Biological and Behavioural Basis of Delayed Sleep-Wake Phase Disorder

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ABSTRACT

Delayed Sleep-Wake Phase Disorder (DSWPD) is a debilitating sleep disturbance, associated with significant morbidity and reduction in patients' quality of life. It is the most common circadian rhythm sleep disorder and particularly prevalent in adolescents and insomnia patients with prevalence ranges of 7-16% (American Academy Of Sleep Medicine; AASM, 2014, Gradisar et al., 2011; Lack et al., 2009). DSWPD arises when patients' sleep-wake behaviour is timed significantly later relative to their necessary or conventional sleep times. Due to recurrent complaints of difficulty falling asleep and waking up at appropriate times, currently assumed aetiology for DSWPD suggests a chronic delay of the circadian system (AASM, 2014).

The use of morning bright light therapy and evening exogenous melatonin administration have been shown to advance circadian rhythms and have the potential to treat the disorder (Cajochen et al., 2005; Dawson, Lack, & Morris, 1993; Lack et al., 2009). However, limited empirical literature is available regarding their efficacy in treatment of DSWPD (Barlett, Biggs & Armstrong, 2013; Auger et al., 2015) with patient relapse reportedly common in clinical settings (Alvarez, Dahlitz, Vignau, & Parkes, 1992; Saxvig et al., 2014; Wilhlemsen-Langeland et al., 2013a). Poor treatment outcomes have stimulated speculation that the disorder may be caused by other hypothesized aetiologies, such as abnormally longer circadian period oscillations or *tau* (i.e., the time taken to complete one circadian cycle).

Using an 80-hour "forced-desynchrony" protocol, the main aim of the present study was to investigate the proposed explanation that DSWPD is driven by a longer *tau*. This protocol involves a series of 1-hour long 'days' whereby participants are given 20-minute sleep opportunities alternating with 40-minutes of enforced wakefulness. Twenty-six patients with DSWPD (N = 17 male, mean age = 21.9 ± 5.0 years) according to diagnostic criteria (AASM, 2014) and 18 healthy, control sleepers (N = 10 male, mean age = 23.7 ± 5.1 years) were selected from a community-based sample. An opportune sample of 4 patients did not meet DSWPD criteria, rather met Non-24-Hour

Sleep-Wake Rhythm Disorder (N24SWD) diagnosis and were also included in the study (N = 3 male, mean age = 25.75 ± 4.99 years).

Dim Light Melatonin Onset (DLMO) and core body temperature were measured across the 80-hour protocol to identify the timing of biological circadian rhythms and the circadian period length. Furthermore, behavioural rhythms were monitored via subjective sleepiness ratings, sleep latency measures and the psychomotor vigilance task. As a secondary aim, relationships between personality profiles using the Big Five Factors (NEO-Personality Inventory-Revised), circadian rhythms and sleep disturbances were examined to further understand if personality types as associated with circadian misalignment.

Consistent with hypotheses, circadian rhythms were generally longer for DSWPD patients relative to controls. Specifically, measures of melatonin and core temperature depicted significantly longer *taus* in DSWPD patients relative to controls. Melatonin and core temperature circadian rhythm period lengths were longer still in N24SWD patients compared to DSWPD and controls. Further scrutiny of biological (i.e., melatonin, core body temperature) and behavioural rhythms (i.e., subjective/objective sleepiness, vigilance) indicated significantly greater phase angle differences between DSWPD patients' sleep propensity and core temperature circadian rhythms compared to controls. Additionally, DSWPD patients had reduced rates of melatonin secretion during the first half of their nocturnal period implying that there may be a deficiency of early melatonin production in the melatoin profile of DSWPD patients. Personality profiles were also different between groups largely consistent with recent reports (Wilhlemsen-Langeland et al., 2013b). Particularly, patients who indicated lower scores on the Conscientiousness dimension were of greater DSWPD severity. These findings help explain the persistent tendency of DSWPD to delay, along with their frequent failure to respond to treatment and high relapse rate. They suggest that the disorder is not homogenous and that various approaches to therapy are necessary to target different causes of sleep delays.

DECLARATION

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Signed..... Date.....

Gorica Micic, B.BehavSci. (Hons)

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GLOSSARY OF ABBREVIATIONS

ADHD	Attention Deficit Hyperactivity Disorder
ANCOVA	One-Way Analyses of Co-Variance
ANOVA	One-Way Analyses of Variance
AUC	Area Under Curve
BLT	Bright Light Therapy
BMI	Body Mass Index (kilograms / meters^2)
CBT	Cognitive Behavioural Therapy
CRSWD	Circadian Rhythm Sleep-Wake Disorders
$DASS_{21}$	Depression Anxiety Stress Scale-21, short form
DLMO	Dim Light Melatonin Onset
DLM^{Off}	Dim Light Melatonin Offset
DSWPD	Delayed Sleep-Wake Phase Disorder
DSWPD-STQ	Delayed Sleep-Wake Phase Disorder - Sleep Timing Questionnaire
EEG	Electroencephalography
ECP	Endogenous Circadian Pacemaker
EMG	Electromyography
EOG	Electrooculography
GHMQ	General Health and Medical Questionnaire
ICSD-2	International Classification of Sleep Disorders-2nd Edition
ICSD-3	International Classification of Sleep Disorders-3rd Edition
M	Mean
MANOVA	Multivariate Analysis of Variance
Max	Circadian Phase Maximum *or* Acrophase
MCTQ	Munich Chronotypes Questionnaire

MD	Mean Difference
MEQ	Morningness-Eveningness Questionnaire
Min	Circadian Phase Minimum *or* Nadir
ms	milliseconds
MSF_{SC}	Mid Sleep Factor (compensating for workday sleep duration)
Mτ	Melatonin rhythm period length or melatonin *tau*
n	sample size (number of participants within study)
N24SWD	Non-24-Hour Sleep-Wake Rhythm Disorder
NEO-PI-R	NEO-Personality Inventory-Revised
OCD	Obsessive Compulsive Disorder
OTC	Over the counter
η^2	Partial Eta Square
PAE	Phase Angle of Entrainment
PSG	Polysomnography
pM	Picomolar
PRC	Phase Response Curve
PSG	Polysomnography
PSQI	Pittsburgh Sleep Quality Index
PVT	Psychomotor Vigilance Task
RIA	Radioimmunoassay
RHT	Retinohypothalamic Tract
RT	Reaction Time
SCN	Suprachiasmatic Nuclei
SD	Standard Deviation
SDS	Sheehan Disability Questionnaire

SE	Standard Error
SP	Sleep Propensity
SS	Subjective Sleepiness
SWS	Slow Wave Sleep
τ	*tau or* circadian rhythm period length
tau	Circadian rhythm period length
T	Core Body Temperature
Tτ	Temperature rhythm period length or temperature *tau*
Tmax	Temperature maximum / Acrophase
Tmin	Temperature minimum / Nadir
TTFL	Transcription-Translation Feedback Loop
V	Vigilance
WMZ	Wake-Maintenance Zone
WUZ	Wake-up Zone

LIST OF MANUSCRIPTS AND PUBLICATIONS

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G. Micic, N. Lovato, M. Gradisar, S.A. Ferguson, H.J. Burgess, L.C. Lack. Circadian Period Lengths of Core Body Temperature and Salivary Melatonin in Delayed Sleep Phase Disorder and Non-24-hour Sleep-Wake Disorder. Paper presented at 27th Annual Scientific Meeting of the Australasian Sleep Association, Melbourne, October 22-24, 2015.

G. Micic, N. Lovato, M. Gradisar, S.A. Ferguson, H.J. Burgess, D.J. Kennaway, L.C. Lack. Nocturnal melatonin profiles in Delayed Sleep Phase Disorder compared with normal sleepers. Paper presented at 27th Annual Scientific Meeting of the Australasian Sleep Association, Melbourne, October 22-24, 2015.

G. Micic, N. Lovato, M. Gradisar, L.C. Lack. Circadian *tau* differences in biological, behavioural and sleepiness rhythms in Delayed Sleep-Wake Phase Disorder and Non-24-hour Sleep-Wake Disorder patients. Paper presented at 27th Annual Scientific Meeting of the Australasian Sleep Association, Melbourne, October 22-24, 2015.

G. Micic, N. Lovato, M. Gradisar, L.C. Lack. Personality differences in Delayed Sleep-Wake Phase Disorder and Non-24-hour Sleep-Wake Disorder patients relative to healthy sleepers. Paper presented at 27th Annual Scientific Meeting of the Australasian Sleep Association, Melbourne, October 22-24, 2015.

N. Lovato, G. Micic, & L. Lack. (2015). Predicting phase timing in delayed sleep phase disorder: The accuracy of melatonin and self-reported sleep timing. 27th Annual Scientific Meeting of the Australasian Sleep Association, Melbourne, October 22-24, 2015.

G. Micic, N. Lovato, M. Gradisar, S.A. Ferguson, H.J. Burgess, L.C. Lack. Circadian Period Lengths of Core Body Temperature and Salivary Melatonin in Delayed Sleep Phase Disorder and Non-24-hour Sleep-Wake Disorder. In 29th Annual Meeting of the Associated Professional Sleep Societies, Seattle, Washington, June 6-10, 2015.

G. Micic, N. Lovato, M. Gradisar, S.A. Ferguson, H.J. Burgess, D.J. Kennaway, L.C.

Lack. Nocturnal melatonin profiles in Delayed Sleep Phase Disorder compared with normal sleepers. In 29th Annual Meeting of the Associated Professional Sleep Societies, Seattle, Washington, June 6-10, 2015.

G. Micic, N. Lovato, M. Gradisar, S.A. Ferguson, H.J. Burgess, L.C. Lack. Core temperature and melatonin circadian rhythm period lengths are longer in delayed sleep phase disorder patients than controls. 22nd Congress of the European Sleep Research Society, Tallinn, Estonia, September 2014.

G. Micic, N. Lovato, M. Gradisar, S.A. Ferguson, H.J. Burgess, L.C. Lack. Sleepiness and behavioural circadian rhythm period lengths of delayed sleep phase disorder patients and controls. 22nd Congress of the European Sleep Research Society, Tallinn, Estonia, September 2014.

G. Micic, N. Lovato, M. Gradisar, S.A. Ferguson, H.J. Burgess, D.J. Kennaway, L.C. Lack. Nocturnal melatonin profiles in Delayed Sleep Phase Disorder compared with normal sleepers. 22nd Congress of the European Sleep Research Society, Tallinn, Estonia, September 2014.

G. Micic, N. Lovato, M. Gradisar, S.A. Ferguson, H.J. Burgess, L.C. Lack. Predictors of core temperature and melatonin circadian phase timings. 22nd Congress of the European Sleep Research Society, Tallinn, Estonia, September 2014.

G. Micic, L. Lack, N. Lovato, P. Turvey, S.A. Ferguson, H.J. Burgess (2013). Circadian temperature and melatonin period lengths in Delayed Sleep Phase Disorder, Sleep Medicine 14S, e175-6. (5^{th} World Congress on Sleep Medicine, Valencia, Spain, September, 2013).

G. Micic, L., Lack, N., Lovato, S.A., Ferguson H.J., Burgess, and D., Kennaway (2013). Circadian temperature and melatonin period lengths in Delayed Sleep Phase Disorder, Sleep Medicine. Paper presented at 25th Annual Scientific Meeting of the Australasian Sleep Association, Brisbane, October 17-19.

G. Micic, L., Lack, N., Lovato, H., Wright, and M., Gradisar (2013). Circadian period lengths of core temperature and sleepiness rhythms in patients with delayed sleep phase disorder. In 27th Annual Meeting of the Associated Professional Sleep Societies.

G. Micic, L., Lack, N., Lovato, P., Turvey, S. Ferguson, and H. Burgess (2013). Endogenous circadian temperature and melatonin period lengths in patients with delayed sleep phase disorder. In 27th Annual Meeting of the Associated Professional Sleep Societies.

G. Micic., L. Lack., & N. Lovato (2012). Endogenous core temperature and melatonin *taus* of healthy individuals and people with Delayed Sleep Phase Disorder. 3rd Annual Scientific Meeting of the Adelaide Sleep Retreat: Adelaide, Australia.

G. Micic, L. Lack, N. Lovato, H. Wright, & P, Turvey (2012). Circadian rhythms of core body temperature and melatonin in persons with Delayed Sleep Phase Disorder. Paper presented at 24th Annual Scientific Meeting of the Australasian Sleep Association, Darwin, October 10-13.

G. Micic, M. Fordyce, L, Lack., & N, Lovato. (2012). Circadian rhythms of central executive functioning in good sleepers and people with Delayed Sleep Phase Disorder. Paper presented at 24th Annual Scientific Meeting of the Australasian Sleep Association, Darwin, October 10-13.

CHAPTER 1 OVERVIEW AND RESEARCH AIMS

1.1 Overview

1.1.1 Defining Delayed Sleep-Wake Phase Disorder

Delayed Sleep-Wake Phase Disorder (DSWPD) is characterised by a 2- to 6-hour delay in sleep patterns, compared to conventional or socially desirable sleep times (American Academy Of Sleep Medicine; AASM, 2014). It is assumedly a body clock sleep disorder due to the misalignment of patients' sleep/wake schedule to the 24-hour solar day. This is coupled with patients' persistent inability to retime, or shift the sleeping pattern to an earlier desirable time (AASM, 2014; Regestein & Monk, 1995; Sack et al., 2007). Although, patients with DSWPD have difficulty falling asleep at night and awakening in the morning at conventional times, they show a stable and solid sleep structure at their habitual delayed time (AASM, 2014). To ensure early, conventional awakenings in the morning in order to meet work or social obligations, alarm clocks and forced awakenings are usually necessary.

There is a markedly negative impact of DSWPD on patients' quality of life. One consequence of forced awakening, coupled with the inability to initiate sleep at a conventional bedtime, is reduced total sleep time and cumulative sleep loss, which lead to impairments in daytime functioning (Millman, 2005). These include poor concentration and alertness, as well as performance dysfunction, daytime sleepiness, mood swings and fatigue (Gradisar et al., 2011; Lovato et al., 2013; Regestein & Monk, 1995; Saxvig et al., 2012). Further, the disorder has been associated with somatic complaints, depression, anxiety, attention deficit hyperactivity disorder (ADHD) symptoms and decreased resilience, likely related to daytime impairments (Gradisar et al., 2011; Silversen et al., 2015).

At present it is estimated that approximately 0.5 - 16% of the population are affected by DSWPD, with greatest prevalence among adolescents/young adults (16%) and those suffering insomnia (10%; AASM 2014; Gradisar et al., 2011; Hazama et al., 2008; Lovato et al., 2013; Regestein & Monk, 1995; Saxvig et al., 2012; Schrader et al., 1993). According to Gradisar et al., (2011) this prevalence estimate equates to approximately 50,000–190,000 Australian adolescents and more than 1,000,000 US adolescents dealing with the associated impairments of DSWPD. In 2004, Australian health care costs related to sleep disorders were estimated at A$10.3 billion, with absenteeism and productivity losses accounting for the majority of this cost (Access Economics, 2005). Given DSWPD is the most prevalent Circadian Rhythm Sleep-Wake Disorder (CRSWD; AASM, 2014), the economic burden associated with DSWPD in Australia is likely to be substantial.

