients with DSPD in a randomized, four-armed, double blinded, placebo controlled design. The second aim was to investigate long-term treatment effects of bright light and melatonin in combination in a randomized, two-armed follow-up design. Main end points were subjective and objective measures of sleep as

recorded by one week of sleep diary and actigraphy prior to treatment, the last week of the two-week intervention and the last week of the three-month follow-up, as well as measures of circadian phase (DLMO) before and after the two-week intervention.

3. METHODS

Publications from two different studies are included in the theses. Paper 1 is based on a cross sectional survey conducted amongst high school students in Hordaland County, Norway (Study 1). Papers 2 and 3 are based on a combined case control study/clinical trial (ClinicalTrials.gov NCT00834886) conducted on adolescents and young adults in Bergen, Norway (Study 2).

3.1 Study 1

3.1.1 Sample

The sample consisted of 1285 high school students (610 girls and 669 boys, 6 unanswered) from 115 school classes in Hordaland County, Norway. The students were between 16 and 19 years, with a mean age of 17.3 years (SD = 0.9).

3.1.2 Procedure

The study was conducted in collaboration with the central Regional School Administration of Western Norway during spring 2004, and participation was incorporated into the school day activities. The survey was internet based and students participated by logging anonymously onto a given internet address where they responded to a questionnaire composed of items on demography, gambling, alcohol use/abuse, drug use/abuse, and anxiety and depression. Paper 1 presents data from items relating to sleep and daytime functioning, demography as well as validated questionnaires on alcohol use/abuse and anxiety and depression. Other results from the survey are not part of this thesis.

3.1.3 Instruments

Demography

The items covering demography and background information were age, gender, weight, height, smoking (yes/no) and self-reported average school grade (scale from 1, lowest to 6, highest).

Sleep questions

A total of 15 items about sleep and sleep habits were included in the questionnaire, of which 11 were included in the present paper. Five of these items were particularly relevant, as they were used to operationalize delayed sleep phase (DSP) and to identify the students with DSP who also reported related daytime impairment and problems advancing the sleep phase:

- How many days per week do you have difficulties falling asleep before 2 a.m.? (0-7)
- On average, how many days per week are you late for school, work or appointments due to oversleeping? (0-7)
- Do you usually feel sleepy at school or work during weekdays? (not at all, a little, moderate, much, very much)
- Do you have difficulties waking up in the morning on weekdays? (not at all, a little, moderate, much, very much)
- Would it be easy for you to go to bed earlier, fall asleep earlier and wake up earlier in the morning than what you now usually do on weekdays? (not at all, a little, moderate, much, very much)

We operationalized delayed sleep phase (DSP) as difficulties falling asleep before 2 a.m. at least 3 nights per week together with much or very much difficulties waking up in the morning. DSP together with much or very much problems advancing the

sleep phase and at least one daytime consequence was also assessed. A daytime consequence was defined as oversleeping at least two days per week and/or reporting much or very much sleepiness at school.

The remaining six items concerned bed time, sleep onset latency (SOL) and wake up time on weekdays and weekends, respectively. Time in bed (TIB) was defined as the time interval from bed time to wake up time. Total sleep time (TST) was defined as TIB minus SOL. Students with TST weekend minus TST weekday > 120 minutes were considered to have weekend rebound sleep.

Alcohol Use Disorders Identification Test (AUDIT)

AUDIT is a self-report instrument measuring use and potentially misuse of alcohol (Saunders *et al.*, 1993). It consists of ten items; the first eight are rated on a 5 point scale (0-4), whereas the two last items are rated on a 3 point scale (0-2-4). Higher scores are associated with higher levels of drinking problems. A score above 10 suggests harmful drinking. Cronbach's alpha for AUDIT was 0.84 in the present study.

The Hospital Anxiety and Depression Scale (HADS)

HADS is a self-report instrument measuring symptoms of anxiety and depression (Zigmond and Snaith, 1983). It consists of two subscales with 7 items each, assessing non-vegetative symptoms of anxiety and depression, respectively. Each item is rated on a four point scale (0-3). Higher scores are associated with higher symptom levels. A score of 8 or more on each scale suggests possible cases of anxiety/depression. Chronbach's alpha for the anxiety and depression subscales were 0.69 and 0.64, respectively.

3.1.4 Ethics

The study was anonymous and a passive consent procedure was applied. The study was approved by the Regional Ethics Committee of Western Norway (REK-West) and Norwegian Social Data Service (NSD).

3.2 Study 2

3.2.1 Sample

Adolescents and young adults with DSPD and healthy controls were recruited from high schools, colleges and the University of Bergen between 2008 and 2011. A total of 264 potential DSPD participants and 55 healthy controls volunteered for participation. They were screened through a short telephone interview and one week of sleep diary. Altogether 60 potential DSPD participants and 31 healthy controls fulfilled the basic criteria for inclusion and were scheduled for a meeting at the sleep laboratory (Faculty of Psychology, University of Bergen). A total of 10 potential DSPD participants and 7 healthy controls withdrew prior to or during this meeting, at which the potential participants were screened with SCID-I (First *et al.*, 1995), Raven's matrices (Raven, 2000; Raven *et al.*, 2000) and a pregnancy test (females), and further set up for a polysomnographic screening. A total of 40 participants with DSPD and 21 healthy controls were included for participation in the study. Paper 2 includes data from the 54 participants with valid polysomnography recordings, 35 with DSPD and 19 healthy controls. Paper 3 includes all the 40 participants enrolled to participate in the treatment study (ClinicalTrials.gov NCT00834886). Fig.3 illustrates participation flow of the study.