There is a clear need to decipher the causes of DSWPD. Although researchers are endeavouring to identify its aetiology (Campbell & Murphy, 2007; Wagner, Moline, Pollack, & Czeisler, 1986; Watanabe et al., 2003), we do not yet fully understand the condition. The present thesis aims to further investigate the underlying causes of DSWPD and understand how the rhythms become and tend to remain delayed. This information will target the critical aetiological factors and hopefully provide more effective, durable therapies for the condition. Improving long-term DSWPD treatment will increase quality of life for patients. Furthermore, by alleviating daytime sleepiness and fatigue we can reduce societal costs of accidents, sickness absence and decline in productivity in workplace and school settings (Akerstedt, Kecklund, Alfredsson, & Selen, 2007).

1.1.2 Sleep Regulation

To understand mechanisms underlying DSWPD, it is important to understand the processes involved in the regulation of sleep and wakeful states. The natural environment cycles on a 24-hour period and sleep/wakefulness cycles have biologically adapted to follow this cycle rate. In varying

ways, the sleeping patterns of all organisms have evolved to correspond with the environment (e.g., with respect day/night sequence, ambient temperatures, access to food and safety). For mammals, this sleep/wake pattern is controlled by intrinsic brain mechanisms proposed in the two-process model of sleep (Borbely, 1982). The two main processes underpinning this model are represented in Figure 1-1 and consist of Circadian Process (Process C) and the Homeostatic Process (Process S). Although these mechanisms are theoretically independent of each other in their effects on sleep/wake states, generally they operate in unison for most individuals who are entrained to the 24-hour world (Dijk & Lockley, 2002). Both processes are subsequently described, and while both are essential for proper regulation of sleep and wakefulness, the circadian process is predominantly associated with DSWPD and its aetiology.

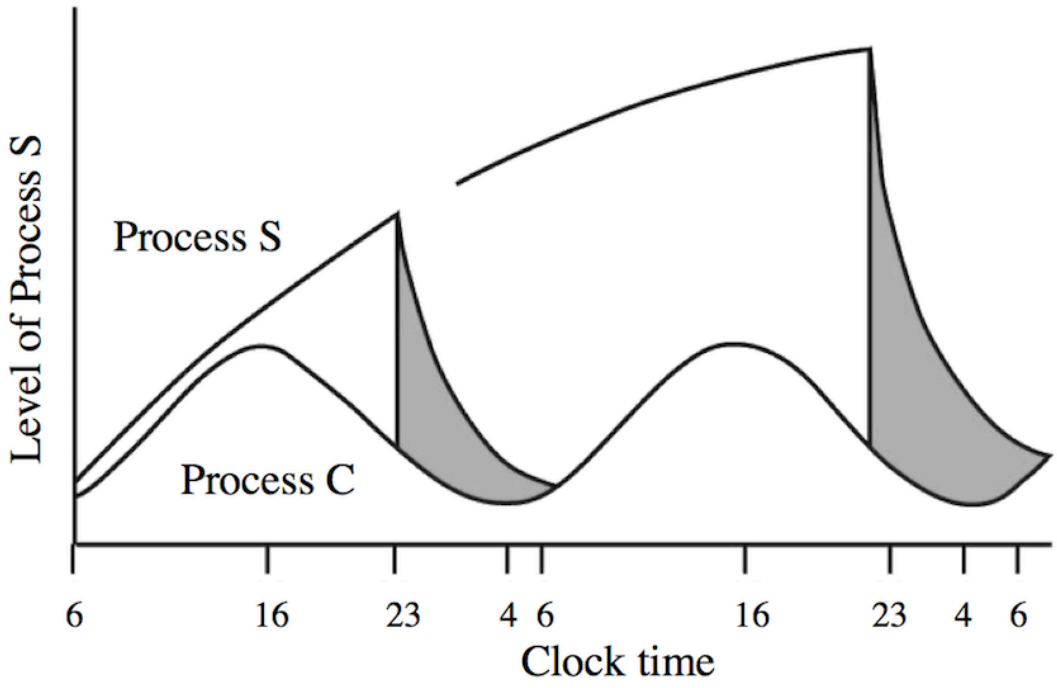

Figure 1-1. Schematic representation of the two process model of sleep and sleep regulation. Process S (S) builds up during wakefulness and dissipates during sleep (depicted in the shaded areas). In the absence of sleep or during sleep deprivation, Process S builds exponentially, while Process C (C) continues to oscillate at a near-24 hour rhythm. Typically sleep onset occurs during the downward slope of Process C and sufficient accumulation of Process S. Sleep offset ensues when Process S unites with the upslope of the (C) curve. Adapted from Voderholzer, U. (2003). Sleep deprivation and antidepressant treatment. *Dialogues in Clinical Neuroscience, 5,* 366–369.

1.1.2.1 Homeostatic Sleep Drive (Process S).

The term 'homeostasis' defines the physiological process involved in maintaining stable bodily states. The homeostatic sleep drive, therefore, sustains a balance between the time a person spends awake and the time spent asleep. Conceptually, sleep propensity accumulates during periods of wakefulness and dissipates during sleep - as time spent awake increases, the need for sleep increases, and, as sleep time increases, the pressure to sleep diminishes. This process is considered pivotal for sleep quality because extended wakefulness accumulates sleep propensity, increasing the likelihood of sleep. That is, as sleep pressure increases sleep onset is faster, sleep is deeper, more consolidated and therefore sleep is of better quality. Nevertheless, while homeostatic sleep drive is an important determiner of sleep, the circadian process is believed to be central to DSWPD aetiology (AASM, 2014; Weitzman et al., 1981) and will be discussed in more detail.

1.1.3 Circadian Rhythms (Process C)

Circadian rhythms define the cyclic changes of the biological body clock every 24-hours. They explain why diurnal mammals' sleep intervals are typically longer during the biological night and shorter during the biological day (Aschoff, 1965). Hence, while Process S supports the quality of sleep, the circadian process strongly determines the timing of sleep and helps to sustain sleep duration at. This is most easily observed in the typical daytime activity and night-time inactivity (i.e., sleep/wake behaviour) of most organisms. Circadian rhythms are ubiquitous and these daily changes can be measured in virtually all behavioural (e.g., vigilance), physiological (e.g. body temperature), hormonal (e.g. melatonin, growth factor), and biochemical (e.g. neurotransmitter, genetic transcription) variables (Czeisler & Khalsa, 2000; Moore-Ede et al., 1982). A master clock within the mammalian brain regulates these rhythms keeping them synchronised to maintain optimal physiological and behavioural functioning.

1.1.3.1 Circadian Regulation.

The master clock, also called the Endogenous Central Pacemaker (ECP), or the Suprachiasmatic Nucleus (SCN)[1] regulates all peripheral circadian rhythms (Aschoff, 1965; Welsh, Takahashi, & Kay, 2010). The ECP synchronises circadian rhythm alignment with the 24-hour solar Earth days. This internal clock is located in the hypothalamus of the brain, directly above the optic chiasm and the brainstem, approximately 3cm behind the eyes. The ECP receives neural inputs from retinal photoreceptors via the optic chiasm and the neural pathways involved in this regulation (depicted in Figure 1-2).

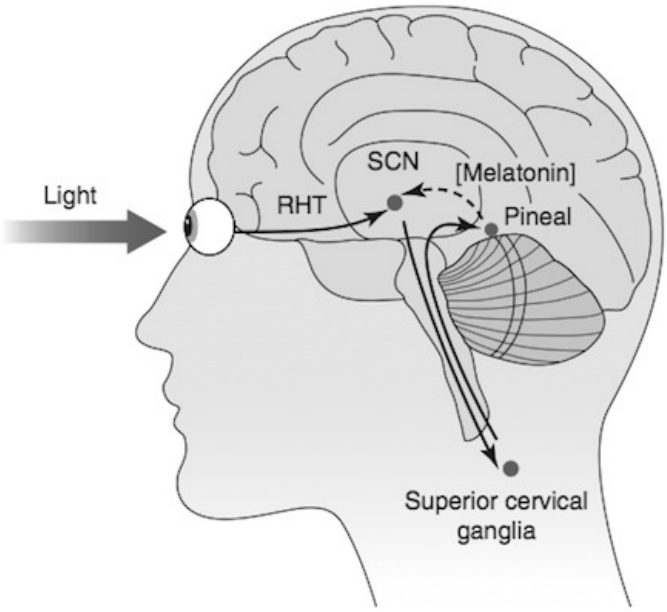

Figure 1-2. An illustration of the light/dark influence on circadian rhythms and related biological and behavioural factors. In the presence of light, photic input travels via the retinohypothalamic tract (RHT) to the suprachiasmatic nucleus (SCN). Neural signals from the SCN activate the pineal gland, sending nerve impulses via the superior cervical ganglion to inhibit melatonin production. In the absence of light and photic signals, inhibiting signals are reversed and melatonin secretion then acts on the SCN to decrease the alerting signal. *From Reid, K.J., Zee, P.C. (2009). Circadian rhythm disorders. Seminars in Neurology, 29, 393–405.*

[1] **Note.** Terms Endogenous Central Pacemaker (ECP) and Suprachiasmatic Nucleus (SCN) are synonyms and used interchangeably throughout this thesis.

Although the ECP is fundamental to the synchrony of the circadian rhythms with the environment, in the absence of light input it exhibits a spontaneous period length that is slightly greater than 24 hours (Aschoff, 1965; Czeisler et al., 1989). The period length (or *tau*) refers to the length of time taken to complete one rhythmic cycle, and an entire cycle is complete when the rhythm begins to repeat itself for the second time. In healthy individuals the timing of ECP is adjusted daily (known as *entrainment*) to near-24 hours via external stimuli (Reppert & Weaver, 2002; Saper, Scammell & Lu, 2005). These external stimuli are called *zeitgebers*, stemming from a German word, *zeit* meaning 'time' and *gebers* meaning 'givers'. Thus, zeitgebers are environmental stimuli that entrain the ECP and peripheral circadian rhythms to the solar 24-hour light/dark cycle (Aschoff, 1965). Light is a paramount zeitgeber, while ambient temperature, social interactions, exercise, daily habits and pharmacological manipulation have been demonstrated to also influence ECP timing usually to a lesser extent (Czeisler et al., 1989; Duffy et al., 1996).

1.1.4 Measuring Circadian Rhythms

Proper alignment of circadian rhythms to the external environment is necessary for health and wellbeing (Vogel et al., 2012). Knowledge of individuals' specific ECP timing helps to inform about their entrainment, sleep/wake cycle and circadian disorders (Pandi-Perumal et al., 2007). The ECP cannot be measured directly, hence 'phase marker' variables are used to quantify the ECP and measure its response to environmental stimuli. Considering the ECP controls the timing of most physiological processes, circadian rhythms can be measured using any circadian-driven variable (e.g., core body temperature, melatonin, etc.). Typically, circadian phase, period length (*tau*) or amplitude are measures of interest when investigating the ECP. To effectively quantify circadian rhythms, it is essential to 'unmask', or remove, the immediate effects of zeitgebers (Hanneman, 2001; Minors & Waterhouse, 1984; Minors & Waterhouse, 1989; Wever, 1985). Chronobiologists do this in carefully controlled, time-free environments, by simulating sleep/wake cycles that are

beyond the range of ECP entrainment (i.e., either much longer or shorter than 24h; Folkard, 1989; Minors & Waterhouse, 1984). In such extreme sleep/wake environments, circadian systems cannot entrain and instead take on spontaneous, endogenous paces. In this manner, sleep occurs at every circadian phase over the entire protocol (see Figure 1-3).

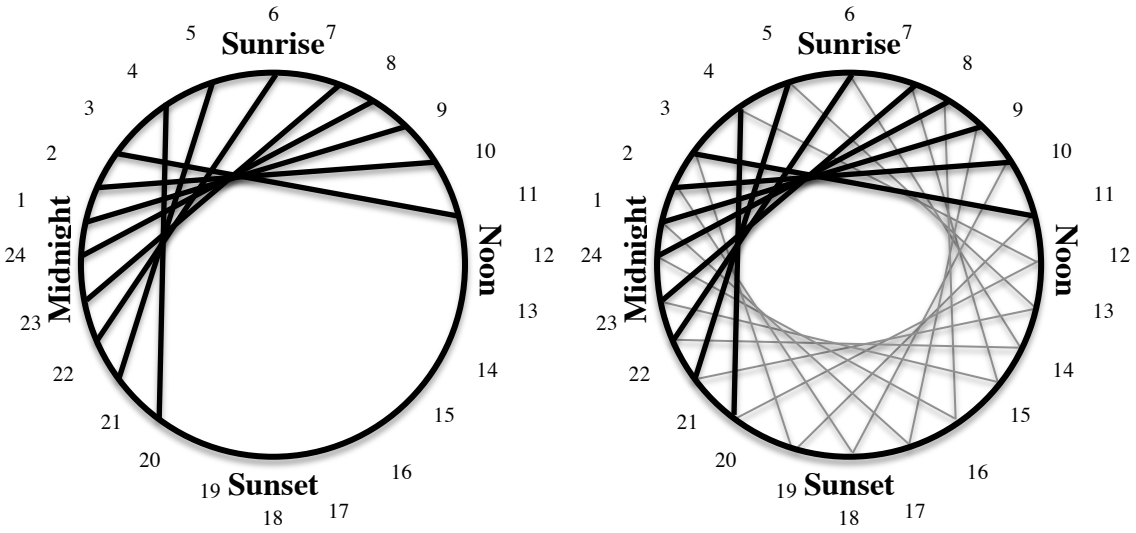

Figure 1-3. A schematic diagram of the forced desynchrony protocol. During natural entrainment (left) sleep/wake timing (bold black lines) is determined by congruence between circadian rhythms and the solar light/dark cycle. During forced desynchrony (right) sleep/wake behaviour is pushed outside the natural boundary such that sleep and wakefulness occur at every circadian phase (black and grey lines), enabling the endogenous period to be measured. *Adapted from Dijk, J.D., (2015, June). Circadian Regulation of the Human Sleep-Wake Cycle Revisited. In Sleep 2015. Symposium conducted at the 29th Annual Meeting of the Associated Professional Sleep Societies, Seattle, WA. Retrieved from http://www.sleepmeeting.org/docs/default-source/attendee-documents/sleep-2015-abstract-supplement.pdf?sfvrsn=2*

A frequently used marker of circadian phase has been the core body temperature rhythm (Tmin; Czeisler et al., 1986; Folkard, 1989; Minors & Waterhouse, 1984). Core body temperature capsules and rectal temperature probes are commonly used to quantify circadian temperature. It is a useful measure, since the Core Body Temperature rhythm (i.e., *tau* or Tτ) follows Process C in the Two-Process Model of Sleep closely (Figure 1-1). During the subjective day, temperature is

elevated and begins to decrease as one becomes physiologically prepared for sleep. Tmin occurs ~2h before spontaneous awakening from nocturnal sleep, equating to around 4-5am in healthy individuals (see Figure 1-4; Duffy et al., 1998). Although Tmin is widely used as the phase marker or timing indicator for the temperature rhythm, it is still 'masked' by a number of factors, including activity levels, eating, secretion, posture and sleep (Minors & Waterhouse, 1989; Wever, 1985).

The circadian rhythm of melatonin production is also used as a marker of circadian phase (Lewy et al., 1999; Rosenthal, 1991). Research suggests melatonin may be the gold-standard measure of circadian rhythms since there is evidence of less masking and greater stability in the measure. This stability may be due to tightly and directly controlled synthesis by the ECP (Benloucif et al., 2005; Klein et al., 1983; Klerman et al., 2002; Minors & Waterhouse, 1989; Moore, 1996; von Treuer et al., 1996).