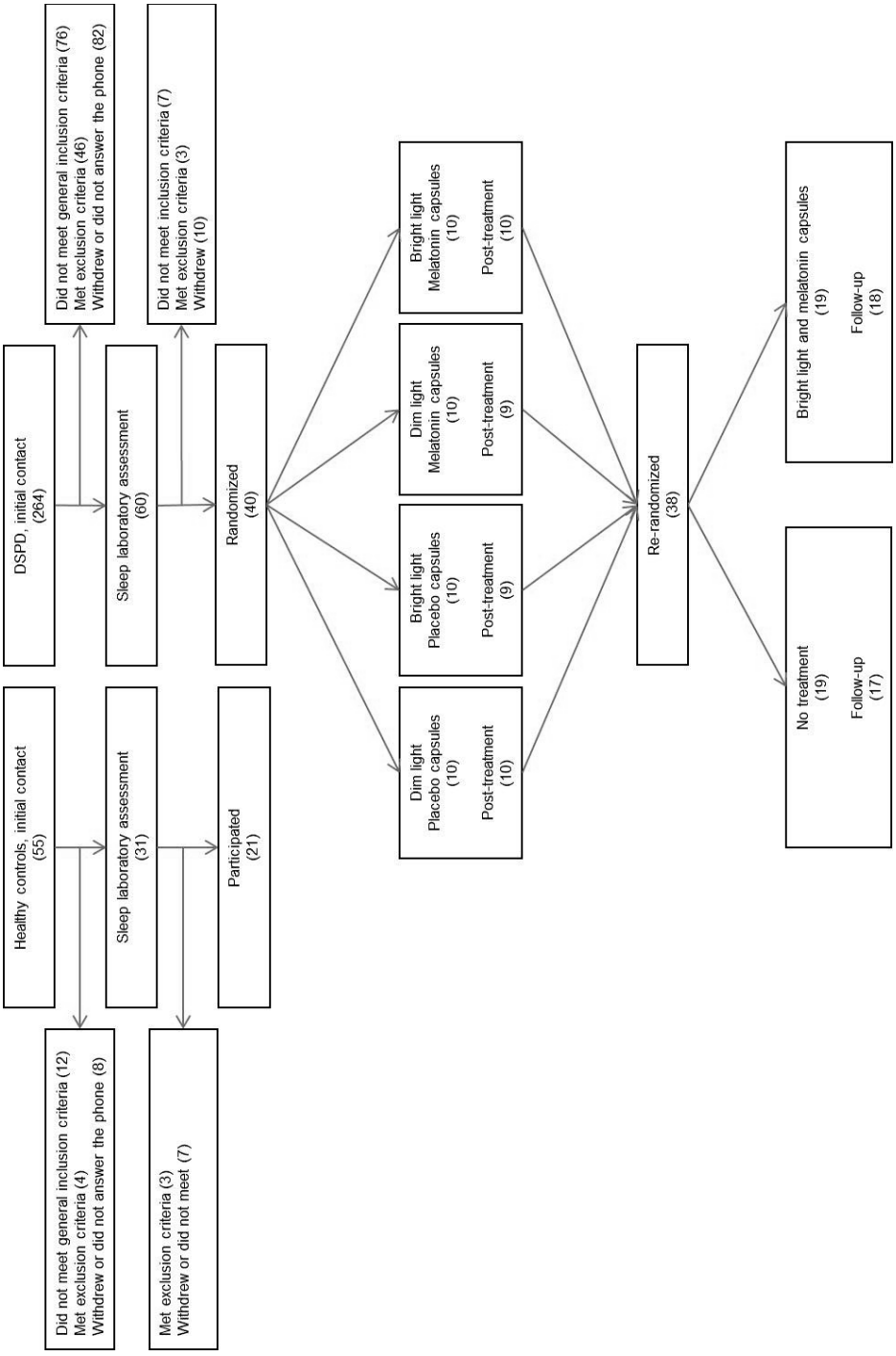

Figure 3. Participant flow of study 2

Inclusion and exclusion criteria

Inclusion criteria for the study were 1) living in Bergen, Norway, 2) age 16-25 years, 3) good general health as specified by the exclusion criteria and 4) DSPD diagnosis OR fulfilling the criteria for healthy controls. DSPD was diagnosed according to the diagnostic criteria of the ICSD-2 (American Academy of Sleep Medicine, 2005), operationalized as 1) problems falling asleep in the evening, 2) falling asleep after 2 a.m. at least 3 days a week, 3) ability to sleep until early afternoon, 4) problems waking up in time for school/studies, 5) early wake-up times associated with extreme daytime sleepiness, 6) good subjective sleep quality and duration when given the opportunity to sleep at preferred times and 7) reporting the abovementioned sleep problems for more than 6 months. The diagnosis was confirmed by one week of sleep diary showing sleep onset later than 2 a.m. at least 3 days per week. Criteria for healthy controls were responding "no" to item 1 through 5, confirmed by one week of sleep diary showing sleep onset before midnight at least three days per week, later than 2 a.m. no more than 2 days per week, and sleep onset latency > 30 minutes less than 3 days per week.

Exclusion criteria were sleep disorders other than DSPD based on subjective reports and polysomnography (apnea-hypopnea index > 5 and periodic limb movement index > 15), moderate to severe psychopathology or treatment for psychopathology within the last 4 weeks (based on SCID-I interviews), somatic disorders or conditions assumed to affect sleep (i.e. migraine, B12 deficiency), all serious somatic disorders (i.e. rheumatoid arthritis, diabetes), medications assumed to affect sleep (i.e. sedative anti-histamines, antidepressants, hypnotics), substance abuse, night work, IQ < 70 (Raven's matrices), breast feeding and pregnancy.

3.2.2 Procedure

Study design

The study was partly at home based and partly laboratory based. The first part of the study (baseline assessment) was identical for all participants (DSPD and controls).

The study schedule is illustrated in Fig.4. Participants in both groups kept a sleep diary and wore an actigraph for 7 days prior to intervention. Subsequently, participants slept according to a self-chosen sleep schedule for 4 consecutive nights/days. On nights 3 and 4, participants underwent PSG. On night 5, saliva samples for estimation of DLMO were collected. On day 5 participants were to rise at 7 a.m. for daytime testing in the sleep laboratory (8 a.m. – 3 p.m.). In the sleep laboratory, participants were tested on different aspects of daytime functioning and completed several questionnaires including the Pittsburgh Sleep Quality Index (PSQI) and the Bergen Insomnia Scale (BIS). The controls had by this completed the study, whereas participants with DSPD entered the clinical trial.

In the clinical trial, starting on day 6, the DSPD participants were randomized into one of 4 treatment conditions each lasting for 2 weeks in a double blinded, placebo controlled design. The 4 treatment conditions were: dim light and placebo capsules (placebo group), bright light and placebo capsules (bright light group), dim light and melatonin capsules (melatonin group) and bright light and melatonin capsules (combination group). In the follow-up study, participants were re-randomized into one of 2 groups to receive treatment with the combination of bright light and melatonin (treatment group) or no treatment (no-treatment group) in an open label trial for approximately 3 months. The participants kept a sleep diary and wore an actigraph during the last 7 days of the two-week intervention (two-week assessment) and during the last 7 days of the three-month follow-up study (three-month assessment). At the end of the intervention periods, saliva samples were collected for estimation of DLMO (not reported for three-month assessment due to small sample size), and on the subsequent day participants came to the sleep laboratory for daytime testing. Capsules were not administered on the evening prior to DLMO assessment. Compliance, possible side effects and adverse events were logged.

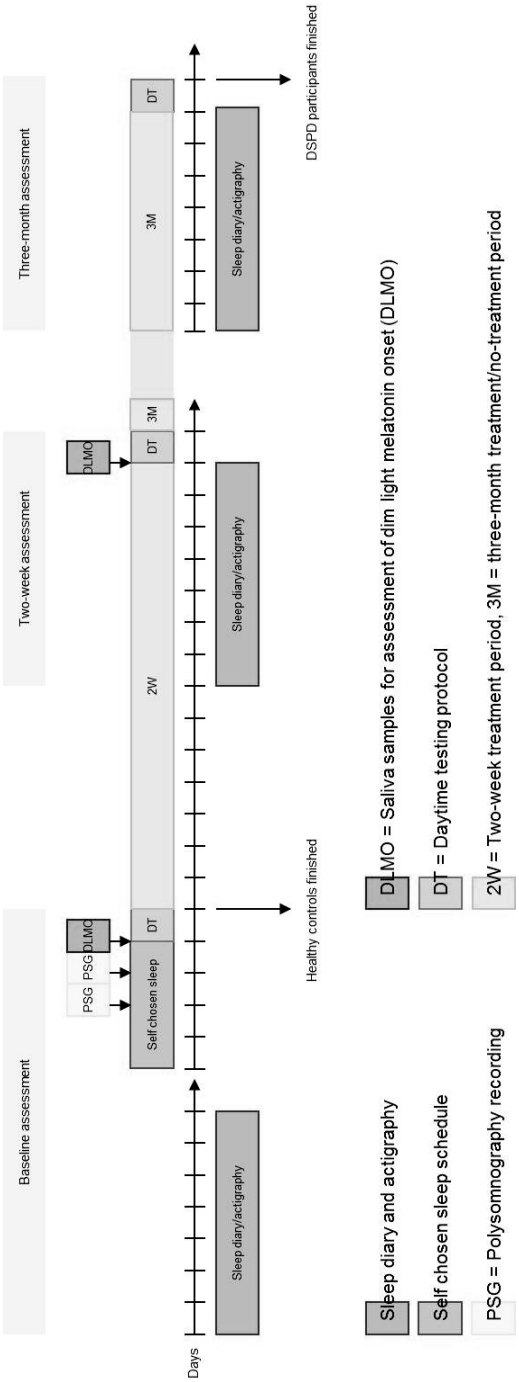

Figure 4. Study schedule of study 2

Paper 2 presents results from the case control study with respect to the polysomnographic measures of sleep from night 4 and DLMO estimates from night 5. Paper 3 presents results from the clinical trial at all three assessment points in terms of sleep (sleep diary, actigraphy, PISQI and BIS). DLMO estimates from the first two assessment points are also presented in this paper. Other results from the study are not part of the thesis.

Treatment protocol

Dim/bright light and placebo/melatonin capsules were administered together with adjunct behavioural instructions. Participants were instructed to sleep until spontaneous awakening on the first day of treatment (if they woke much earlier than their habitual wake up time they were to stay in bed and try to go back to sleep). Rise time was then to be advanced with one hour each day until preferred rise time was achieved (as chosen by the individual participant). This preferred rise time was to be maintained throughout the treatment period. Light was administered every day immediately upon awakening, for 30 – 45 minutes, with eyes directed towards the lamp and in a distance providing approximately 10 000 lux. In the evenings, 12 hours after awakening, participants were to take a capsule. However, for safety reasons related to the soporific properties of melatonin, capsules were not to be taken before 8 p.m. In the case of oversleeping, participants were to take light immediately upon awakening, melatonin 12 hours later and to advance rise time with one hour on the following days. No information was given regarding bed time. Use of alcohol was not allowed during the 2 week intervention. No restrictions for alcohol use were given for the 3 month follow-up study.

Bright light and dim light

The light source was ML-10 000 (47 x 17.5 x 29 cm) from Miljølys Inc., Norway. In the two-week intervention, lamplight was either bright (approximately 10 000 lux on 50 cm distance) containing three fluorescent bulbs (Philips, Ecotone, P1-L, RA-index=80, light temperature 4000 K) with a transparent cover screen or dim

(approximately 400 lux on 50 cm distance, placebo) with 1 fluorescent bulb and a dark red cover screen. In the three-month intervention, all participants received bright light.

Melatonin capsules and placebo capsules

The melatonin (5-methoxy-N-acetyltryptamine) 3 mg was purchased from Nature's One, Asman Inc, USA. For the two-week intervention, hard capsules containing either melatonin 3 mg or 3 mg of Maydis Amylum (maize starch, placebo) were packed by Kragerø Tablettproduksjon Inc., Norway. For the 3 month follow-up study, the original capsules from Nature's One were used.

Blinding

The 2 week intervention was double blinded. Lamps were packed in boxes concealing the colour of the cover screen and sealed by two PhD students at the Faculty of Psychology, University of Bergen, Norway. Participants received information that light could be either white or red, but not that light intensity was different or that the red light was regarded a placebo condition. To ensure double blinding, participants were thoroughly instructed not to reveal the colour of the lamp to the study personnel upon return. Capsules were packed and blinded by Kragerø Tablettproduksjon Inc., Norway. The codes were not broken until all data were collected and analysed. The 3 month follow-up study was not blinded.

Randomization

Prior to study start, two randomization lists were produced (4 groups x two-week intervention, 2 groups x three-month follow-up), using the internet based program Research Randomizer (http://www.randomizer.org.form.htm). Upon enrolment participants were chronologically assigned to the respective groups according to these lists.

3.2.3 Instruments

Polysomnography (PSG)

Electrodes were montaged and recordings analysed according to 'The AASM Manual for the Scoring of Sleep and Associated Events' (Iber *et al.*, 2007). Embla Titanium® recorder and the Somnologica™ Studio 5.1 (Embla Systems Inc., USA) were used for data acquisition and analysis. All PSG recordings were ambulatory. Participants were prepared with electrodes in the sleep laboratory at a self-chosen time between 6 p.m. and 10 p.m. to suit their daily routines. They then retired to their homes to sleep in their own beds. Two nights of PSG were performed (nights 3 and 4). The first night was regarded as an adaptation night to account for confounding first night effects and also served as a screening night for sleep apnea and periodic limb movement disorder. On the second night, sensors for respiratory events were omitted to minimize discomfort for the participants. Only data from the second night were used in the analyses. A description of sleep parameters included in analyses can be found in paper 2.

Actigraphy

Actiwatch recorder was used for data acquisition and Actigraphy Sleep Analysis software for data analyses (Cambridge Neurotechnology Ltd, England). Data were collected with an epoch length of 1 minute. Sensitivity was set to medium. A description of sleep parameters included in analyses can be found in paper 3.

Sleep diary

The sleep diary items included bed time and rise time, sleep onset latency (SOL), number and length of awakenings, wake up time and sleep quality (scale from 1=very light to 5=very deep). A description of sleep parameters included in analyses can be found in paper 3.

Pittsburgh Sleep Quality Index (PSQI)

The Pittsburgh Sleep Quality Index is a 19 item, self-rated questionnaire which assesses sleep quality and disturbances over a 1-month time interval (Buysse *et al.*, 1989). Global score range from 0 – 21, higher scores indicating more sleep problems. The cut off for problematic sleep is set to 5.

Bergen Insomnia Scale (BIS)

BIS is a 6 item self-report instrument, constructed based upon current formal and clinical diagnostic criteria for insomnia (Pallesen *et al.*, 2008). Each item is rated along an 8-point scale, ranging from 0 to 7 days per week, yielding a total score ranging from 0 to 42.

Dim light melatonin onset (DLMO)

Saliva was collected using Salivette® tubes from Sarstedt, Germany and analyzed with enzyme-linked immunosorbent assay (ELISA) from Bühlmann laboratories, Switzerland. Analytical sensitivity of this kit is 0.5 pg/ml and functional sensitivity is 1.6-20.5 pg/ml with an inter-assay coefficient of variation of less than 30%. Samples were analyzed with Wallac plate reader from Perkin Elmer Inc., USA. The melatonin sampling procedure was based on the protocol for a partial melatonin curve as advised by Pandi-Perumal *et al.* (2007), but with modifications due to logistic reasons related to the design of the study. Participants were instructed to stay at home on the evening of the DLMO assessment (night 5). Saliva was sampled hourly from 7 p.m. and until self-chosen bed time. One hour prior to sampling start (6 p.m.) participants put on dark sunglasses (Uvex athletic ISO 9001, Uvex winter holding, Germany) reducing light intensity to less than 1% (for example 690 lux was reduced to 5.6 lux). During the collection period participants were instructed to avoid drinks with artificial colorants, alcohol or caffeine, and to avoid tooth brushing, lipstick/lip gloss, chewing gum, lemons and bananas. Participants were instructed not to eat, drink or use tobacco for the last 30 minutes before sampling. Samples were labelled, kept in the refrigerator and brought to the sleep laboratory the next morning. In the sleep

laboratory, samples were kept in a -22ºC freezer before they were transported to the immunoassay laboratory where they were stored in a -80ºC freezer until time of analysis.

DLMO was calculated by interpolation between the last sample before and the first sample after the saliva concentration reached 4 pg/ml, or extrapolation from the last 2 values when concentration reached 3, but not 4 pg/ml (Keijzer *et al.*, 2011). The calculations were done based on the scheduled sampling times (7 p.m., 8 p.m., etc). Phase angle relationship was calculated as the time interval from DLMO to sleep onset and from DLMO to sleep offset (times for sleep onset and offset were derived from the PSG recording on night 4).

3.2.4 Ethics

The study was conducted according to Good Clinical Practice (GCP), and the PhD-students conducting the trial were GCP certified. Internal monitoring procedures were incorporated in the design. Participants received written and oral information about the study before consent forms were signed. When participants were < 18 years, written and verbal consent from both the adolescent and the parents were obtained. Participants received a compensation fee (approximately 60 EURO for participants with DSPD, approximately 25 EURO for healthy controls) for the time invested. The study was approved by the Regional Committee for Medical and Health Research Ethics, the Norwegian Social Data Service and the Norwegian Medicines Agency. The clinical trial was registered at ClinicalTrials.gov with registration number NCT00834886.

3.3 Statistics

Paper 1

Means were compared between subjects with DSP and subjects without DSP using t-tests for independent samples. Frequencies were compared using Pearson chi-square test with continuity correction for 2 x 2 tables. Logistic regression analyses were conducted using DSP/not DSP as dependent variable and age, gender, smoking, alcohol use, BMI, self-reported average school grade, anxiety score and depression score as independent variables. The variables were entered both separately (crude logistic regression analysis) and simultaneously (adjusted logistic regression analysis).

Paper 2

Means for the different sleep parameters, DLMO and phase angle were compared between participants in the DSPD group and the control group using t-tests for independent samples. Effect sizes (Cohen's d = M_1-M_2/SD pooled) were calculated using an online calculator: http://easycalculation.com/statistics/effect-size.php.

Paper 3

In cases of drop outs/missing data, values were moved forward from the baseline assessment (intention to treat). Due to the smaller sample, intention to treat was not used for DLMO analyses. Background and demographic variables were compared between groups using one-way ANOVA, t-tests for independent samples and Pearson chi square tests. Intervention effects were compared between the groups using two-way ANOVA for repeated measures (baseline vs. two-week assessment, baseline vs. three-month assessment, two-week assessment vs. three-month assessment). Interaction effects were further assessed using t-tests for paired samples within each group. Cohen's d was calculated using the formula d=M1-M2/(sqrt(SD_1^2+SD_2^2)/2).