Melatonin levels are regulated by the SCN and Pineal Gland output. Melatonin is negligible during most of the subjective daytime and begins to rise during the biologically determined night. Given light is the major zeitgeber, the presence of light inhibits melatonin production and in dim light, spontaneous endogenous melatonin production will occur. Reduction in core body temperature is associated with the elevation of melatonin (or what is termed dim light melatonin onset; DLMO). DLMO tends to occur about 8 hours prior to Tmin and ~1-2 hours prior bedtime (Brown, Choe, Shanahan, & Czeisler, 1997a; Duffy et al., 1998). To determine DLMO (and circadian timing), interval samples of salivary or plasma melatonin are taken in the evening and under dimly lit conditions. Partial protocols for salivary DLMO assessment have been validated that typically occur over an ~8h period, during the biological evening (Burgess, Wyatt, Park, & Fogg, 2015; Pandi-Perumal et al., 2007).

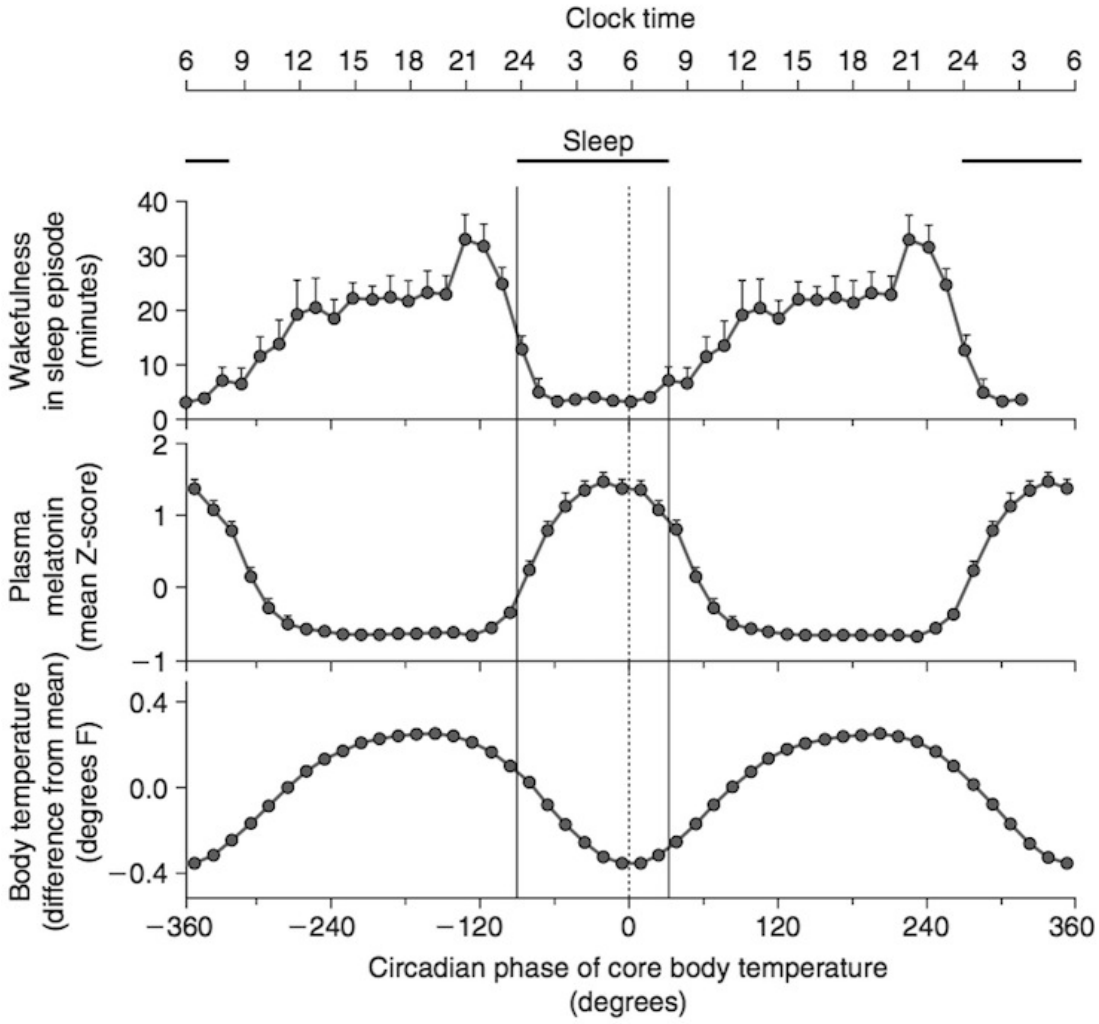

Figure 1-4. A young adult's typical sleep period and relative measures of circadian rhythms. The core body temperature minimum (Tmin - vertical dotted line) occurs about 2 hours before the end of the sleep period and approximately 1-2h after the midpoint of melatonin secretion. The amount of wakefulness (an inverse measure of sleep propensity) increases just before the sleep period, then falls with the onset of melatonin secretion. *Adapted from Duffy, J.F., Dijk, D.J., Klerman, E.B., Czeisler, C.A. (1998). Later endogenous circadian temperature nadir relative to an earlier wake time in older people, American Journal of Physiology, 275, R1478–R1487.*

1.1.5 Effects of Circadian Rhythms on Sleep

Circadian rhythms exert a strong effect on individuals' sleep propensity. Sleep propensity specifically refers to individuals' feelings of sleepiness, the time it takes them to fall asleep, and the likelihood of awakening (Dijk & Lockley, 2002; Lavie, 1985; Lavie, 1997; Lavie, 2001). Allowing

sleep opportunities and measuring how quickly one falls asleep at any given opportunity gives indication of their sleep propensity (Lack & Lushington, 1996; Uchiyama et al., 2000b). The Multiple Sleep Latency Test (MSLT; Carskadon et al., 1986) is based on this notion and the most universally used objective measure of sleep propensity. The underlying concept of its use is that shorter sleep onset latency (SOL) reflects a higher sleep need, while long SOL, indicates lower sleep propensity (Carskadon et al., 1986). Hence, MSLTs use a protocol that imposes a series of timed sleep opportunities to measure SOL objective using polysomnographic (PSG) recordings.

The interaction between circadian rhythms and sleep propensity (using 40min nap MSLTs) is depicted in Figure 1-4 (i.e., the top illustration in which sleep propensity is illustrated inversely as amount of wake time in hourly sleep opportunities). In healthy individuals, an increase in sleep propensity and subjective feelings of sleepiness are associated with DLMO (Duffy et al., 1998; Lavie, 1985; Uchiyama et al., 2000b). Typical nocturnal sleep periods and maximum sleep propensity approximately coincide with Tmin and peak melatonin secretion (Duffy, Rimmer, & Czeisler, 2001; Tozawa et al., 2003). Wake-up time and feelings of alertness are associated with the rise in core body temperature and dissipation of melatonin. Hence, delaying the timing of ECP (Tmin/DLMO) will also move sleepiness and sleep to a later clock time. This represents a '*phase delay*' of the circadian rhythms. Conversely, shifting the ECP to an earlier clock time will result in a '*phase advance*' of the ECP and associated sleep periods (Khalsa et al., 2003; Shirani & St. Louis, 2009).

1.1.6 Entrainment Using Light and Melatonin

As previously mentioned, human circadian timing system is most sensitive to light as a zeitgeber, particularly during times at which people are normally asleep. Light exposure affects the magnitude and the direction of ECP entrainment. The phase-response curve (PRC) illustrates the magnitude and direction of ECP entrainment as a result of light exposure at various times relative to the

existent circadian phase. Figure 1-5 illustrates that exposure to light before Tmin evokes a phase delay (i.e., moves the ECP to a later clock time), and light exposure after Tmin evokes a phase advance (i.e., moves the ECP to an earlier clock time). The magnitude of ECP entrainment depends on the proximity of exposure relative to the timing of Tmin. Exposure to light closer to Tmin will generally produce a greater phase shift compared to light exposed temporally further from Tmin. For example, exposure to light during the subjective midday will have little or no phase-shifting effect on the ECP, while exposure to light half an hour before habitual wake up time will produce a significant phase advance.

Furthermore, recent work suggests that the intensity and wavelength also affect the entrainment of ECP. Higher intensity light correlates positively with greater phase shifting effects. High energy, short-wavelength light (i.e., blue-green spectrum 470-500nm) has greater phase-shifting properties compared to any other colour of the visible spectrum, particularly low-energy, red light (Lockley et al., 2003; Wright & Lack, 2001; Wright, Lack, & Kennaway, 2004). This knowledge of entrainment has been applied to optimise light therapy for the treatment of DSWPD, circadian rhythm disorders and sleep problems (Barion & Zee, 2007; Bartlett, Biggs & Armstrong, 2013; Wirz-Justice, 2003).

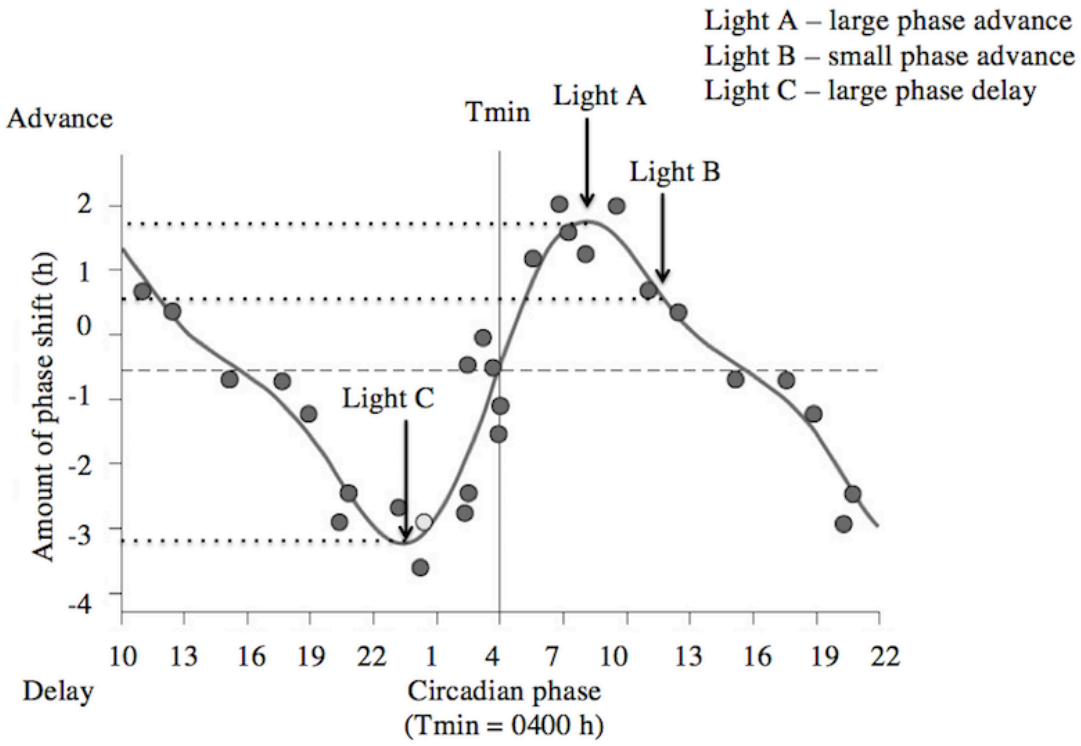

Figure 1-5. Schematic depiction of the effect of light on the ECP represented by the phase-response curve (PRC). Light exposure before Tmin will cause the ECP to move to a later clock time and light exposure after Tmin will cause a phase advancement of the ECP. Maximum phase shifts occur closer to Tmin (Light A and Light C) and light exposure temporally further from Tmin (Light B) have a smaller affect on the ECP. *Adapted from Khalsa, S.B.S., Jewett, M.E., Cajochen, C., Czeisler, C.A. (2003). A phase response curve to single bright light pulses in human subjects. Journal of Physiology, 549, 945–952.*

Melatonin also relays photic information regarding the time-of-day to the body and back to the ECP, and consequently is used as another method of entrainment (Zeitzer et al., 2000). However, melatonin production is inhibited in the presence of light and released during the night in the absence of light, therefore acts in opposition to light. Hence, melatonin signals information regarding darkness and melatonin administration generates a PRC that is inverse and ~12h out of phase to the light PRC (Lewy et al., 1992; Lewy et al., 1998). Exogenous melatonin, just like light administration can be used to change circadian timing. Melatonin can be administered orally in the form of a capsule and in this form is classified as a *chronobiotic* because of its phase-shifting

properties (Sack et al., 2000; Zeitzer et al., 2000). In essence, exposure to light in the evening will cause a phase delay while ingesting melatonin in the early evening will stimulate a phase advance. Inversely, light stimulation in the morning will result in a phase advance but melatonin administration at that time will result in a phase delay. Oral melatonin administration is often used to treat DSWPD and other circadian rhythm disorders (Barion & Zee, 2007; Bartlett, Biggs & Armstrong, 2013; Sack et al., 2000).

1.1.7 Circadian Rhythms in DSWPD

Drawing from empirical evidence surrounding circadian rhythms and their effects on sleep propensity, it is important to highlight that the currently assumed aetiology of DSWPD is a delay in circadian timing (AASM, 2014). In most measures of circadian rhythms, DSWPD patients portray a ~2-6h lag in the timing of circadian rhythms (DLMO/Tmin) compared to healthy controls (Micic et al., 2013; Regestein & Monk, 1995; Sack et al., 2007; Saxvig et al., 2013; Uchiyama et al., 2000a). The timing of sleep is associated with circadian timing and thus DSWPD patients exhibit sleep that is significantly delayed by ~2-6h relative to controls[2]. As such, methods of ECP entrainment, such as selective exposure to morning bright light and orally administered melatonin in the early evening, are readily used to re-time the ECP in DSWPD patients (Barion & Zee, 2007; Bartlett, Biggs & Armstrong, 2013; Bjorvatn & Pallesen, 2009).

1.1.7.1 Non-Physiological Factors of Sleep.

In addition to the biological factors (i.e., homeostatic and circadian processes), individual differences, habits, personality and behaviours also contribute significantly to sleep regulation (Cavallera, Labyak, Acebo, & Seifer, 2011; Wright et al., 2005). According to a large study conducted in Great Britain, 80% of the population (16-95 year olds) had bedtimes between 2200h-

[2] Terms ‘controls’, ‘good’ sleepers and ‘normal’ sleepers are used interchangeably throughout this thesis to signify individuals without difficulty maintaining similar sleep-wake times. A brief description of their characteristics is presented in Chapter 2: “Circadian rhythms”.

0100h (Groeger et al., 2004). This substantial variability within the population suggests that biological mechanisms do not solely determine individuals' sleep times. For example, night-shift workers work across the night in opposition to circadian and homeostatic forces. Hence, sleeping patterns can be overridden by external factors such as societal demands (e.g., nightshifts, social engagements; Wittman et al., 2006), exposure to stimulants and sedatives (e.g. caffeine, ethanol; Peuhkuri, Sohvola & Korpela, 2012; Sherman et al., 2011) and physiologically alerting aspects of lifestyles (e.g., bedroom lights, electronic light-emitting devices; Dijk, 2013; Gooley et al., 2011; Wright et al., 2013). With progressive developments toward 24h societies (see Figure 1-6), particularly in industrial countries, use of light at all times of the natural light/dark cycle can have profound implications for sleep and health. Therefore they should be considered in circadian and sleep investigations (Dijk, 2013; Wright et al., 2013). Research suggests that behaviourally, DSWPD patients tend to avoid light in the morning and gain greater exposure to light in the evening (Auger Burgess, Dierkhising, Sharma, & Slocumb, 2011; Dagan & Eisenstein, 1999). In this case, exogenous factors can perpetuate delays in DSWPD and cause further disruptions to sleep. Due to 'around-the-clock' societal demands, it is little wonder that incidence of DSWPD has increased since earliest estimates (Pelayo, Thorpy & Govinski, 1988).