4. SUMMARY OF RESULTS

Paper 1

A sample of 1285 high school students (aged 16-19 years) participated in an internet based study answering questions about sleep habits, height, weight, smoking, alcohol use, school grades, and anxiety and depression symptoms. Delayed sleep phase (DSP) was operationalized as difficulties falling asleep before 2 a.m. at least 3 nights per week together with much or very much difficulties waking up in the morning. The prevalence of students reporting DSP in the present study was 8.4%. The students with DSP had shorter TST during weekdays due to later bed times and longer sleep onset latencies whereas TST during weekends did not differ from students without DSP. Students with DSP more frequently reported weekend rebound sleep (76% compared to 52 % in students without DSP). Logistic regression analyses showed that average school grade was negatively whereas smoking, alcohol use and scores on anxiety and depression were positively associated with DSP. DSP was not associated with age, gender or BMI. Students with DSP who also reported daytime consequences and problems advancing the sleep period constituted 5.7% of the total sample (68% of all students reporting DSP). In conclusion, DSP appeared to be common amongst Norwegian adolescents and was associated with negative outcomes such as lower average school grades, smoking, alcohol usage and elevated anxiety and depression scores.

Paper 2

Polysomnographic data from 54 adolescents and young adults (age range 16-25 years) sleeping on a self-chosen schedule were analysed, 35 diagnosed with DSPD and 19 healthy controls. Salivary dim light melatonin onset (DLMO) was measured on the evening following the PSG recording. Results showed delayed timing of sleep in participants with DSPD in terms of bed time (2:32 ± 124 min compared to 23:50 ± 69 min in the control group, $p<.0005$) and rise time (12:55 ± 113 min compared to 09:01

± 70 min in the control group, p<.0005). Participants with DSPD had longer sleep onset latency, but once sleep was initiated no group differences in sleep parameters were observed with respect to sleep duration or architecture, as illustrated by two representative hypnograms in Fig.5. DLMO was delayed with more than 3 hours in participants with DSPD, but no difference in phase angle was observed between the groups. In conclusion, both sleep and DLMO were delayed in participants with DSPD. The sleep period appeared to occur at a similar phase angle in both groups, and once sleep was initiated no differences in sleep parameters were observed.

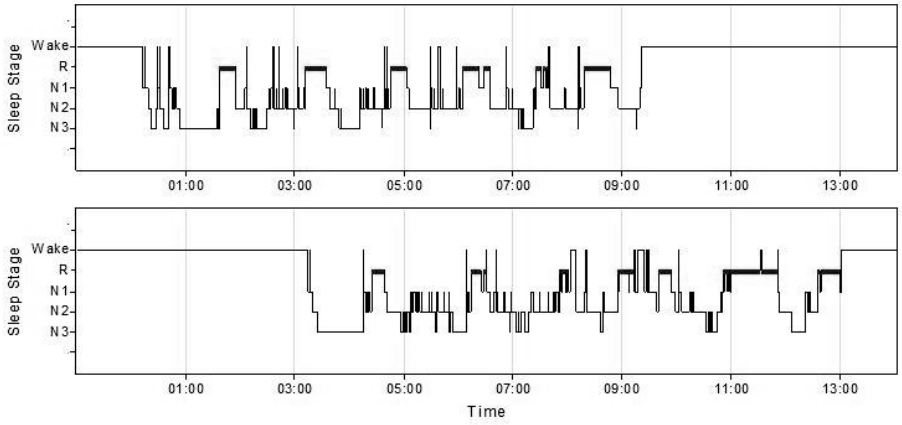

Figure 5. Two representative hypnograms showing the sleep stage distribution in a healthy control (top) and a participant with DSPD (bottom). Differences were only related to the timing of sleep.

Paper 3

A total of 40 adolescents and young adults (age range 16-25 years) diagnosed with DSPD were randomized to receive two weeks of treatment in one of four treatment conditions: dim light and placebo capsules, bright light and placebo capsules, dim light and melatonin capsules, bright light and melatonin capsules. Subsequently, participants were re-randomized into one of two groups to receive treatment for three months (bright light and melatonin) or no treatment. Light and capsules were administered alongside gradual advancement of rise times. Main end points were

subjective and objective measures of sleep as recorded by sleep diary and actigraphy, and estimation of DLMO (at baseline- and two-week assessment). Self-reported sleep problems was addressed using the Pittsburgh Sleep Quality Index (PSQI) and the Bergen Insomnia Scale (BIS). At two-week assessment the timing of sleep and DLMO were advanced in all four treatment conditions as seen by about 1 hour advance of bed time, 2 hours advance of rise time and 2 hours advance of DLMO in all groups. TST was reduced with approximately 1 hour. Scores on the PSQI and the BIS improved across the groups. At three-month follow-up, only the treatment group had maintained an advanced sleep phase whereas the no-treatment group had relapsed to baseline levels. TST had returned to baseline levels in both groups. Scores on the PSQI were further improved in the treatment group but not in the no-treatment group. In conclusion, gradual advancement of rise time produced a phase advance during the two-week intervention, irrespective of treatment condition. Termination of treatment caused relapse into delayed sleep times, whereas long-term treatment with bright light and melatonin (three months) allowed maintenance of the sleep rhythm.

5. DISCUSSION

5.1 Discussion of findings

Adolescent sleep and prevalence of DSP

Research has consistently shown sleepiness and delayed and irregular sleep patterns in adolescents, and it is likely that a large proportion of adolescents have a delayed sleep phase (DSP) with potentially severe negative consequences (Crowley *et al.*, 2007). In our sample of Norwegian high school students, 8.4 % reported DSP operationalized as difficulties falling asleep before 2 a.m. at least 3 nights per week together with much or very much difficulties waking up in the morning (paper 1). With less than 7 hours sleep duration on weekdays it appears that the students with DSP experience weekday sleep curtailment (Carskadon *et al.*, 1980). In line with this, students with DSP frequently reported weekend rebound sleep. Maintaining delayed sleep times during weekends may have prevented these students from achieving a stable sleep phase advance, and it is possible that some of the students with DSP were "trapped" in a vicious cycle. This notion is supported by the fact that sleep onset latency on weekdays was long (more than 1 hour) in students with DSP despite the late bed times, possibly indicating an internal delay of the endogenous rhythm. Moreover, almost 80% of students with DSP reported problems advancing the sleep period. We note, however, that also students without DSP reported short weekday sleep duration (less than 8 hours during weekdays) and weekend rebound sleep. Hence, it appears that also students without DSP developed a sleep debt during weekdays. As such, paper 1 confirms previous reports of delayed and irregular sleep habits in adolescents.

Correlates of DSP

DSP was associated with lower school grades, more smoking, more alcohol use and higher scores on anxiety and depression (paper 1). Previous studies have shown that

students with better school grades report longer and more regular sleep on school nights and less sleep schedule delay during weekends than students with lower grades, and that students with adequate sleep perform better at school than students with less than adequate sleep (Wolfson and Carskadon, 1998; Wolfson and Carskadon, 2003; Dewald *et al.*, 2010). Short sleep in adolescents has also been linked to elevated scores on anxiety and depression (Wolfson and Carskadon, 1998; Pasch *et al.*, 2010; Regestein *et al.*, 2010) and to the use of cigarettes, alcohol and illicit drugs (Pasch *et al.*, 2010). The results from paper 1 are as such in agreement with previous findings of negative associations of delayed and irregular sleep patterns.

Due to the cross sectional design, the study does not yield information of the causal relationship between sleep and the outcome variables. It is, however, plausible to assume that the associations found in the present study may be related to the observed weekday sleep curtailment. Students who get more sleep may be more alert during the day and thus able to achieve better grades. In studies where school start times have been delayed, adolescents sleep longer on school nights, report less daytime sleepiness, obtain better grades and experience less mood disturbances (Wolfson and Carskadon, 2003; Owens *et al.*, 2010; Vedaa *et al.*, 2012).

The associations reported in paper 1 may also be explained in terms of diurnal preference. The adolescent sleep phase delay is associated with increased evening preference (Carskadon *et al.*, 1993), and although chronotype was not assessed in the present study, students with DSP were likely high scorers on eveningness. Evening- and morning types have different optimal time windows for cognitive performance, as reviewed by Cavallera *et al.* (2011), and accordingly the relationship between DSP and academic performance may be attributed to circadian variations affecting performance in ways not mediated through sleep debt (Carrier and Monk, 2000). Several studies have linked eveningness to personality features such as neuroticism and conscientiousness, in particular low self-control (Digdon and Howell, 2008; Tonetti *et al.*, 2009). Sensation seeking, impulsivity and low self-control may explain some of the problems associated with eveningness in terms of smoking and alcohol

use (Tonetti *et al.*, 2010; Negriff *et al.*, 2011; Prat and Adan, 2011; Urban *et al.*, 2011). Depression seems to be associated with an evening preference, independent of sleep-wake conditions (Hidalgo *et al.*, 2009; Kitamura *et al.*, 2010; Randler, 2011).

It is, however, likely that the negative associations of DSP are of a more complex and multi-directional nature. Whereas insomnia is a common symptom of depression, poor sleep has at the same time been shown to affect mood (Riemann, 2007). Smoking is known to affect sleep architecture (Zhang *et al.*, 2006) and may cause problems initiating sleep (Soldatos *et al.*, 1980). At the same time, delayed sleep onset may provide an increased opportunity for smoking during late evening hours (Negriff *et al.*, 2011). Similarly, alcohol usage may cause adolescents to stay awake late, leading to a delay in the sleep/wake rhythm. On the other hand, late bed times may endorse engagement in social evening events where alcohol is involved. As an example of more complex associations, findings by Singleton and Wolfson (2009) indicated that the effect of alcohol on sleep quality may lead to reduced academic performance.

Prevalence of DSPD

DSPD has been described as the most common circadian rhythm disorder (Dagan and Eisenstein, 1999; Lack and Wright, 2007). Still, prevalence rates in adult populations appear to be low and little is known about its prevalence in adolescents and young adults (Gradisar *et al.*, 2011b). Survey studies have yielded prevalence rates of collections of symptoms resembling the disorder in adolescents and young adults, but results have not been univocal. Discrepancies are likely due to the use of different criteria to identify DSPD-like symptoms. In one end Lack (1986) reported that 17% of his student sample matched the criteria for at least a mild form of DSPD, whereas in the other end Hazama *et al.* (2008) estimated the prevalence of "probable DSPD" to 0.48% in their sample of Japanese students. In view of these discrepancies, results from paper 1, although not addressing DSPD directly, may add importantly to the field. In our sample, 5.7% reported DSP in addition to daytime consequences and

problems advancing the sleep phase, symptoms representing the essential features of DSPD (American Academy of Sleep Medicine, 2005).

Sleep in DSPD

Polysomnographic data presented in paper 2 revealed that the major differences between patients with DSPD and healthy controls in terms of sleep were related to timing, whereas sleep duration and sleep stage distribution were essentially similar in the two groups. These findings support the statement in the ICSD-2 that sleep in DSPD is normal when patients are allowed to sleep on a self-preferred schedule (American Academy of Sleep Medicine, 2005), but contrast findings in the only previous controlled polysomnography study performed on patients with DSPD, where sleep quality was found to be reduced in the patient group (Watanabe *et al.*, 2003). In our study participants were allowed to go to bed at a self-chosen time, whereas a habitual sleep time protocol (which possibly may reflect influences of external factors) was used by Watanabe *et al.* (2003). Hence, slight methodological dissimilarities can account for some of these inconsistencies.

Interestingly, despite the fact that participants in our study were allowed to sleep on a self-chosen schedule, SOL was slightly prolonged in the DSPD group. It is possible that patients with DSPD had earlier habitual bed times with respect to their endogenous circadian phase, which might have influenced bed time decision also upon instructions of a self-chosen sleep schedule. Moreover, the sleep onset process in patients with DSPD may be more vulnerable to external disturbances (i.e. the PSG montage) than healthy controls. Lack and Wright (2007) have proposed that patients with DSPD may develop conditioned sleep onset insomnia based on previous experiences of not being able to fall asleep. In such case, future treatment protocols should incorporate elements to address this issue, e.g. stimulus control. In a study by Gradisar *et al.* (2011a), bright light therapy was used in combination with cognitive behavioural therapy, addressing amongst others the associated insomnia. However, findings by MacMahon *et al.* (2006) give no indication of attention bias for sleep related stimuli (which is common in primary insomnia) in patients with DSPD.

Circadian phase and phase angle relationship in DSPD

In line with previous findings (Shibui *et al.*, 1999; Wyatt *et al.*, 2006; Chang *et al.*, 2009), DLMO was delayed in participants with DSPD (paper 2). Moreover, similar to what was reported by Wyatt *et al.* (2006) and Chang *et al.* (2009), no difference in phase angle relationship was observed between the groups. Prolonged phase angle relationship has, however, been reported in other studies of patients with DSPD (Ozaki *et al.*, 1996; Uchiyama *et al.*, 2000a; Watanabe *et al.*, 2003). Hence, the issue of a possible disassociation between circadian phase and sleep timing in DSPD is unresolved. Discrepancies in findings may in part be explained by the impact of external constraints, as suggested by Mongrain *et al.* (2004). It is also possible that DSPD may arise from different pathophysiological mechanisms, e.g. phase angle may be prolonged in patients where the delayed sleep phase is related to abnormalities in homeostatic processes (Uchiyama *et al.*, 2000b) but not in patients with particularly long τ (Czeisler *et al.*, 1981). This, however, remains speculations and larger scaled studies are needed in order to elucidate this issue.

Taken together, results from paper 2 support the argumentation by Chang *et al.* (2009) that when allowed to sleep on a schedule free from social restraints, sleep in DSPD takes place at a normal phase angle allowing normal sleep quality.

Short-term treatment of DSPD with bright light and melatonin

Most current treatment approaches for DSPD aim to correct the sleep phase delay by means of bright light and/or melatonin. However, no consensus exists with respect to timing, dosage, duration and adjunct behavioural instructions. Placebo controlled treatment studies are scarce both in terms of bright light (Rosenthal *et al.*, 1990; Cole *et al.*, 2002; Lack *et al.*, 2007; Gradisar *et al.*, 2011a) and melatonin (Dahlitz *et al.*, 1991; Nagtegaal *et al.*, 1998b; Kayumov *et al.*, 2001; Mundey *et al.*, 2005; Rahman *et al.*, 2010a), in particular in adolescents and young adults (Gradisar *et al.*, 2011a). In paper 3 we reported that two weeks of treatment involving administration of bright light and exogenous melatonin alongside gradual advancement of rise time in a four-armed, randomized, placebo controlled design, produced a phase advance irrespective

of treatment condition. Hence, no differences were found between the placebo group and the treatment groups, and no additive effects of bright light and melatonin could be observed, contrasting previous findings in healthy persons (Burgess *et al.*, 2003; Wirz-Justice *et al.*, 2004; Revell *et al.*, 2006). The finding that a similar phase advance was produced in the placebo group who was instructed to gradually advance rise time was unexpected. It is known that the timing of the sleep period itself may act as a zeitgeber, but its influence on the circadian phase is believed to be weak (Duffy *et al.*, 1996; Danilenko *et al.*, 2003). Still, some previous studies have suggested that sleep timing may be advanced in patients with DSPD through behavioural instructions, at least in less severe cases (Cole *et al.*, 2002; Sharkey *et al.*, 2011). Findings in paper 3 indicate that gradual advancement of rise time schedules may effectively produce a phase advance also in more severe cases of delayed sleep phase. In our study, sleep duration was reduced across groups, likely due to the imposed rise time advancement. Advanced rise times may contribute to a sleep phase advance through homeostatic processes by inducing a sleep debt which in turn instigates earlier sleep onset (Borbely, 1982). The early rise times may also have promoted exposure to outdoor light during the optimal phase advancing portion of the PRC also in the dim light conditions. In addition, the possibility of a phase advancing effect of the 300 lux dim light cannot be ruled out. Hence, phase advancement may be attributed to light also in the placebo light groups. In the present study, sleep diary scores on sleep quality improved across the groups and taken together with reduced scores on the PSQI and the BIS it appears that short-term effects of the present treatment protocol were beneficial, also in the face of reduced sleep duration.