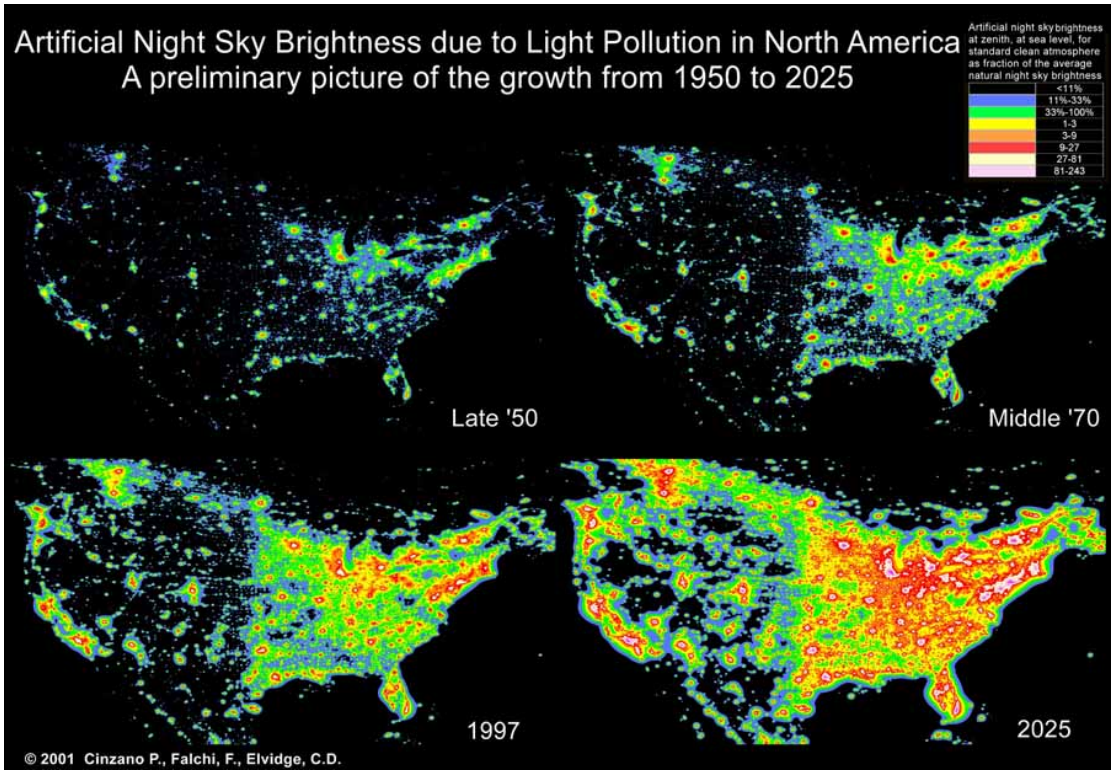

Figure 1-6. Schematic illustration of artificial night sky brightness in North America as a result of non-stop artificial light illumination. The night sky in all areas that are in colours other than black is considered polluted by artificial light at night. Top-left hand-side depicts occurrence of artificial light in the 1950s, top-right hand-side shows increasing amounts in 1970s and even more so in 1997 (bottom-left hand-side). The bottom-right hand-side represents projected light pollution in 2025. *From Cinzano, P. (2002). The growth of the artificial night sky brightness in North America in the period 1947-2000: a preliminary picture. In H. Schwarz ED. Light Pollution: a Global View. (pp. 39-48). Dordrecht: Kluwer.*

1.1.8 Sleep Preference

Additionally, among people with normal circadian rhythms, there are some "morning-type people" who prefer to wake early and go to bed early, and there are “evening-type people” who prefer to wake later and stay up later at night (Horne & Ostberg, 1976). This factor is called one’s *diurnal preference* or *chronotype*. Similar to sleeping patterns, these differences are malleable and tend to be either biologically driven (Hur, 2007) or a behavioural adaptation to lifestyle requirements (Korczak et al., 2008). However, recent evidence suggests that they may also be related to

personality traits (Cavallera et al., 2011). DSWPD patients are thought to be extreme evening-types, however there are some patients who report not being morning- or evening-types. Nevertheless, a significant distinction between evening-types and DSWPD patients is that, when necessary, evening-types can sleep at a different, required time, while DSWPD patients cannot override the circadian delay.

1.1.9 Phase Angle of Entrainment

The phase angle of entrainment is defined as the relationship between a marker of the ECP (e.g., DLMO or Tmin) and the timing of external light exposure (Pittendrign & Daan, 1976). Since the timing of exposure to light is mainly determined by the timing of the sleep period, the phase angle of entrainment is usually measured by the timing of the sleep period relative to a circadian phase marker (Emens et al., 2009). If the currently assumed aetiology of DSWPD is circadian-based and patients report having evening-type sleep preferences, then there would be differences in the phase angle of entrainment to the light-dark cycle between normal sleepers and those with circadian delays (Emens et al., 2009). It may be that circadian rhythms drive delays in sleep timing, thus if patients attempt to sleep earlier relative to their circadian phase, they would have a large phase angle between their sleep onset time and Tmin (or a short phase angle between DLMO and sleep onset) compared to normal sleepers with neither morning- or evening-type sleep preferences. However, the opposite could also be true. Hypothetically, due to a later timed diurnal sleep preference, DSWPD patients could postpone sleep to a later circadian phase. Hence, they could exhibit a shorter interval between their sleep onset and Tmin, or a large phase angle between their DLMO and sleep onset. Research findings in this area are inconsistent and these discrepancies are further discussed in Chapter 2.

Furthermore, Lack and colleagues (2009) indicate that factors such as subjective sleepiness are important in determining the sleep period. They found that individuals with evening-type sleep

preferences have circadian markers (i.e., Tmin and DLMO) that are timed approximately 2-3h later relative to morning-types. However, the subjective sleepiness rhythm markers were timed 9 hours later in evening-type individuals compared to morning-types. They suggest that this significant delay in the timing of minimum feelings of sleepiness may be the result of significantly longer subjective sleepiness period lengths that drive a delay in the timing of the sleep period and thus, with more exposure to evening light and less to morning light, drive the endogenous circadian period later. If DSWPD stems from extreme evening-type preference, these findings could apply to the aetiology of the disorder and the differences in their phase angles of entrainment. It would suggest that DSWPD patients might have quite late sleep periods relative to circadian markers.

1.1.10 DSWPD Treatment Options

Various hypotheses regarding DSWPD aetiology have been proposed and are fully elucidated in Chapter 2. Nevertheless, while many questions about the aetiology of DSWPD remain, the generally assumed, primary cause of DSWPD is a general delay in circadian rhythm phase. As such, three major treatment techniques are presently developed and used to phase-shift circadian rhythms and sleep in DSWPD patients. These include chronotherapy, scheduled bright light therapy (BLT; also known as phototherapy) and exogenous melatonin administration (Lack & Wright, 2007).

1.1.10.1 Morning Bright Light Therapy.

From the available treatment options Bright light therapy (BLT) is most commonly used (Barion & Zee, 2007). Scheduled timing ensures light is received by the SCN during the phase-advance portion of the PRC, thus shifting circadian phase and thus sleep periods to desired, earlier times. Although BLT is deemed a ubiquitous DSWPD treatment option, a standardized management regimen does not exist (Bartlett, Biggs & Armstrong, 2013). This is likely due to extended debate in

the empirical literature regarding efficacy of the dimensions of light intensity and duration, optimal wavelengths and timing of light exposure (Mundey et al., 2005).

In practice, broad-spectrum white light of 2,000-10,000 lux each morning immediately after awakening is generally administered to DSWPD patients for 1-3 h (Chesson et al., 1999), although recent work suggests blue/green light is most effective in phase-advancing melatonin rhythms (Lockley et al., 2003; Wright & Lack, 2001; Wright, Lack, & Kennaway, 2004). It would seem blue/green light also depicts slightly different phase responses with broader advance and delay portions of the curve (Revell et al., 2006). If used in place of white light, blue/green light should be administered somewhat later following Tmin to achieve best outcomes. Where possible, it is useful to determine the timing of DLMO or Tmin to optimize the timing of BLT relative to the patients' circadian phase and ensure the correct phase of response is targeted (Barion & Zee, 2007; Bjorvatn & Pallesen, 2009; Rahman et al., 2009). For example, an ambitious, early light pulse occurring before Tmin, that is not followed by continued adequate light intensity, has the potential to further phase delay and exacerbate the DSWPD condition. Light therapy is typically conducted over several weeks during which patients are asked to gradually advance their wake up time and immediate light administration time until their desired wake up time is achieved.

In clinical settings, patients are typically advised to remain outdoors at specified, adequate times of the day in order to gain exposure to light and acquire phase shifts. Light therapy lamps are also available for purchase, and more recently portable devices with light sources mounted on glasses and visors have been developed. However, regardless of delivery medium, low compliance to BLT tends to truncate treatment outcomes (Barion & Zee, 2007).

1.1.10.2 Exogenous Melatonin Administration.

Like light, the timing of melatonin administration is crucial to its efficacy. Melatonin is administered at the opposite time of day to bright light therapy. Prior work demonstrates 0.3-3 mg of melatonin administered approximately 6-7 h prior to sleep onset produced the greatest phase-

advances (Mundey et al., 2005). However, empirical evidence regarding optimal dose, number of administrations and timing of exogenous melatonin for treatment of DSWPD is scarce. Only recent investigations are elucidating effects of exogenous melatonin administration alone on DSWPD outcomes (Mundey et al., 2005; Rahman et al., 2009; Saxvig et al., 2014; Wilhelmsen-Langeland et al., 2013a). Research suggests that lower doses (0.1-0.5mg) administered closer to sleep onset time (4-5 hours before) can be equally effective to higher doses (1-3mg) at earlier times (6-7 hours before sleep onset; Burgess et al., 2010).

Optimal treatment to advance sleep schedules is deemed a combination of morning bright light therapy and evening low dose melatonin all gradually advancing with the scheduled sleep period at a rate of 15-20 minutes/day until the desired sleep period is attained. It is then wise to continue treatment for at least another two weeks to stabilize the sleep period to the target period. It may be necessary in some cases to present periodic treatment to prevent relapse or treat mild relapse (Burke et al., 2013; Wirz-Justice et al., 2004).

1.1.10.3 Chronotherapy.

Chronotherapy was initially proposed as an effective management for DSWPD (Weitzman et al., 1981), although, it is recommended only for severe cases (e.g. sleep period > 5 hours delayed from desired sleep period). In such circumstances forcing a further daily delay is less distressing than attempting phase advances for patients and ultimately synchronizes sleep rhythms to preferred, earlier times but with movement around the clock in the delay direction. This is achieved by progressively delaying sleep periods, in increments determined by a baseline sleep/wake measure (e.g., sleep diary or an activity monitor; Barion & Zee, 2007; Bartlett, Biggs & Armstrong, 2013; Lack & Wright, 2007). Typically, two-three hour delays in bed and rise times are imposed and treatment takes 1-2 weeks, until the timing of sleep synchronizes with the desired target schedule. Since the entrainment period (i.e., during which sleep is being delayed), leads to extreme disturbances to sleep/wake schedules, Lack and Wright (2007) recommend use of early evening

melatonin administration, morning BLT, and sleep hygiene education, after target sleep is reached. The use of these will help to stabilize and maintain sleep times, and prevent relapse once desired sleep period timing is achieved.

1.1.10.4 Non-Physiological Factors to Treatment.

Additional cognitive behavioural therapy (CBT) is effective for correcting behaviours and cognitions, as well as teaching good sleep hygiene particularly in adolescents with DSWPD (Gradisar et al., 2011). Treatment should also be tailored based on degree of phase delay, severity of associated symptoms, lifestyle circumstance, feasibility of adherence, as well as motivation to comply (Barion & Zee, 2007; Bartlett, Biggs & Armstrong, 2013; Dagan & Eisenstein, 1999; Lack & Wright, 2007; Lack, Wright & Bootzin, 2009).

Although light is ubiquitous in all lifestyles, general knowledge of circadian systems, and the effects of light on the timing of those systems is rare. With constant emergence of technological devices that transmit light and have potentially alerting and phase delaying affects (Chang et al., 2015), it is becoming increasingly more important to educate individuals about circadian systems and give insight about factors that exacerbate delayed sleep. It may prove beneficial to inform patients about circadian timing and teach them that lifestyle factors (e.g., exercising, consuming caffeine/energy dense food and keeping irregular sleep schedules; Bonmati-Carrion et al., 2014; Czeisler et al., 1989; Duffy et al., 1996) may aggravate their DSWPD.

1.1.11 Problems with Current DSWPD Aetiology

Clinical treatment studies have evaluated the efficacy of DSWPD interventions such as morning BLT, melatonin administration, chronotherapy and behavioural strategies for treatment of DSWPD (Lack, Wright, & Bootzin, 2009; Richardson & Malin, 1996; Saxvig et al., 2014). Initially, these interventions usually prove successful. However, patient follow-ups frequently indicate short-term efficacy that abates once treatment ceases (Alvarez et al.,1992; Regestein, & Monk, 1995; Saxvig et

al., 2014). Since DSWPD treatments are rarely permanent, it is important to investigate why patients with DSWPD have such a strong tendency to become phase delayed and relapse even after seemingly efficacious treatment.

1.1.12 Alternative DSWPD Aetiology

Since the identification of DSWPD as a sleep disorder, researchers have suggested that abnormally long *taus*, may explain the strong tendency to delay and relapse after treatment (Campbell & Murphy, 2007; Regestein & Monk, 1995; Weitzman et al., 1981). For healthy individuals the time taken to complete one full circadian cycle (i.e., *tau*) has been estimated to be approximately 24.2 hours (Czeisler et al., 1999). Therefore most humans already have a slight inbuilt tendency to delay 0.2 hours or 12 minutes/day. Due to external entraining stimuli, most individuals are able to resist the delay tendency and adhere to a conventional 24-hour day by advancing their circadian rhythm by ~12 minutes daily. This is generally achieved quite easily and passively, by exposure to light in the morning. However, if DSWPD patients have a longer *tau*, for example 25.2 hours, it would be more difficult to advance rhythms sufficiently to remain normally entrained, in this example by 1 hour, 12 minutes, as opposed to just 12 minutes daily, therefore causing a great tendency to delay in those with DSWPD. Theoretically, these patients must advance their circadian rhythm timing by up to 1.2 hours every day to remain stabilised to the 24-hour world. If such patients allowed their circadian rhythm to free-run in absence of zeitgebers, their rhythm and its associated sleep cycle might delay by 1.2 hours every day (see Figure 1-7). A longer than normal *tau* may therefore explain why the sleep cycle of a DSWPD patient has an extreme and persistent tendency to delay (Alvarez et al., 1992).

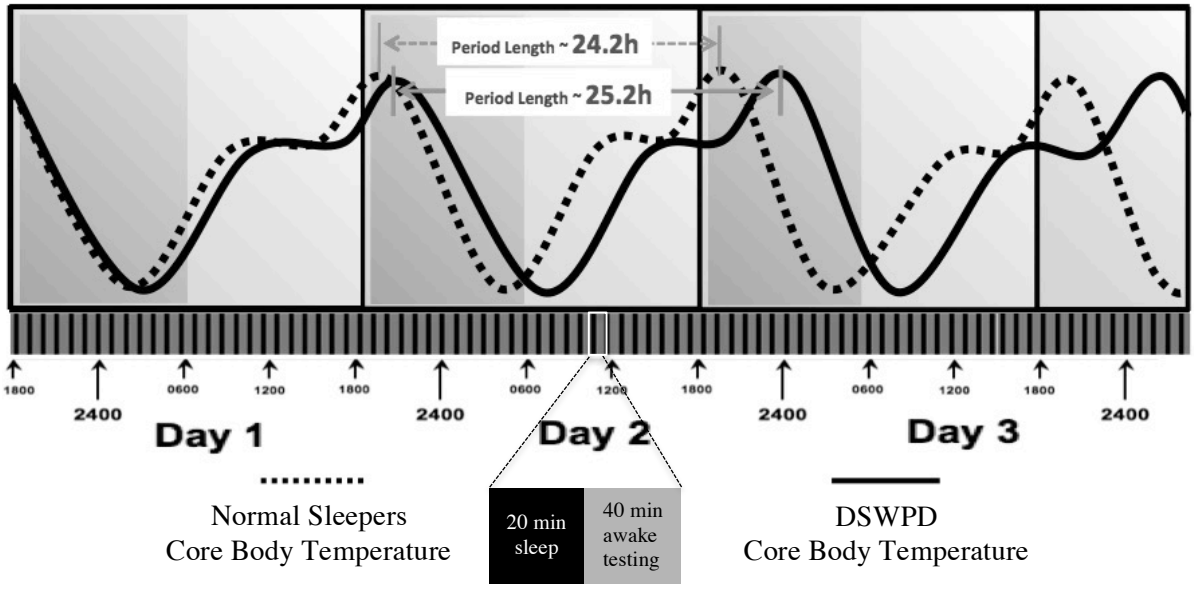

Figure 1-7. Schematic representation of the proposed aetiology in DSWPD. The endogenous variation of core body temperature for an individual with normal circadian rhythm timing. The period length (*tau*) of about 24.2 hours is shown by the perforated curve and a DSWPD patient's circadian core temperature rhythm is depicted by the solid curve with a hypothetically longer *tau* of 25.2 hours. With an endogenous *tau* only slightly longer than 24 hours the normal individual would delay only 0.6 hours over the three-day protocol while the DSWPD would delay about 3 hours. Black vertical bars indicate sleep opportunities of 20 minutes in darkness (<1 lux). Grey vertical bars indicate 40-minute intervals of wakefulness in very dim illumination (10-15 lux). That timing is shown in the enlarged box beneath Day 2.

1.2 Research Aims

This project will investigate how circadian rhythms and sleep/wake patterns in DSWPD become delayed and are resistant to the current treatment options. We aim to examine suggestions that the circadian oscillator is abnormally slow in DSWPD, taking longer to complete a cycle and therefore leading to an unrelenting tendency to delay in the 24-hour world.

By sampling body rhythms (e.g., temperature, hormones, sleepiness, vigilance, etc.) in isolation from environmental time indicators such as light, the intrinsic phase timing and period length of circadian cycles can be determined (Burgess & Eastman, 2008; Czeisler et al., 1999; Clodore et al., 1986; Duffy et al., 2001; Gradisar & Lack, 2004; Lack & Lushington, 1996; Wever,

1975). Measures of core body temperature have been considered reliable phase markers of the circadian system (Aschoff, 1970). Melatonin phase markers are also regarded as reliable and stable indicators of circadian timing. Therefore, they are accepted as reliable and valid practical circadian phase-assessments (Benloucif et al., 2005; Klerman et al., 2002) and measured over a number of consecutive circadian cycles can elucidate the measure of period length or *tau* of the circadian rhythms.

Our secondary objectives are to investigate if behavioural patterns, such as the tendency to choose later bed times, drive the delay of circadian rhythms. We hope to elucidate the potentially important role that biological, behavioural and psychological factors have in this disorder and contribute to building a diagnostic profile. These outcomes can then be applied to devising optimum intervention options for more effective DSWPD therapy.

1.2.1 Justification of Methodology

Although these possible causes of DSWPD, including longer *tau* and behavioural factors have been previously proposed, they have not yet been fully investigated. This is likely due to the time-consuming nature and expenses required to measure endogenous circadian rhythms. Constant, time-free environments and a lot of time have been assumed to be necessary to effectively execute protocols that allow circadian rhythms to 'run free' (20-30 continuous days of laboratory confinement).

The most appropriate experimental approach to accurately measure circadian rhythms has been a topic of deliberation among circadian rhythm researchers (Burgess & Eastman, 2008). Classic investigations of circadian rhythms used month-long isolation protocols to unmask the circadian cycles that help to regulate the sleep-wake patterns. This was achieved by allowing participants to self-select sleep in temporal isolation (i.e., free-running routine; Aschoff, 1970; Wever, 1979) or by imposing extreme sleep/wake cycles to which the circadian pacemaker cannot entrain and thus expose the underlying circadian rhythms (e.g., 28-hour 'day' forced desynchrony;

Czeisler et al., 1999; Hiddinga et al., 1997). Researchers have recently devised more economical and time-efficient protocols that shorten the sleep/wake cycle to ultra-short sleeps and enforced wakefulness states to unmask the circadian rhythm over only a few days (i.e., ultradian routine; Kripke et al., 2005; Lack & Lushington, 1996). It is apparent that different experimental paradigms yield somewhat different measurements of human *tau* and reasons for these differences still stand to be investigated. In free-running experiments, the measured *taus* of normal-sleeping humans tend to be longer, (~24.5-26h; Campbell & Murphy, 2007; Wever, 1979). They are shorter in ultradian routines (at ~24.5h; Kripke et al., 2005; Micic et al., 2013) and even shorter in 28-hour forced-descynchrony studies (at ~24.2h; Czeisler et al., 1999; Hiddinga et al., 1997). Nevertheless, comparisons of circadian rhythms between DSWPD and controls should be valid when using the same methodology.

In the present study the ultradian routine is used to measure biological and behavioural rhythms of DSWPD patients relative to controls. Periods of sleep and wakefulness were enforced to simulate a series of 1-hour long "days" (i.e., 20-minute sleep opportunities alternating with 40-minutes of enforced wakefulness). These simulated "days" are too short for the ECP to entrain. Since the ECP cannot synchronise to the timing of the ultra-short cycles circadian rhythms (i.e., core temperature) become desynchronised from the enforced sleep-wake cycle (Czeisler et al., 1999; Hanneman, 2001). Researchers suggest that this allows the endogenous components of the circadian rhythm alone to drive changes in core temperature (Czeisler et al., 1999). This relatively new methodology has been implemented in previous circadian rhythm investigations, which have supported its validity (Carskadon & Dement, 1975; Czeisler et al., 1999; Duffy & Dijk, 2002; Lack et al., 2009; Wyatt, Cecco, Czeisler, & Dijk, 1999; Weitzman et al., 1974).

Given this methodology is theoretically justified, cost-effective and practical, it was implemented in the present study. This will be the first study to use rigorous methodology and a large sample size to examine the biological and behavioural circadian rhythms of DSWPD

compared to normal sleeping individuals. Subsequent sections give a brief overview of the structure of the thesis and the specific focus of each chapter before conclusions are drawn in the General Discussion, Chapter 7.

1.2.2 Chapter 2

This paper thoroughly examined the available literature pertaining to DSWPD aetiology. We critically reviewed genetic, psychological, behavioural and physiological evidence related to the disorder to outline gaps in empirical evidence and suggest future research. In addition to its scientific significance, the paper has important clinical implications as an effective tool to inform clinicians about other causes of DSWPD and guide them to consider alternative therapeutic strategies for DSWPD treatment.

1.2.3 Chapter 3

This study investigated whether endogenous circadian *taus* (core temperature, melatonin) are significantly longer, as well as delayed, in those with DSWPD compared to healthy individuals.

1.2.4 Chapter 4

The aim of this chapter was to test the hypothesis that circadian *taus* of subjective and objective sleepiness and vigilance are longer than biological circadian *taus* in DSWPD individuals. A secondary aim was to investigate phase angle differences between behavioural rhythm period lengths and biological circadian *taus* in DSWPD patients relative to healthy controls.

1.2.5 Chapter 5

This chapter examined differences in overall melatonin secretion profiles between adults diagnosed with DSWPD and healthy controls. Differences in the nocturnal expression of melatonin, and not

just a general circadian delay in melatonin secretion may make DSWPD patients more susceptible to circadian delays.

1.2.6 Chapter 6

The main aim of chapter 6 was to compare the NEO-PI-R personality profile of patients with DSWPD with healthy controls. The secondary aim was to identify personality factors that were associated with sleep and biological rhythm delays as well as lifestyle impairments.

CHAPTER 2 LITERATURE REVIEW

The etiology of Delayed Sleep Phase Disorder

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Author Contributions

GM proposed, organized and wrote the manuscript. LL, MG and NL contributed to proposal of the paper and helped draft the manuscript. SAF and HJB also contributed to the draft of the final paper and along with LL, developed the primary study design for the overall investigation.

Micic, G., Lovato, N., Gradisar, M., Ferguson, S., Burgess, H.J, Lack, L. (2016). The etiology of Delayed Sleep Phase Disorder. *Sleep Medicine Reviews, 27,* 29-38.

2.1 Summary

According to classification manuals for sleep disorders, nine disorders are directly related to biological clock timing misalignments. Of all, Delayed Sleep-Wake Phase Disorder (DSWPD) is the most commonly diagnosed, predominantly affecting adolescents, young adults, and insomnia patients. It is a persistent inability to fall asleep at earlier, more desirable and socially conventional times, coupled with extreme difficulty awakening in the morning. Considerable evidence shows a delay in the circadian clock to be associated with DSWPD. Therefore, treatments have mainly focused on advancing the biological clock and sleep timing through pharmacotherapy, phototherapy and behavioural therapies. The clinical evidence indicates that these treatments are efficacious, at least in the short term. However, follow up studies show frequent patient relapse, leading researchers to speculate that alternative etiologies may be contributing to sleep and circadian clock delays in DSWPD. The aim of the present paper is to review and collate current literature related to DSWPD aetiology in order to outline gaps in current knowledge and suggest future research.

Keywords: delayed sleep phase; aetiology; treatment; circadian rhythm sleep disorders; phase markers; homeostatic sleep drive; circadian rhythm period; light; diagnosis; adolescence

2.2 Delayed Sleep-Wake Phase Disorder

Delayed Sleep-Wake Phase Disorder (DSWPD) is typically characterized by significantly later sleep onset times compared to social convention and long sleep latencies when attempting sleep at more conventional bedtimes. According to two globally recognized diagnostic manuals for sleep and mental disorders, DSWPD is identified by normal and stable sleep cycle that occurs 2-6 h later relative to patients' desired and socially conventional sleep and rise times (AASM, 2014; American Psychiatric Association; APA, 2013; see Figure 2-1). Consistently late sleep onsets coupled with the need for regular sleep quota, lead to difficulty spontaneously awakening at earlier, more 'conventional' times in the morning to meet commitments. Major complaints emergantoye when necessary early awakenings are forced by alarm clocks and thus shorten sleep duration. Over several nights of insufficient sleep, excessive sleep need accumulates causing impairments to daytime cognitive functioning, irritable mood, and, over time, reducing life prospects (Regestein & Monk, 1995). Although falling asleep at conventional times is problematic, when free of daytime commitments and able to self-select their sleep schedule, patients are said to display stable sleep of normal quality and duration at the delayed time (AASM, 2014; Campbell, Murphy, van den Heuvel, Roberts, & Stauble, 1999; Regestein & Monk, 1995; Saxvig et al., 2014).

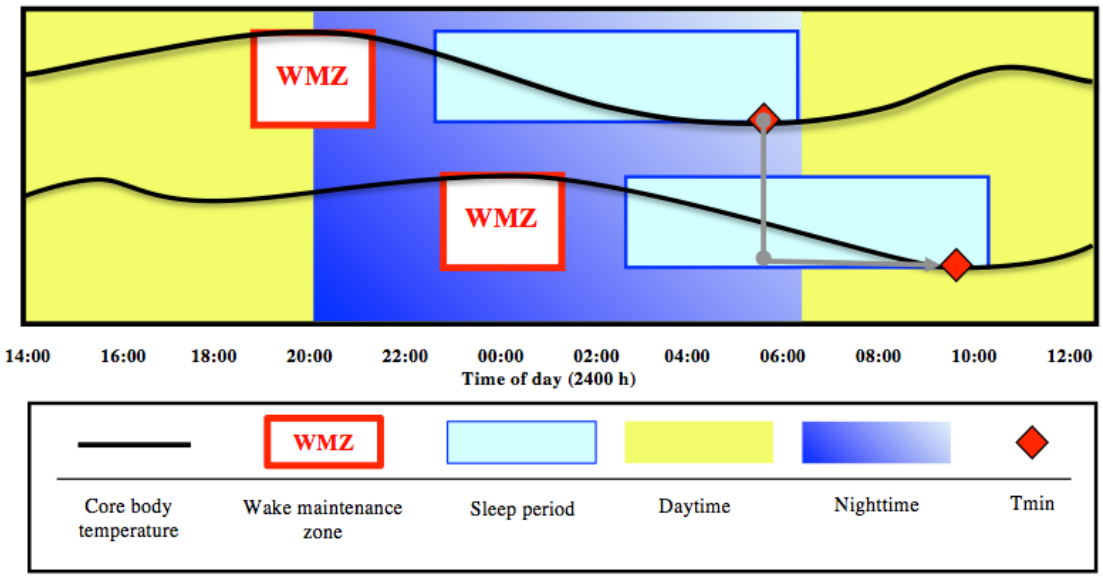

Figure 2-1. An example of the temporal distribution of Delayed Sleep-Wake Phase Disorder (DSWPD) patients' sleep pattern relative to good sleepers. Patients with DSWPD report difficulty initiating sleep, usually before 01:00 h, and have difficulty awakening in the morning, while good sleepers tend to fall asleep between 22:00 h e 23:00 h and have sleep periods in line with social convention and the solar day. Sleep periods are the large blue shaded areas and temperature minimum (Tmin) times are represented by diamonds. Core body temperature minimum in good sleepers and DSWPD track a similar phase-delay in their timing. The wake-maintenance zones (WMZ) are depicted by the small red and white colored sections. Sleep mechanisms of normal sleeping individuals are depicted in the top half of the figure and the sleep mechanisms of DSWPD patients are illustrated in the bottom half.

2.3 Review Aims

Current DSWPD treatments have a strong focus on advancing circadian phase and sleep timing to an earlier time in order to increase sleep duration and ameliorate associated daytime impairments (Alvarez et al., 1992; Campbell et al., 1999; Gradisar et al., 2011; Lack, Wright, & Bootzin, 2009; Wlihelmsen-Langeland et al., 2013). Although empirical evidence regarding DSWPD remission is scant, it appears that relapse following current DSWPD treatments is common and often quick (Dagan & Eisenstein, 1999; Sack et al., 2007). A simple correction of the circadian delay in

DSWPD rarely provides a permanent cure. Therefore, the causes of the strong tendency to delay should be thoroughly investigated with implications for clinical treatment. Etiologically tailored treatments are more likely to produce long-term improvements in patients' daily functioning and overall wellbeing. With the emergence of several recent studies the aim of this review is to summarize the current empirical literature pertaining to the aetiology of DSWPD in order to propose efficacious and durable treatments. Circadian rhythms and their association with DSWPD will be discussed, as well as DSWPD prevalence and current diagnostic criteria. Furthermore, we will consider gaps in current knowledge of DSWPD aetiology that contribute to difficulties in precision of diagnosis and uncertainties about treatment.