Long-term treatment of DSPD with bright light and melatonin

Several clinical reports indicate that the effect of light and melatonin fades out upon termination of treatment (Alvarez *et al.*, 1992; Dagan *et al.*, 1998b; van Maanen *et al.*, 2011). Accordingly, treatment protocols should address the sleep phase delay in a two-step manner by 1) achieving and 2) maintaining a phase advance. However, few controlled trials have reported long-term treatment effects of DSPD. In paper 3 we

reported that three months of treatment with bright light and melatonin in an open label follow-up study allowed the patients to maintain the advanced sleep phase achieved during the two-week intervention. Hence, there was a continued effectiveness of bright light and melatonin over three months with no signs of tolerance development or adverse outcomes. At three-month assessment sleep duration had increased to baseline levels, indicating that although gradual advancement of rise time induces a sleep phase advance at the cost of sleep duration in the initial phase, normal sleep duration is resumed when treatment is maintained over sustained periods of time. Since sleep duration relies on the timing of the sleep period in relation to the endogenous circadian phase (Borbely, 1982; Dijk and Czeisler, 1995) the resumption of normal sleep duration can be taken as support for a well-adapted sleep schedule/circadian phase relationship. At three-month follow-up, the PSQI and the BIS scores were reduced in the treatment group, not only with respect to the baseline levels but also with respect to the two-week assessment, indicating that the beneficial treatment effect increased as the sleep schedule/circadian phase stabilised. In contrast, patients not receiving treatment relapsed into delayed sleep times despite the fact that participants had become familiar with the principles of the gradual rise time advancement and maintenance protocol. Accordingly, although behavioural interventions in the form of rise time schedules appear capable of producing a sleep phase advance in patients with DSPD, information and training related to these concepts do not appear to suffice for sustained effects. Motivational issues may have strong impact on compliance to the treatment regime since it involves structuring the daily schedule, in particular in young patients with DSPD. It is possible that motivational therapy, such as Motivational Interviewing, can increase adherence to the advanced sleep schedules. In the study by Gradisar *et al.* (2011a) an advanced sleep schedule was more or less maintained for six months in adolescents with DSPD after the initial sleep phase advance.

5.2 Methodological considerations

5.2.1 Study design

Study 1

Study 1 was an internet-based, cross sectional survey. A cross sectional design allows for large sample sizes, and is therefore well suited to calculate prevalence rates. In the present study, data were collected in a large and representative sample of Norwegian high school students with a relatively high response rate of 69.8%. Still, results must be interpreted with caution since we have no information about the non-responders (30%) and potential selection bias. Using a cross sectional design it is also possible to address associations between variables such as risk factors for the outcome of interest. It is, however, important to note that cross sectional studies yield information only with respect to a given time, hence no conclusions can be drawn concerning cause and effect relationships. In order to determine prognoses or outcome, large studies of longitudinal designs (participants followed up over time) are required. All data in the present study were self-reported, and relationship between constructs may therefore be influenced by the common method bias.

Study 2

Study 2 comprised both a case control study and a randomized, placebo controlled clinical trial (RCT) with a randomized, controlled open label follow-up study. The sample included a group of 40 patients with DSPD and 21 healthy controls in the age range 16-25 years. Mean age did not differ between the groups. Most participants were students attending high school, college or university. High school students more often have early hour obligations than college and university students, but since the proportion of students from each institution was similar in the different groups any major effect on the outcome of the study is unlikely. The study was conducted in Bergen, Norway, at 60.39°N, yielding large differences in solar day length between

midsummer and midwinter. In the present study, we did not systematically account for effects of season which potentially may have affected the trial outcome. However, the project ran for almost 4 years with continuous enrolment across seasons, with no obvious difference between the groups with respect to season of enrolment. Participants in the DSPD group had volunteered to participate in a treatment study, causing a potential selection bias towards help-seeking patients. Participants in the control group, however, received only a small compensatory fee. Hence, there is a potential selection bias in this group towards resourceful and socially engaged youngsters.

In the case control study, polysomnographic measures of sleep were compared between 35 patients with DSPD and 19 controls (paper 2). For the remaining participants (5 DSPD, 2 controls), PSG recordings from night 2 were either missing due to technical issues (5 participants) or considered not valid due to non-compliance with respect to the protocol (i.e. forced early awakening, 2 participants). The missing PSG data were thus not expected to represent a selection bias. Case control designs are well suited to address characteristics of a patient group with respect to healthy individuals. To reduce the impact of confounding factors, we carefully sought that participants in both groups followed the exact same study protocol. In particular, the impact of external influence on the sleep schedule prior to and during PSG recording was reduced by instructing the participants to sleep on a self-chosen sleep schedule. Still, in case control designs it is impossible to adequately account for all potential confounding factors and apparent relationships may thus not be causal. The four-armed clinical trial was randomized and double blinded. Since randomization and blinding reduce potential bias, such trials are considered to yield reliable measures of intervention effects. The two-armed follow-up study was not blinded or placebo controlled, hence differences between groups may be explained in terms of expectation bias. Few participants dropped out from the trial during treatment (Fig.3) and analyses were carried out according to intention to treat, hence reducing potential bias.

5.2.2 Procedures

Operationalization of DSP

In paper 1, DSP was operationalized as problems falling asleep before 2 a.m. at least 3 nights per week together with much/very much difficulties waking up in the morning. These criteria were based on descriptions in the first edition of the international classification of sleep disorders (ICSD-1) (American Academy of Sleep Medicine, 1990) that patients with DSPD typically complain primarily of chronic difficulty in falling asleep before 2 a.m. or difficulty awakening at the desired or necessary time in the morning to fulfil social or occupational obligations. Using criteria related to descriptions of DSPD we aimed to identify students with a severe and potentially debilitating delayed sleep phase. The prevalence rate of DSPD was not addressed in the study. We report, however, the prevalence of students with DSP together with related daytime consequences and problems advancing the sleep/wake cycle, which may indicate at least a mild form of DSPD. No sleep diary was included to show a stable, delayed sleep pattern, and students were not screened for other potential disorders. Accordingly, no DSPD diagnoses could be made, and the practical value of the prevalence rate obtained may thus be questioned.

Screening and diagnostic criteria

In study 2, patients with DSPD were diagnosed according to the criteria of the ICSD-2 (American Academy of Sleep Medicine, 2005), confirmed by one week of sleep diary. In diagnosing DSPD there are no requirements with respect to clock times. Patients should experience a phase delay in relation to the desired rhythms. However, in order to ensure that the DSPD group and the control group did not overlap with respect to sleep timing, we included only patients with DSPD with sleep onset later than 2 a.m. at least 3 days per week and controls with sleep onset before midnight at least 3 days per week, later than 2 a.m. no more than 2 days per week and sleep onset latency >30 min <3 days per week. All participants were thoroughly screened for other medical or psychiatric disorders, and PSG was used to screen for sleep apnea

and periodic limb movement disorder. Thus, our sample consisted of otherwise healthy young people with DSPD and healthy young people with normal sleep schedules.

Sleep assessment

In paper 2 we compared polysomnographic measures of sleep between patients with DSPD and controls. The self-chosen sleep schedule protocol allowed participants to sleep until spontaneous awakening for 3 days prior to the PSG night used for data analyses and 4 days prior to saliva sampling (see Fig.3), hence ruling out effects of chronic sleep debt or enforced asynchrony. Although the results are in line with descriptions of DSPD in the ICSD-2 (American Academy of Sleep Medicine, 2005), they contrast findings in the only previous controlled polysomnographic study performed on patients with DSPD (Watanabe *et al.*, 2003). It is likely that slight differences in protocols may be the cause of these discrepancies. In the habitual sleep times protocol used by Watanabe *et al.* (2003), it appears that patients were instructed to go to bed at their usual bed time. Usual bed times may be affected by external factors such as work, school or input from parents and partners, and the long phase angle may thus reflect an influence of external restraints on habitual sleep time rather than underlying pathophysiology. Moreover, the control group in that study slept at a fixed time (23 p.m. – 7 a.m.), whereas both groups in our study received instructions of a self-chosen bed time.

In paper 3, sleep was assessed by means of sleep diary and actigraphy recordings. Prior to intervention, sleep was assessed on a habitual sleep schedule. At two-week assessment (the last intervention week) sleep was assessed on a gradual advancement (and subsequent maintenance) of rise time schedule. At three-month assessment (the last intervention week), sleep was assessed on a habitual sleep schedule or a gradual advancement of rise time schedule in the no-treatment and treatment groups, respectively. Seven day average (5 weekdays and 2 weekend days) was used for analyses. In terms of sleep diary, items missing on a weekday were replaced by the average of the remaining weekdays (27 days at baseline, 6 days at

two-week assessment, 2 days at three-month assessment were replaced), whenever an item was missing on a weekend day it was replaced by the other weekend day (3 days at baseline, 0 days at two-week assessment, 1 day at three-month assessment were replaced).

Assessment of circadian phase

By measuring melatonin in saliva sampled at hourly intervals during the period of assumed DLMO, we were able to perform at home based assessment of circadian phase (Pandi-Perumal *et al.*, 2007). Saliva was sampled from 7 p.m. to self-chosen bed time to allow assessment of circadian phase without restricting sleep. However, in several cases DLMO could not be calculated due to consistent low melatonin levels in all samples. In paper 2, DLMO could be calculated in 20 participants in the DSPD group and 8 controls. In paper 3, DLMO could be calculated at both baseline- and two-week assessment in 16 participants. Saliva samples were also collected at three-month assessment, but DLMO could be calculated at both baseline- and three-month assessment in only 5 participants, which were considered too low for analyses. Consequently, DLMO is reported only for baseline and two-week assessment. It is possible that by allowing a self-chosen bed time, some participants went to bed earlier than usual and therefore before DLMO. Participants were scheduled for rise time at 7 a.m. on the following morning, which may have augmented the tendency to go early to bed. Also, the sunglasses worn by the participants were very dark and probably limited the number of activities participants could engage in. In participants where no rise in melatonin concentration was observed, the time for collection of the last saliva sample was more than 2 hours earlier than their bed time on the previous night (PSG night) compared to less than an hour in participants where DLMO could be calculated, indeed indicating that several participants went to bed earlier than usual. It is, however, also possible that behavioural factors temporarily suppressed evening melatonin. Since sampling was at-home based it was not possible to confirm that all instructions were followed. Inconsistent use of the sunglasses may have caused light exposure and thus led to a suppression of melatonin. The participants received no

specific instructions regarding posture or physical activity and occasional use of non-steroidal anti-inflammatory drugs was not prohibited. Both non-steroidal anti-inflammatory drugs and physical activity may suppress melatonin secretion. Saliva samples collected at 7 and 8 a.m. on the morning after DLMO assessment showed increased melatonin levels in all but two participants (data not included in the theses), hence arguing against the possibility that participants were low melatonin secretors (Rahman *et al.*, 2010b). In estimating phase angle relationship, PSG was obtained on the night prior to DLMO sampling (night 4 and 5, respectively). Hence, there was no direct temporal relationship between DLMO and sleep onset/offset parameters. It is, however, not likely that DLMO changes much over such a short time interval (one day), considering the findings by Wyatt *et al.* (2006) of temporal stability of DLMO.

Randomization, placebo and blinding procedures

Upon enrolment in the clinical trial, participants were chronologically allocated to the respective treatment conditions according to randomization lists. The four-armed clinical trial was double blinded, preventing allocation bias. In contrast, the two-armed follow-up study was not blinded, and strict adherence to the lists was required to avoid allocation bias. Adherence to the lists was ensured by the internal trial monitoring procedures.

In the clinical trial we faced a challenge with respect to the placebo light condition since it involved exposure of 300 lux intensity light at an optimal circadian phase. Dim light has been shown to have significant impact on the circadian rhythm (Boivin *et al.*, 1996; Zeitzer *et al.*, 2000), dependent on the duration of exposure and background illumination. Furthermore, although participants were blinded with respect to light intensity and the expected effects, it is likely that some participants realized that the red light represented the placebo condition (this was not assessed in the study). Hence, participants may not have been blinded with respect to the light condition. Conducting the study, there was also a risk that participants would reveal the colour of their lamp to the study personnel. However, no such events were reported to take place, likely due to the emphasis put on this issue when instructing

the participants. In the case control study, PSG scoring was performed blind with respect to the group identity of the participants. However, clock times were not concealed and group identity often obvious from late bed- and rise times.

Treatment protocol

In the clinical trial, timing for light/capsule administration was based on times for spontaneous awakening on the first day of treatment, which is assumed to be approximately 2 hours after CTmin (Bjorvatn and Pallesen, 2009). This aspect of the treatment protocol was essential, since we aimed to investigate a treatment protocol suitable for use in primary health care. However, by not estimating CTmin or DLMO prior to treatment, there was a risk of initiating treatment at a phase delaying portion of the PRC. Moreover, the protocol used in the present study was based on the assumption that patients were able to advance the circadian phase at least 1 hour per day. It is possible that some participants may have achieved smaller phase advances and, consequently, had scheduled rise times prior to CTmin on subsequent days. Although sleep timing and DLMO were advanced in all treatment conditions, we cannot rule out the possibility that some participants on occasions were exposed to bright light prior to CTmin which may produce a phase delay, counteracting the phase advance achieved by bright light after CTmin and possibly explaining the lack of difference between the bright light and dim light groups. In clinical practice, this pitfall may be avoided by tailoring treatment to the individual patient, for example by advancing rise time by only 30 minutes each day. The risk of administering melatonin at a phase delaying time was lower. However, research has shown that different doses of melatonin have different time windows for optimal effects (Burgess *et al.*, 2010). It is possible that melatonin administered at late times wash over to the phase delaying part of the curve, counteracting the phase advancing effect of melatonin at the phase advancing part of the curve. Accordingly, we cannot rule out the possibility that treatment with bright light and/or melatonin at more optimal times or doses may produce larger treatment effects than what is reported in paper 3. Treatment in the present study was based on administration of full spectrum light of 10 000 lux

intensity and 3 mg exogenous melatonin, and results may not be directly transferable to different doses of melatonin and/or light of different wavelengths/intensities/durations.

During the two-week intervention, compliance to treatment was high in all groups whereas compliance was somewhat lower during the three month intervention (data not included in the thesis). No serous or long lasting adverse events were reported in the trial. During the two-week intervention, mild possible side effects such as eye itch, and headaches were reported by approximately one third of the participants, with no differences between the treatment conditions. During the three-month intervention one sixth reported such side effects.

5.2.3 Instrument validity

Sleep assessment

Yielding objective information about both quality and quantity of sleep, PSG represents the gold standard for measuring sleep (Rechtschaffen and Kales, 1968; Iber *et al.*, 2007; Carskadon and Dement, 2011). In study 2, PSG was performed ambulatory following an adaptation night, minimizing potential first night effects of the procedure on sleep (Agnew *et al.*, 1966). Still, PSG is invasive and may influence sleep duration and quality. Despite the fact that PSG is regarded the classical objective measure of sleep, with standardized criteria for sleep stage assignment (Iber *et al.*, 2007), the scoring of PSG protocols is highly subjective and rather large inter- and intra-rater variability have been described (Stepnowsky *et al.*, 2004). In the present study, all PSG protocols were scored by a single, experienced, registered polysomnographic technologist (RPSGT), hence increasing the reliability of the results.

PSG is invasive and costly and yields information about one night of sleep rather than habitual sleep pattern. Since DSPD seems to reflect a sleep phase misalignment rather than abnormal sleep, sleep and treatment effects are likely better

described by less invasive procedures used over longer periods of time. This is also acknowledged in the ICSD-2 (American Academy of Sleep Medicine, 2005) where sleep diary or actigraphy rather than PSG is required for diagnosing DSPD. In the clinical trial, objective and subjective measures of sleep were therefore assessed by means of actigraphy and sleep diary. Although the objectivity of actigraphy recordings may be questioned, sleep diary and actigrapy are complimentary may be considered the recommended measurements in this kind of sleep research (Ancoli-Israel *et al.*, 2003; Morgenthaler *et al.*, 2007)

Assessment of circadian phase and phase angle relationship

DLMO is considered a robust marker of circadian phase, and at-home based DLMO assessment appears to correlate well with laboratory assessment in most patients (Pullman *et al.*, 2012). However, in some cases, at home sampling deviates from laboratory samples likely due to differences in behaviour or environment (Pullman *et al.*, 2012). Although participants in study 2 received thorough instructions with respect to the saliva sampling procedure, we cannot confirm that all instructions were followed. The saliva samples were analysed using an enzyme-linked immunosorbent assay (ELISA) with high sensitivity in concentration ranges around the DLMO threshold which was set to 4 pg/ml (Nagtegaal *et al.*, 1998a). Samples were analysed in duplicates by spectrophotometry. Samples from each participant at one assessment point were analysed with the same kit. Intra-assay variance was kept low, and in cases of discrepancies between duplicates a third analysis was performed. DLMO estimates by ELISA technique have been validated as an accurate tool in patients with DSPD (Rahman *et al.*, 2009), hence we consider the output from analyses to be reliable. In estimating DLMO, scheduled times for sampling (7 p.m., 8 p.m. etc) were used since few participants reported the accurate sampling times. Since it is not possible to confirm that participants adhered to the scheduled sampling times, inaccuracies in sampling times may have influenced the results.