2.4 Correlates of the Disorder

The onset of puberty and adolescence is typically characterized by a biological delay in the habitual sleep pattern (Carskadon, Vieira, & Acebo; 1993). In a cross-sectional study of 25,000 individuals, Roenneberg et al. (2004) identified a significant delay in sleep timing during adolescence, followed by a reversal towards earlier sleep times at the start of adulthood. The point of inflection is apparent at ~20 years of age, where delayed sleep tends to advance. However, delayed sleep can be maintained in some individuals well into adulthood. DSWPD diagnosis has previously been documented to last an average of 19.2 years following onset in adolescence, with chronic DSWPD patients experiencing symptoms beyond 60 years of age (AASM, 2014; Alvarez et al., 1992; Schrader, Bovim, & Sand, 1993).

Although a tendency for eveningness and delayed sleep timing increases across adolescent development (e.g., 11-18 years; Gradisar et al., 2011), it is important to note that a DSWPD diagnosis can be provided during adolescence irrespective of the severity of eveningness, just so as long as their sleep timing results in some form of significant impairment (e.g., school performance, attendance, etc.; Gradisar et al., 2011). Thus, it is feasible that two different adolescents of the same age could have the same evening preference and delayed sleep timing, but one attributes their poor

school performance to their sleep pattern (i.e., DSWPD) and the other does not (evening preference and delayed sleep timing).

Nevertheless, long lasting DSWPD can cause permanent health and social disruptions, thus significantly impact the quality of life (Alvarez et al., 1992; Crowley, Acebo, & Carskadon, 2007). Though only a few studies have investigated the impact of DSWPD on health (Baron & Reid, 2014), results suggest that depression, as well as the use of medication (esp. antacids and hypnotics), tobacco, alcohol and caffeine is greater in patients than controls (Kripke et al., 2008, Lovato et al., 2013). DSWPD patients report less sport participation in school (Lovato et al., 2013), as well as social difficulties and daytime sleepiness. Job loss, truancy and school failure are quite often reported (Czeisler et al., 1981; Thorpy, Korman, Spielman, & Glovinsky, 1988), and could likely be related to health and lifestyle issues.

2.5 Circadian Rhythms

Due to the Earth's solar rotation, organisms have adapted to living in a cycling 24h environment and humans are no exception (Lowrey & Takahashi, 2004). Internal mechanisms generate rhythms of physiological human processes such as temperature, hormonal, behavioural, biochemical fluctuations and other biotic factors (Leproult, van Reeth, Byrne, Sturis, & Van Cauter, 1997; Moore, 1999). Rhythms that oscillate with a period (completed cycle) of approximately 24h are called circadian rhythms; the origin of the word stemming from the Latin words "circa" meaning *about*, and "dies" meaning *day*. The circadian system helps to ensure that our sleep/wake cycle remains congruent with the environment's 24 h light/dark cycle. In most organisms, this self-sustained rhythm regulation is synchronized at the molecular level by complex systems that operate via positive and negative transcription-translation feedback loops (TTFL; Lowrey & Takahashi, 2004; Moore, 1999). In these loops, circadian clock genes are transcribed into product protein structures that translate genetic material into neural and hormonal signals and set circadian rhythms to ~24h. However, the outcome period length of the rhythms (i.e., the time taken to complete one

full circadian cycle) varies slightly between individuals. The population average appears to be slightly longer than 24h, and thus, for most individuals, persistent adjustment from environmental time cues, or *zeitgebers* is necessary in order to avoid becoming delayed relative to the 24h world. Photic information is a major zeitgeber or circadian entraining stimulus and plays an important role in synchronizing the endogenous central pacemaker (ECP) to the 24h light/dark cycle. Compensatory signals such as light from the environment help to correct and synchronize the circadian cycle to 24h through photic input, predominantly processed in the Suprachiasmatic Nuclei (SCN) of the brain. The cells of the SCN also have self-sustained rhythms involving a feedback loop of mRNA transcription.

The presence or absence of external light in the environment is relayed to the SCN from the retina of the eye and through the optic chiasm located in close proximity to the SCN (Freedman et al., 1999; Hattar, Liao, Takao, Berson, & Yau, 2002; Meijer & Rietveld, 1989; Morin, 1994. In response to photic transmission the SCN triggers hormonal and protein changes that affect and adjust circadian processes controlled by the SCN. Although absence of zeitgebers can lead to desynchrony of circadian rhythms from the 24h environment, circadian rhythms continue to persist via the internal feedback loop. As such, in constant confinement free from external factors, human circadian rhythms continue to cycle at a period length that is, on average, 24.2h long (Burgess & Eastman, 2008; Czeisler et al., 1999). 'Normally-timed circadian rhythms' are those that can adequately phase-delay or -advance daily, such that individuals' ECPs remain synchronized or 'entrained' to conventional 24h solar days (Baron & Reid, 2014; Czeisler & Gooley, 2007; Gronfier, Wright, Kronauer, & Czeisler, 2007; Wright, Hughes, Kronauer, Dijk, Czeisler, 2001). Although there is large variability between societies regarding 'conventional' sleep times (Soldatos, Allaert, Ohta, & Dikeos, 2005), sleep is considered 'normal' when typical sleep architecture is exhibited and sleep occurs at societal conventional times. Sleep disorders that arise from a misalignment due to changes in the timing of the ECP, its synchronizing mechanisms, or alteration

in the timing of circadian rhythms and the external 24h environment are classified as Circadian Rhythm Sleep-Wake Disorders (CRSWD; AASM, 2014). It has been reported that 83% of CRSWD are diagnosed as DSWPD and it is the most commonly reported CRSWD in clinics (Dagan & Eisenstein, 1999).

2.6 Prevalence

While prevalence of DSWPD is poorly documented in empirical literature, depending on the diagnostic sample, it is estimated to range from 0.2% to 10% (Lack, Wright, & Bootzin, 2007) or 0.5-16%(Gradisar et al., 2011). Two recent DSWPD prevalence studies reveal 3.3% out of a sample of 10,220 Norwegian high school students presented with DSWPD diagnosis (Silvertsen et al., 2013) while 8.4% out of a sample of 1285 had delayed sleep timing (Saxvig et al., 2012). In an effort to further refine prevalence estimates, Lovato and colleagues (2013) specifically assessed the frequency of a school-based adolescent population of 374 students who met one, two, or all criteria for DSWPD from the International Classification of Sleep Disorders-2 (ICSD-2). Results suggest 1.1% of the Australian adolescents attained a full DSWPD diagnosis, while a further 14% met two out of three criteria and 51.9% of adolescents met one criterion.

Recognizably, there is significant variability in prevalence estimates of DSWPD. Aforementioned studies may have a sampling bias in surveying high school students who, across the lifespan, are developmentally and biologically most susceptible to sleep delays (Carskadon, Vieira, & Acebo, 1993). Additionally, higher prevalence tends to be calculated when criteria are less stringent (e.g., Saxvig et al., 2012; Sivertsen et al., 2013), while considerably fewer individuals meet full diagnostic criteria (Lovato et al., 2013). These discrepancies are elucidated in most recent estimates from the New Zealand population where, based on subjective responses, 0.34 and 1.53% of a sample of 4386 respondents were deemed as having DSWPD but rates were higher when participants did not meet full criteria (Paine, Fink, Gander, & Warman, 2014). The prevalence in this most recent study (Paine et al., 2014) was even lower compared to Lovato et al.'s strict criteria,

perhaps due to a more representative sample of the overall population and a larger sample size. Lower prevalence estimates are also more in line with a 1993 paper reporting prevalence of DSWPD to be 0.17% among 10,000 individuals, aged 16-67 y, surveyed in the general Norwegian population (Schrader, Bovim, & Sand, 1993).

Given discussed findings, figures estimated by sampling large numbers of individuals, from the general population seem most representative of the overall prevalence of DSWPD, thus likely range between 0.17 and 1.53% (Paine, Fink, Gander, & Warman, 2014; Schrader, Bovim, & Sand, 1993). Prevalence tends to be higher in adolescent population with estimates of approximately 3.3-7.3% in this sample (Lovato et al., 2013; Pelayo, Thorpy, & Glovinsky, 1988; Saxvig et al., 2012; Sivertsen et al., 2013) and lower later in life, such that 0.7% of middle-aged adults meet criteria for DSWPD (Ando, Kripke, & Ancoli-Israel, 2002). Highest prevalence rates are reported in clinical samples where 6.7% (Van Maanen et al., 2013) to 16% (AASM, 2014) of patients who present with insomnia symptoms are diagnosed with DSWPD.

2.7 Aetiology of DSWPD

Several publications have identified possible mechanisms driving delayed sleeping patterns observed in DSWPD (e.g. Carskadon, Vieira, & Acebo, 1993; Crowley, Acebo, & Carskadon, 2007), some of which include a delay in the timing of circadian rhythms, a slower circadian oscillator (longer period, *tau*), abnormal relationships between the circadian phase and sleep timing (phase angle of entrainment), slower build up of homeostatic sleep drive, and abnormal phase response curves to light. These proposed mechanisms are illustrated in Figure 2-2 and will subsequently be discussed. Empirical work suggests that genetic, behavioural, cognitive, personality and emotional (Bartlett, Biggs, & Armstrong, 2013; Gradisar et al., 2011; Hiller, Lovato, Gradisar, Oliver, & Slater, 2014; Jones, Huang, Ptacek, & Fu, 2013; Regestein and Monk, 1995; Wilhelmsen-Langeland et al., 2013b) differences also potentially contribute to, or cause DSWPD.

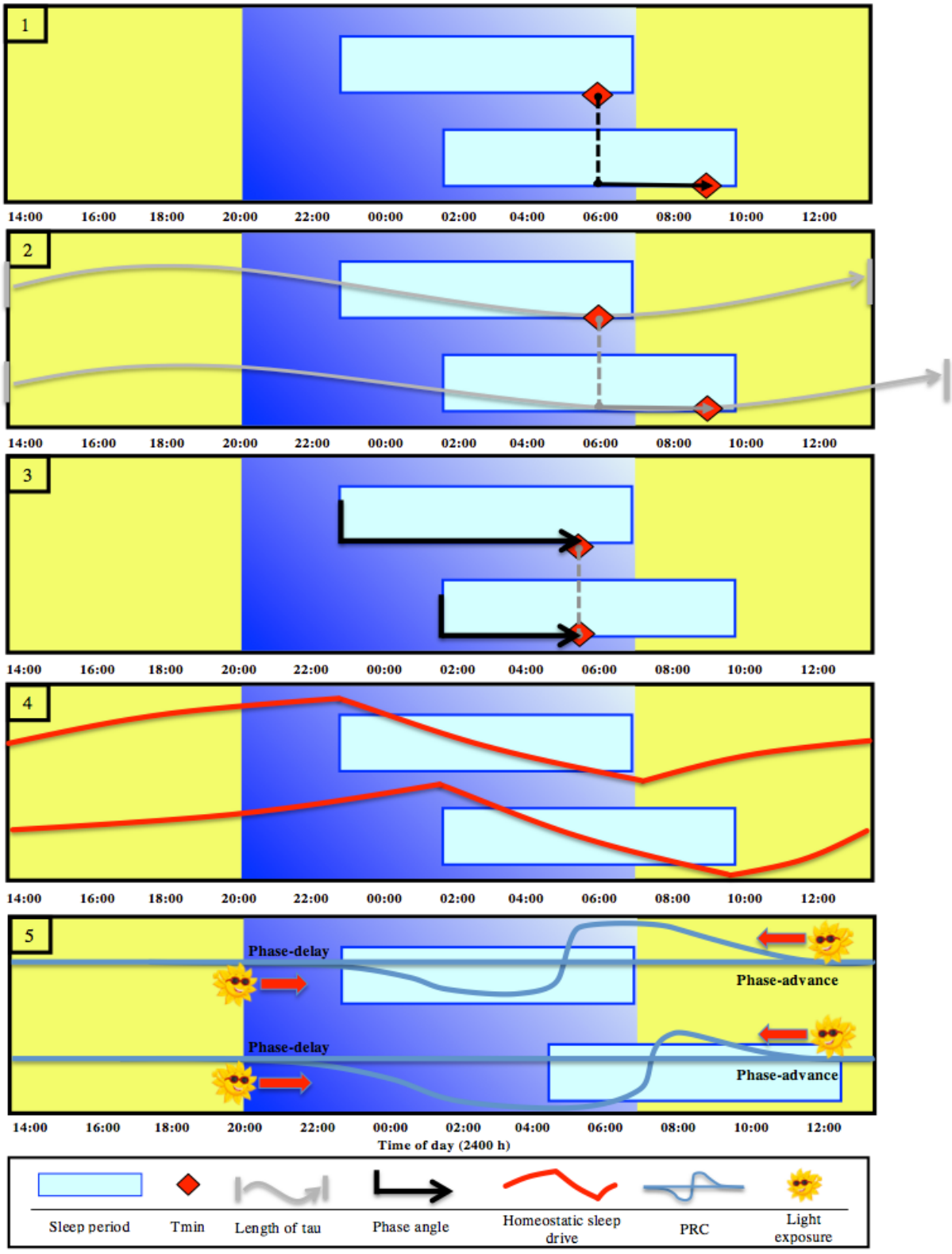

Figure 2-2. Physiological factors that are hypothesized to contribute to delayed sleep in DSWPD. (1) Phase delay of circadian markers such as temperature minimum (Tmin); (2) slower circadian oscillator (longer *tau*); (3) phase angle of entrainment; (4) homeostatic sleep drive dysfunction; (5) responsivity to zeitgebers at each end of the phase response curve (PRC). In each example the proposed sleep mechanisms of normal sleeping individuals are depicted in the top halves of the figure and the sleep mechanisms of DSWPD patients are illustrated in the bottom halves.

2.7.1 Current Aetiology: A Delay in Circadian Phase

The current aetiology of DSWPD is believed to be a significant delay in the timing of circadian rhythms (AASM, 2014; APA, 2013; Oren, Turner, & Wehr, 1995; Ozaki, Uchiyama, Shirakawa, & Okawa, 1996; Shibui, Uchiyama, & Okawa, 1999; Uchiyama et al., 2000a; Watanabe et al., 2003; Weitzman et al., 1981; Wyatt, Stepanski, & Kirkby, 2006). The circadian timing system exerts a strong effect on individuals' feelings of sleepiness, the time it takes them to fall asleep and the likely time of awakening. Figure 2-2 indicates the typical sleep timing of a normal sleeper. In individuals with normally timed circadian rhythms, sleep may occur, for example, from approximately 23:00 h to 7:00 h and maximum circadian drive for sleep is associated with minimum core body temperature (Tmin; Duffy, Rimmer, & Czeisler). Wake-up time usually occurs soon after core body temperature begins to rise. Melatonin is a hormone secreted by the pineal gland and is also under the control of the SCN. In healthy individuals, melatonin levels are negligible during the day and begin to rise in the early evening. Melatonin secretion onset time (or Dim Light Melatonin Onset; DLMO) usually occurs 1-2 hours before typical bedtime. Core body temperature minimum and melatonin onsets, are thus gold standard measures for identifying phase timing of circadian rhythms and are known as *phase markers* (Benloucif et al., 2005).