Questionnaires

All questionnaires used in the study (the AUDIT, the HADS, the PSQI and the BIS) are commonly used and of high validity (Buysse *et al.*, 1989; Bjelland *et al.*, 2002; Reinert and Allen, 2007; Pallesen *et al.*, 2008). They are, however, in nature based on self-report and designed to screen for collections of symptoms. Hence clinical status with respect to alcohol abuse, anxiety, depression and sleep problems cannot be confirmed.

5.2.4 Statistical analyses

Sample size and statistical power

Data reported in paper 1 were collected in a large and representative sample of Norwegian high school students with a relatively high response rate. The case control study reported in paper 2 is, to our knowledge, the largest controlled study assessing objective measures of sleep in patients with DSPD. It is, however, worth mentioning that rather large descriptive differences on several parameters reported in paper 2 (e.g. TST and phase angle) may have failed to reach statistical significance due to large inter-individual differences in the present study (high variance). It is possible that DSPD may arise from different pathophysiological sources, and that larger scaled studies may be needed to detect and to differentiate between them. Results from the clinical trial reported in paper 3 were based on 40 patients with DSPD. Pre-calculations indicated 60 participants to be enrolled for adequate statistical power in the four-armed intervention. However, this was not possible due to recruitment challenges. Hence, a potential limitation of the clinical trial was reflected by the small group sizes, which may account for the fact that no differences were observed between the treatment conditions. The rather smaller sample in which DLMO could be calculated is a particular limitation to the study. In paper 2 and 3, group differences were reported in terms of statistical significance as well as effect size calculations. Effect size yields information about the size of the difference observed between groups/sessions, hence it extends to the clinical interpretation of the findings.

Intention to treat

Analysing the results from the clinical trial (paper 3), values were moved forward from baseline in cases of drop outs and missing data (intention to treat). Intention to treat is a conservative approach commonly used in trials to reduce bias, and provides an estimate of treatment effectiveness. Of the 40 participants enrolled in the clinical trial, two participants dropped out during the two-week intervention (one from the bright light condition, one from the melatonin condition) whereas three participants withdrew from the study during the three-month follow-up period (2 from the no-treatment condition, 1 from the treatment condition) (see Fig.3). In addition, sleep diaries at two-week assessment were missing from 2 participants in the placebo group and 1 in the bright light group, actigraphy was missing from 1 in the placebo group, PSQI was missing from 2 in the placebo group, 2 in the bright light group and 1 in the melatonin group, and BIS and HADS were missing from 1 participant in the melatonin group. At three-month assessment, sleep diary was missing from 1 participant in the no-treatment group. PSQI was missing from 1 in the no-treatment group and from 1 in the treatment group. Analyses where missing data post-treatment were excluded from the data set yielded similar results as the intention to treat analyses (data not presented in the theses). Due to the smaller sample size, intention to treat was not used in DLMO analyses.

5.2.5 Ethics

The studies in this theses were conducted in accordance with the Helsinki declaration and approved by adequate instances. In study 1, participants and their parents had received written information about the study prior to participation. A passive consent procedure was applied, which was considered appropriate since data were collected anonymously. In study 2, participants (and parents when participants were < 18 years) received written and oral information about the study and gave both written and oral consent. All participants in study 2 received a compensation fee proportional to the time invested. The clinical trial was conducted in accordance with good clinical practice (GCP) of which the PhD students conducting the trial were certified, and

internal monitoring procedures were incorporated in the design. Since no guidelines exist with respect to treatment of DSPD and adequate treatment is often not provided these patients, participants in the three-month no-treatment group were not offered treatment after follow up. During the two-week intervention, also participants in the placebo group received behavioural instructions which could potentially benefit the sleep phase through altered sleep practices (Brown *et al.*, 2002).

5.3 Implications and future perspectives

The overarching goal of this theses was to contribute to increased understanding and ultimately to better clinical management of patients with DSPD. In paper 1 we estimated a prevalence of DSP together with problems advancing the sleep phase and at least one daytime consequence of 5.7% in our sample of Norwegian high school students. Although it is important to clarify the true prevalence of DSPD in adolescents and young adults, also severe DSP is associated with a wide range of negative outcomes. Statistics Norway reports that a total of 324 990 adolescents between 15 and 19 years were living in Norway at January 1st 2012. Accordingly, more than 16 000 adolescents in Norway may have a debilitating delayed sleep phase. It is essential that effective strategies to prevent and overcome a delayed sleep phase are communicated to adolescents, parents and teachers. As shown by Brown *et al.* (2002), increased knowledge of sleep hygiene has beneficial effects on sleep practices and leads to improved sleep quality, and fixed advanced sleep times have been shown to phase advance the circadian rhythm in patients with subclinical DSPD (Sharkey *et al.*, 2011). Later school start times may allow adolescents to sleep at more optimal times according to their internal circadian rhythms, and appear to increase weekday sleep duration rather than bring about further sleep phase delay (Epstein *et al.*, 1998; Owens *et al.*, 2010; Vedaa *et al.*, 2012).

In some adolescents and young adults, however, attempts to advance the sleep phase and adhere to societal norms are unsuccessful. The reason why some develop

DSPD is not known, although mechanisms related to circadian and homeostatic abnormalities as well as behavioural factors and factors related to innate traits (i.e. personality factors) have been proposed. In paper 2 we show delayed sleep and delayed circadian phase in patients with DSPD, with no indication of homeostatic dysregulation in terms of prolonged phase angle or altered sleep architecture. Hence, although the cause of circadian misalignment in terms of τ, light sensitivity and contributing behavioural factors needs to be further addressed in the future, results from the present study support the idea that DSPD treatment protocols should aim to advance the internal circadian phase.

In specialised sleep clinics, DSPD is often treated with bright light and/or melatonin. However, no guidelines for treatment exist and management of DSPD in primary health care is often poor. Considering the presumably large number of adolescents with DSPD, it seems crucial that treatment protocols suitable for use in primary health care are established. In paper 3 we demonstrated an effect of a DSPD treatment protocol in which the timing for bright light/melatonin administration was based on behaviour rather than pre-treatment measurements of DLMO or CTmin. The results indicate that an initial phase advance may be achieved through gradual advancement of rise time. The protocol is simple and can be used by anyone with knowledge about circadian rhythms and sleep regulation, and treatment can be successfully initiated even when no specialized equipment or facilities are available. Hence, results from the present study may be directly transferable to clinical practice. In the maintenance phase of DSPD treatment, the use of bright light/melatonin may be necessary to prevent relapse. In the present study, no sign of tolerance development was observed when light and melatonin were used over a period of 3 months with no reports of serious or long lasting adverse events. It therefore seems that adolescents and young adults with DSPD may be able to adhere to work/school schedules over time through sustained use of bright light/melatonin. Results from paper 3 provide a platform clinical management, but also bring forward new research questions. According to current chronobiological knowledge, bright light is the most potent zeitgeber in humans, and the finding that an equal phase advance was produced in the

placebo group calls for further investigations as of the contributions of dim light exposure and sleep schedules. Also the effect of a phase advance on daytime functioning and sleepiness in patients with DSPD needs to be explored. Objective and subjective measures of sleepiness as well as measures of cognitive functioning were included in study 2, and will be analysed and published in the near future.

5.4 Conclusion

In paper 1 we reported a prevalence of DSP operationalized as problems falling asleep before 2 a.m. at least 3 nights per week together with much/very much difficulties waking up in the morning of 8.4 %. DSP was associated with lower school grades, more smoking, more alcohol use and higher scores on anxiety and depression. In paper 2 we reported delayed timing of sleep and DLMO in patients with DSPD. The sleep period occurred, however, at a normal phase angle and once sleep was initiated, no differences in sleep parameters were observed between patients with DSPD and controls. In paper 3 we reported a phase advance with respect to sleep timing and DLMO after short-term treatment involving gradual advancement of rise time, with no additional effect of administration of bright light and/or melatonin. Long-term treatment with bright light and melatonin allowed maintenance of the sleep rhythm whereas termination of treatment caused relapse into delayed sleep times.

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