Of particular relevance to DSWPD is the circadian phase of heightened alertness that precedes the 'sleep-conducive phase' (Lavie, & Zomer, 1984; Strogatz, Kronauer, & Czeisler, 1987). This is indicated in Figure 2-1 as the wake-maintenance zone (WMZ), which lasts 3-4 h and occurs between 18:00 h and 21:00 h in normal sleepers. This particular interval coincides with the circadian maximum of core body temperature (Tmax). Hence, in direct contrast to Tmin, the Tmax that characterizes and occurs during WMZ is associated with the minimum circadian drive for sleep and maximum physiological alertness. Previous empirical research has found 2-6h delays in DSWPD patients' circadian phase timings compared to normal sleepers' (Oren, Turner, & Wehr, 1995; Ozaki et al., Shibui, Uchiyama, & Okawa, 1999; Uchiyama et al., 2000a; Watanabe et al.,

2003). For example, Ozaki and colleagues (1996) found DSWPD patients had core body temperature minimums at 07:17 h ± 00:47 h while controls exhibited core temperature minimums at 04:56 h ± 00:19 h. Similarly, assessments of melatonin phase markers show melatonin increases approximately 4 h later in DSWPD patients (*M* = 04:18 h ± 00:42 h) compared to controls (*M* = 12:36am ± 00:24 h; Strogatz, Kronauer, & Czeisler, 1987). It is important to note, controls in this study had DLMOs almost 3 h later than most studies of normal sleepers (Burgess, & Eastman, 2005). Nevertheless, since phase markers are highly and positively correlated with sleep onsets and offsets, as well as sleep timing preferences DSWPD is hypothesized to arise from a circadian rhythm delay (Benloucif et al., 2005; Duffy, Rimmer, & Czeisler, 2001; Lack & Lushington, 1996; Lavie & Zomer, 1984; Mongrain, Lavoie, Selmaoui, Paquet, & Dumont, 2004; Strogatz, Kronauer, & Czeisler, 1987; Van Someren EJW, Riemersma-Van Der Lek, 2007). It is thought that the circadian delay in DSWPD also delays Tmax and the WMZ so that it coincides with the desired earlier conventional bedtime making it extremely difficult for patients to initiate sleep at that earlier time (Lavie & Zomer, 1984; Regestein & Monk, 1995). Accordingly, both cited studies observed differences in typical sleep timing such that DSWPD patients initiated sleep between 02:43 h – 04:30 h and woke up between 11:20 h – 13:18 h, while controls slept from 12:28 h to about 07:56 h (Ozaki et al., 1996; Shibui, Uchiyama, & Okawa, 1999).

2.7.2 Longer Circadian Period (*tau*)

A longer than normal *tau* indicates a slower circadian oscillator of the ECP and associated rhythms, thus increasing the time taken to complete a full circadian cycle. This mechanism has been hypothesized to contribute to DSWPD (Duffy, Rimmer, & Czeisler, 2001; Van Maanen et al., 2013; Weitzman et al., 1981). The effects of a longer *tau* include an increased tendency for circadian rhythms to delay, as well as concomitant delays of WMZs and sleep timing. These therefore result in later evening light exposure and delayed morning light exposure thus perpetuating the delay. Patients with a longer *tau* (e.g. 25 h) must phase advance a greater amount each day (e.g. 60 min for

individuals with a 25 h *tau* compared to 12min for individuals with an average 24.2 h *tau*), in order to remain synchronized with the 24 h world.

Recent empirical studies suggest patients with DSWPD exhibit significantly longer *taus* compared to normal sleepers (Campbell & Murphy, 2007; Micic et al., 2013). In two experiments, participants were isolated from zeitgebers to assess intrinsic circadian *taus*. In 2007 (Campbell & Murphy), a single DSWPD patient displayed a core body temperature *tau* that was significantly longer (*M* = 25:23 h) than three controls (*M* = 24:26 h). Similarly, six DSWPD patients in a 2013 study had significantly longer *taus* (*M* = 24:54 h ± 00:23 h) compared to seven controls (*M* = 24:29h ± 00:16 h; Micic et al., 2013). Longer *taus* have been associated with delayed circadian phase and later sleep timing (Hida et al., 2013; Lazar et al., 2013), as well as eveningness preference (Duffy, Rimmer, & Czeisler, 2001; Kitamura et al., 2013; Lewy et al., 1983). A longer circadian period has also been observed during adolescence (Carskadon, Labyak, Acebo, & Seifer, 1999) and in people with Non-24-hour Sleep-Wake Rhythm Disorder (N24SWD; Kitamura et al., 2013). Although N24SWD is diagnosed as a distinct CRSWD from DSWPD, they share two predominantly common features in that 1) patients exhibit delayed sleep timing and 2) are unable to initiate sleep at their desired time. Considering only these commonalities, N24SWD can be likened to a severe extension of DSWPD where an extremely long *tau* may result in sleep timing losing synchrony with the 24 h world. This resultant 'drifting around the clock' would therefore pose extreme difficulty advancing sleep and falling asleep at conventional times.

2.7.3 Phase Angle of Entrainment

The relationship between the markers of ECP and the timing of individuals' sleep timing (i.e., sleep onset/offset) is called the phase angle of entrainment and is also hypothesized to be associated with the aetiology of DSWPD. A few studies have investigated the phase angle of entrainment in patients with DSWPD (Ozaki et al., 1996; Uchiyama et al., 2000a; Watanabe et al., 2003). Ozaki et al. (1996) were the first to discover significant differences between sleep onsets/offsets relative to

Tmin in DSWPD patients compared to healthy controls. Participants' circadian systems were measured for 6-10 days in their home environments, using ambulatory core temperature monitoring devices. Results show that DSWPD patients have a significantly extended sleep interval between Tmin and sleep offset compared to controls. These results were replicated in a later study by the same group (Uchiyama et al., 2000a). This tendency may lead to individuals sleeping through the 'advance' portion of the circadian rhythm. Exposure to light during this portion effectively shifts sleep timing to an earlier time, as determined by the phase response curve (PRC; Lewy et al., 1983). Lack of exposure to light during this pivotal phase for advancing sleep releases the "brakes" on the tendency to phase delay for individuals with *taus* longer than 24h (Carskadon et al., 1999; Emens et al., 2009; Okawa & Uchiyama, 2007).

Although this provides a logical explanation of how a long interval between Tmin and wake-up time can contribute to DSWPD, more recent ambulatory studies have not confirmed the earlier findings (Chang, Reid, Gourineni, & Zee, 2009). When patients are not allowed to self-select sleep and wake times in the home environment, but instructed to maintain their 'conventional' sleep/wake routines, the phase angle of entrainment for DSWPD did not differ from controls, questioning this etiological factor in DSWPD. One drawback of most phase-angle difference investigations is the use of ambulatory data, not carefully monitored laboratory studies. Occasional early mandatory awakenings to meet daytime commitments of DSWPD patients may confound phase-angle estimations in the original findings (Ozaki et al., 1996; Uchiyama et al., 2000a), since they expose the circadian pacemaker to light at a relatively early circadian time causing a partial phase advance. This could also explain the observed phase angle differences for the patient sample.

Experimental studies are necessary to resolve this uncertainty. More standardized, laboratory-based experiments that monitor circadian phase and control wake-up times during assessment are warranted to unmask the role of the ECP in timing the sleep episodes of DSWPD patients. In particular, investigations that are more specific about phase angle of entrainment measurements,

and what is meant by a typical sleep episode may more accurately deduce contributions of this factor to DSWPD. That is, should only "free days" be classed as typical sleep or a compromise environment when some early commitments across the week are included?

2.7.4 Homeostatic Sleep Drive Differences

The circadian system assists to appropriately time sleep, however this process is part of a larger model of sleep called the 'two-process model' (Borboley, 1982). The second process in this model is termed the 'homeostatic sleep-wake mechanism' or 'Process S'. Although these two processes work independently, when harmonized, they regulate and generate optimal sleep. An alternative theory is that DSWPD may not originate as a dysfunction of the circadian system, but rather the homeostatic sleep-wake mechanisms. Much like other biological homeostatic regulation (e.g., hunger drive), the sleep mechanism is a function of time spent awake or asleep. The drive for sleep increases with time spent awake and decreases during sleep.

It may be that DSWPD patients accumulate homeostatic drive more slowly during wakefulness, compared to controls (Duffy, Rimmer, & Czeisler, 2001; Uchiyama et al., 2000b; Uchiyama et al., 1999). A group of sleep deprived DSWPD patients and healthy controls were exposed to a constant routine of ultra short 'days' consisting of 10-min napping opportunities and 20-min of enforced wakefulness (Uchiyama et al., 2000b; Uchiyama et al., 1999). DSWPD patients used the 10-min nap opportunities less efficiently compared to sleep deprived controls. During the 26-30 h modified constant routine, controls obtained 5.2 h ± 0.2 h of sleep while DSWPD patients averaged only 3.2 h ± 0.2 h. When nap trials were aligned to individuals' DLMO, compared to controls DSWPD patients had significantly longer sleep latencies from 12 h prior to DLMO to 3 h following DLMO. This would suggest a slower accumulation of homeostatic sleep drive. If one assumes that choice of bedtime occurs after reaching comparable levels of sleep drive, DSWPD would not reach the same level until later, thus delaying bedtime.

If the only difficulty were a slower accumulation of sleep drive, DSWPD patients would initiate sleep later but have the same total sleep time as normal sleepers. However, Uchiyama and colleagues reported DSWPD patients to have longer intervals between habitual sleep onset and offset times (Uchiyama et al., 2000b; Uchiyama et al., 1999). Therefore, it seems possible that DSWPD may have both slower accumulation of homeostatic sleep drive and slower dissipation during sleep. These dual tendencies would contribute even more strongly to the tendency to phase delay. Extension of wake and sleep intervals effectively creates a longer sleep/wake rhythm period, as well as delaying evening- and morning-light exposure, allowing a >24 h *tau* to phase delay. Since light can even induce phase shifts in *taus* that are not significantly longer than 24h (Wright, Gronfier, Duffy, & Czeisler, 2005), delays in those with *taus* considerably >24 h would be greater and more chronic since light would persistently hit the phase-delaying portion of the light-PRC. The findings of Watanabe and colleagues (2003) also support the notion of a difference in the homeostatic sleep drive of DSWPD patients and healthy sleepers. Polysomnography (PSG) recordings showed significant differences in sleep parameters such as reduced sleep efficiency, more total sleep time and less slow wave sleep (SWS) as well as more stage 1 sleep in DSWPD patients relative to controls. The study controlled for sleep history and prior length of wakefulness and circadian phase of the sleep interval. Since SWS is associated with dissipation of the homeostatic sleep drive, researchers hypothesize that slower accumulation of homeostatic sleep drive contribute to delays in the typical sleep timing in DSWPD Watanabe et al., 2003).

Work conducted in morning- and evening-types also suggests that not only circadian phase, but also the chronobiological aspect of homeostatic sleep/wake mechanism can contribute to diurnal preference (Mongrain et al., 2004; Mongrain, Carrier, & Dumont, 2006). While half of the sample in these investigations showed circadian phase shifts relative to diurnal preference, the other half of each subgroup were neither morning- nor evening-types according to DLMO/Tmin measures. However, assessment of overnight PSG while sleeping at self-selected times indicated that both the

initial level and rate of SWS decay was higher in morning- compared to evening-types of this 'intermediate' subgroup. If evening-types have some characteristics of DSWPD patients these results may suggest that DSWPD patients who do not exhibit phase-delays may have reduced homeostatic sleep accumulation.

Moreover, developmental studies assessing longitudinal sleep differences show similar reduced homeostatic drive for sleep and SWS during adolescence. DSWPD may commonly emerge in adolescence because of a reduced rate of accumulating homeostatic sleep drive evident around puberty (Campbell et al., 2011; Gradisar & Crowley, 2013; Jenni, Achermann, & Carskadon, 2005; Tarokh, Van Reen, LeBourgeois, Seifer, & Carskadon, 2013). If so, this mechanism may be contributing to sleep delays typically observed in both adolescents and DSWPD patients. Empirical research with DSWPD patients is necessary to explore rates of accumulation and dissipation of homeostatic sleep drive and provide support for this hypothesis (Gradisar & Crowley, 2013). This is of particular importance since DSWPD investigations and diagnostic criteria describe PSG studies in these patients as presenting 'normal' sleep architecture when they are free to choose their sleep timing (Alvarez et al., 1992; Regestein & Monk, 1995; Sack et al., 2007; Saxvig et al., 2013; Thorpy et al., 1988). Extensive work in this area will be pivotal to clarify this incongruence and answer the question - do anomalies in homeostatic sleep regulation contribute to DSWPD?

2.7.5 Responsiveness to Light

It is well established that the ECP can be reset or retimed using appropriately timed external light stimulation (Czeisler et al., 1989). Greatest evidence of this is that most people remain synchronized to the precise 24h world despite having a longer than 24h *tau* (24.2h on average). While the conventional sleeping time is relatively late in the nighttime, (perhaps a result of the longer than 24h *tau*) normal sleepers awaken to morning light that stops any further delay (Moore & Meltzer, 2008). DSWPD may arise from abnormalities in the phase response curve (PRC) to light in which DSWPD patients may be hypersensitive to light in the phase delay portion of the PRC (in

the late evening) and/or hyposensitive to light in the phase advance portion of the PRC (post awakening in the morning; see Figure 2-2, #5). During clinical interviews with 322 CRSWD patients (83.5% had DSWPD), Dagan and Eisenstein (1999) found that 47% of patients reported subjective sensitivity to light compared to 19.6% of control, healthy sleepers. Although this was posed as a general question, it would be worthwhile to investigate if sensitivity to light was abnormal at particular times of day.

Aoki, Ozeki and Yamada (2001) compared DSWPD and control subjects' sensitivity of melatonin suppression in response to light exposure. The timing of individuals' peak melatonin secretion was determined on night one, then on night two 1000-lux light was administered for 2h prior to, and until estimated peak secretion time was reached. Melatonin suppression was significantly greater in DSWPD patients compared to controls. As such, hypersensitivity to light in the evening is inferred to increase individuals' susceptibility for developing or sustaining delayed sleep phase. Aoki and colleagues's study is the sole published evidence linking this process to DSWPD. Further clinical investigations on light stimulation and phase responses in addition to melatonin suppression are required to support this notion. Furthermore, it is necessary to establish DSWPD phase advancing response to light exposure in the morning compared with normal sleepers.

Similar work has been conducted assessing the response to light of adolescents. If it can be assumed that delayed sleep usually develops in adolescence, conceivably, findings in adolescent sleep could be applied to DSWPD aetiology. Crowley and Carskadon (2010) showed exposure to 1h of early morning bright light immediately following wake-up was ineffective to phase advance 15-17 year old adolescents' sleep schedules on weekends. It appears that the phase-delaying portion of the curve may be more responsive to light compared to the phase-advancing part of the PRC in adolescents and perhaps DSWPD (Hagenauer, Perryman, Lee, & Carskadon, 2009). Still, Crowley and Carskadon's (2010) post hoc tests reveal light may not have been administered close enough to

the phase-advancing portion of the PRC. This limits our understanding of the extent to which lowered responsiveness to morning light is related to DSWPD.

Most recently, Cain, Stanghi, McConchie, & Anderson (2014) supported Aoki and colleagues' findings and showed greater melatonin suppression in four DSWPD patients compared to five controls. In this unpublished, pilot study participants were exposed to regular room light (200-lux), for 3h from habitual bedtime. DSWPD patients also showed heightened alertness responses on both subjective and objective measures compared to healthy controls (i.e., Karolinska drowsiness test, Psychomotor vigilance test, Karolinska sleepiness scale, objective EEG/EOG alertness). Future findings by Cain and colleagues may provide fundamental information about the contribution of evening light sensitivity as a mechanism perpetuating DSWPD.

2.7.6 Genetic Basis of DSWPD

It seems likely that some or all these possible circadian contributors to DSWPD (circadian delay, longer *tau*, altered PRC) have a genetic basis. It is possible with further research that simple clinical measures may emerge that are predictive of some or all of these circadian characteristics. Indeed there is considerable evidence for specific genotypic differences related to *tau* and circadian phase, most predominantly polymorphisms in hPER3 genes (Archer et al., 2010; Ebisawa et al., 1999; Jones et al., 2013). Associations with DSWPD and polymorphisms in hPER1 (Carpen, von Schantz, Smits, Skene, & Archer, 2006), hPER2 (Carpen, Archer, Skene, Smits, & von Schantz, 2005) and hCLOCK genes (Katzenberg et al., 1998) have also been documented. There is also higher incidence of Arylalkylamine N-acetyltransferase (Hohjoh et al., 2003) and HLA-DR1 (Hohjoh et al., 1999) alterations in DSWPD patients relative to controls, while mutations of S408N in hCKIepsilon (Takano et al., 2004) act as protective factors in DSWPD and N24SWD.

2.7.7 Behavioural Tendencies in DSWPD

According to circadian mechanisms a DSWPD patient is essentially jetlagged. Through exposure to light, the jetlagged traveler's new environment is thought to re-time and entrain the circadian clock. Travelers therefore typically recover from jetlag after a few days to a week in their new destination. However, DSWPD circadian delay is surprisingly stubborn to correct even with weeks of treatment. Consequently, it has been hypothesized that variables other than those related to circadian and sleep physiology also contribute to DSWPD manifestation. Although diagnostic criteria for DSWPD suggest that patients exhibit 'normal' sleeping patterns at delayed times (AASM, 2014; APA, 2013), evidence suggests the presence of some insomnia symptoms. According to ICSD-3, insomnia symptoms are a diagnostic feature of CRSWD. Despite the general rule that where sleep/wake disturbance can be better explained by other disorders (e.g. DSWPD) an insomnia diagnosis should not be given, the involvement of insomnia in DSWPD is an important aspect to consider.

According to previous literature, even when allowed to choose unconstrained bedtimes, DSWPD patients tend to exhibit significantly longer sleep latency compared to controls. Campbell and Murphy's lab study (2007) indicated their patient took an average of 38min to fall asleep over 17 nights of self-selected bedtimes, while controls took approximately 17min. In a separate study, 205 DSWPD patients reported 37min to fall asleep compared to 211 controls who estimated 17min latencies (Kripke et al., 2008). DSWPD patients also indicated significantly greater difficulty falling asleep on 2.9 d/wk compared to 0.7 d/wk reported by controls. Sivertsen and colleague's (2013) findings show significant co-morbidity between DSWPD and insomnia, with 55.9% of adolescents who met DSWPD criteria also meeting insomnia criteria. It would not be surprising to find some psychophysiological or learned sleep onset insomnia resulting from the numerous difficulties, worry and frustration associated with trying to fall asleep at conventional bedtimes. Even on nights prior to "free days" patients may also feel pressure to attempt sleep at earlier 'conventional' times before

circadian preparedness for sleep. They may feel social pressure and attempt sleep at earlier times in order to conform or 'do the right thing'. This timing would be too early for their biological clock, likely hitting the WMZ and accordingly leading to problems initiating sleep.

It seems DSWPD patients experience heightened arousal in the evening around the time where controls are falling asleep or preparing for bed. Gradisar et al. (2011) showed evidence of delayed arousal, reporting that 75% of their DSWPD patient sample described feeling alert after dinner. As DSWPD patients tend to be extreme evening types they most probably share the same social rhythm as evening types who show depressed alertness in the first hours after awakening and steady increases across the total waking interval reaching highest levels in the few hours before typical bedtime (Lack et a.l, 2009; Lack & Wright, 2012; Taillard, Philip, Coste, Sagaspe, & Bioulac, 2003; Zilli, Giganti, & Salzarulo, 2007). This may predispose them behaviourally to avoid the mornings by sleeping-in late and extend their positive experiences in the evening by delaying bedtime (Lack & Wright, 2012).

There is also some suggestion that DSWPD individuals may have a behavioural tendency to avoid light in the mornings compared to the evenings. Auger et al., (2011) examined ad libitum light exposure and sleep parameters in DSWPD and controls. DSWPD patients had greater exposure to evening and less to morning bright light, compared to controls. Findings remained congruent, even when adjustments were made relative to sleep onset time (i.e., to control for later sleep times in DSWPD). Similarly, 85% of adolescents diagnosed with DSWPD reported the avoidance of morning light (Gradisar et al., 2011). These clinical findings suggest that in addition to differential circadian sensitivity to light, DSWPD behavioural tendencies may lead to predisposition for greater light in the evening and avoidance of bright light in the mornings.

2.7.8 Personality and Psychosocial Factors

Most recent research has highlighted personality and psychosocial factors in DSWPD. A personality questionnaire assessment (NEO-PI-R) indicated DSWPD patients, compared to

controls, were lower on conscientiousness and extroversion, whilst showing significantly higher neuroticism scores (Wilhelmsen-Langeland et al., 2013b). Patients showed greatest deviation from both norms and controls on the 'conscientiousness' dimension, with lower scores on subcategories of competence, order, dutifulness, achievement striving, self-discipline and deliberation. People with lower conscientiousness scores tend to be less organized, less driven, procrastinate on tasks and are more prone to quitting (Costa & McCrae, 1992a). At present one can only speculate about the possible causal linkages between DSWPD and these personality characteristics. Lower conscientiousness may lead to less consistent sleep/wake schedule, succumbing more to sleeping-in late tendencies and thus resulting in a biologically delayed circadian rhythm. Additionally, a stronger than normal biological predisposition to DSWPD (e.g. longer *tau*, distorted PRC) may be less effectively resisted or treated in those low in conscientiousness.

Alternatively, since these findings are observational, the causal link may be in the reverse direction. A stronger than normal predisposition to circadian delay may cause such frequent sleep/wake disruption that it undermines a pre-existing healthy personality eventually resulting in the characteristics found by Wilhelmsen-Langeland et al. (2012). A better understanding of causal links between circadian physiology and personality may be imperative for understanding aetiology and/or enhancing DSWPD treatment. Future treatment studies can inform this endeavor by investigating changes in personality profiles post-treatment and by investigating personality predictors of treatment efficacy. In addition, longitudinal studies over the pubescent period could reveal if those who develop DSWPD in adolescence had pre-existing personality characteristics of older DSWPD or if their development followed the onset of DSWPD.

Wilhelmsen-Langeland et al. (2012) also highlight the role of psycho-social factors, not only in determining the diagnosis of DSWPD, but in the likelihood of successful treatment. An individual may fit the criteria for delayed sleep pattern but have few commitments early in the day, not see that they have a difficulty and have little motivation to attempt a phase advance treatment.

Thus the number of early commitments per week (e.g. full-time or part-time work, school attendance) and the distress resulting from trying to meet those commitments helps to determine the diagnosis and will likely affect treatment success. Those with few early commitments (e.g. unemployed, supported by parents, out of school) may attempt to seek daytime employment or other commitments but be unconfident in their ability to adhere to phase advance treatment. As such, severity of the disorder, patients' symptoms, complaints and motivation to change are determined not only by the delay in sleep but also situational factors.

2.8 Future Research and Clinical Recommendations

It is clear that the aetiology of DSWPD has not been fully elucidated. A functional interplay, or a combination of proposed factors may explain the underlying aetiology. Further research in the area with well-conducted experiments may reveal different subtypes of the disorder, with variable amounts of contributing biological, behavioural, psychological, and situational factors (Regestein & Monk, 1995).

It would be beneficial for future etiological DSWPD investigations to present clear, well-defined lifestyle factors, symptoms and inclusion criteria used to classify DSWPD samples. Factors that could be assessed include differences in current and preferred sleep time, daytime commitments, motivation to advance current sleep to preferred sleep time, impairments due to their sleep delays/loss, difficulty advancing to preferred sleep time, and outcome of attempted efforts to advance sleep timing. Personality factors may also help to further define severity of DSWPD and predict treatment success. Such screening data may help to identify predictors and mechanisms driving various subtypes of DSWPD. This information could further be used to obtain severity index measures. For example, a tool similar to the Insomnia Severity Index could be developed to better diagnose DSWPD or other CRSWD. Clinically these tools could be used to guide treatment and further assess treatment outcomes.

Given the aetiology of DSWPD remains uncertain, a combination of interventions tailored to individual cases is likely to yield the most successful treatment outcomes. Clinicians are urged to focus not only on correcting the phase-delay in DSWPD but to bear in mind alternative etiological explanations, such as behavioural, situational, and personality factors, and develop customized treatment options for their individual patients (Bartlett, Biggs, & Armstrong, 2013; Dagan & Eistenstein, 1999; Jones et al., 2013; Hiller et al., 2014; Lack, Wright, & Bootzin, 2009; Regestein & Monk, 1995; Wilhelmsen-Langeland et al., 2013b). Irrespective of aetiology, any circadian phase-delay should be initially corrected with the use of scheduled bright light (also known as phototherapy), exogenous melatonin administration, and/or chronotherapy (in severe cases).

Perhaps in cases where DSWPD is the result of sleep homeostatic dysfunction (incl. altered phase angle), rather than circadian misalignment, Sleep Restriction Therapy that sets the sleep interval to a fixed window and reduces total time in bed may also be efficacious. By reducing time in bed and allowing patients' sleep-pressure to build up, therapists may be able to reset sleep intervals and adjust them to more desirable times. Though this may seem intuitive and effective in insomnia patients, empirical studies have not investigated the efficacy of this treatment in DSWPD, which may be grounds for future research. Additional cognitive-behaviour therapy, education regarding sleep hygiene (Gradisar et al., 2011), and motivational techniques (Gradisar, Smits, & Bjorvatn, 2014) may be required to stabilize circadian phase and prevent relapse in many cases.

2.9 Conclusions

The aetiology of DSWPD remains unclear. Circadian phase delays are apparent contributors to delayed sleep schedules in the disorder, however, it is unknown how circadian rhythms become delayed and the processes involved in the maintenance of the chronically delayed but stable sleep timing. Extensive empirical studies continue to elucidate potential drivers of the delays. Current lack of etiological knowledge is limiting developments in diagnosis of DSWPD as well as efficacious treatment options. Though genetic, behavioural and cognitive pre-dispositions have been

associated with DSWPD, we have discussed physiological anomalies that will hopefully provide a basis for the cause of DSWPD, and furthermore, create the foundations for more efficacious and enduring treatment outcomes. Research surrounding circadian delays, longer circadian *taus*, abnormal phase angles, abnormal homeostatic sleep build-up and dissipation, and abnormal PRCs to light are currently the focus of empirical literature seeking to explain the cause of DSWPD. These notions all propose different hypotheses for describing the source of delayed sleeping patterns in DSWPD. While most studies predominantly highlight physiological contributions to DSWPD, recent evidence for genetic, behavioural and psychosocial precursors cannot be overlooked as drivers or at least contributors to delayed sleeping patterns. It is suggested that successful DSWPD treatment outcomes are achieved through a combination of interventions tailored to individual cases. As such, clinicians should be educated about approaches, be up-to-date with emerging etiological and treatment literature, and apply these appropriately to each patient.

2.9.1 Practice Points

1. Aetiology of DSWPD is uncertain.
2. Relative to healthy, normal sleepers, DSWPD patients exhibit a 3-4 h delay in markers of circadian phase position.
3. Compared to normal sleepers, DSWPD patients may exhibit:

- significantly longer *taus*;
- an abnormal relationship between circadian phase relative to their typical sleep times;
- a dysfunction of the homeostatic sleep drive;
- increased sensitivity to evening light and decreased sensitivity to morning light.
- decreased 'conscientiousness' personality trait.
- situational factors that may reduce motivation to comply with phase advance treatments.

4. Focus not only on correcting phase-delays in DSWPD but to consider alternate etiological explanations when developing customized treatments for individual patients.

2.9.2 Research Agenda

To further elucidate the link between DSWPD and proposed etiological models empirical studies should:

1. Use more standardized, laboratory-based experiments that control for prior accumulation of sleep pressure and circadian phase to investigate whether/how positions of circadian phase relative to sleep time could delay sleep.
2. Examine ability to compensate for prior sleep debt to gauge if deficits in sleep recovery could lead to sleep delays in DSWPD.
3. Replicate current studies to assess if DSWPD patients experience reduced sleep pressure as commonly observed in adolescents' delayed sleep.
4. By comparing PRC curves in patients and normal sleepers, establish if, in fact, DSWPD sensitivity to light is increased in the late evening, and/or reduced in the morning.
5. Further investigate behavioural tendency to be active and alert in the evening and avoid morning bright light stimulation.
6. Seek confirmation of early findings about the personality profile of individuals with DSWPD.
7. Help to identify predictors and perhaps subtypes of DSWPD by providing clear descriptions of patients' lifestyles, symptoms, psycho-social or situational factors, and complaints that merit DSWPD diagnosis in investigational samples.
8. Develop a DSWPD severity index based on evidentially confirmed aetiology and that can also be helpful for identifying DSWPD subtypes.

CHAPTER 3 MAIN AIMS

Circadian melatonin and temperature *taus* in Delayed Sleep-Wake Phase Disorder and Non-24-Hour Sleep-Wake Rhythm Disorder patients

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Author Contributions

GM had responsibility for day-to-day administration of the project, supervision of recruitment, screening and scheduling of participants, participation in data collection, data management, analysis and write up of the manuscript. LL had primary supervisory role for the project, overall planning, supervision of the students, data analysis, and manuscript preparation. NL had supervisory role of students and was directly involved in helping to manage the project. She was also significantly involved in written publications and communications of results. SF and HB gave important input to the project and to the proposal. SF, HB and MG continued to assist with advice, written publications, and other modes of communication of results.

Micic, G., Lovato, N., Gradisar, M., Burgess, H.J, Ferguson, S., Lack, L. (Submitted 04/12/2015). Circadian melatonin and temperature *taus* in Delayed Sleep-Wake Phase Disorder and Non-24-Hour Sleep-Wake Rhythm Disorder patients. *Journal of Biological Rhythms*.

3.1 Summary

Our objectives were to investigate the period lengths (i.e., *taus*) of the endogenous core body temperature rhythm and melatonin rhythm in Delayed Sleep-Wake Phase Disorder (DSWPD) and Non-24-Hour Sleep-Wake Rhythm Disorder (N24SWD) compared to normally entrained individuals. Circadian rhythms were measured during an 80-hour ultradian modified constant routine consisting of 80 ultra-short 1-hour 'days' in which participants had 20-minute sleep opportunities alternating with 40-minutes of enforced wakefulness. We recruited a community-based sample of 26 DSWPD patients who met diagnostic criteria (17m, 9f, age: 21.85±4.97 years) and 18 healthy controls (10m, 8f, age: 23.72±5.10 years). Additionally, 4 full-sighted patients (3m, 1f, age: 25.75±4.99 years) were diagnosed with N24SWD and included as a discrete study group. Ingestible core temperature capsules were used to record minute temperatures that were averaged to obtain 80-hourly data points. Salivary melatonin concentration was assessed half-hourly to determine time of Dim Light Melatonin Onset at the beginning and end of the 80-hour protocol. DSWPD patients had significantly longer melatonin rhythm *taus* (24h34m±17m) than controls (24h22m±15m, p=0.03, d=0.70). These results were further supported by longer temperature rhythm *taus* in DSWPD (24h34m±26m) relative to controls (24h13m±15m; p=0.01, d=0.80). N24SWD patients had even longer melatonin (25h±19m) and temperature (24h52m±17m) *taus* than both DSWPD (p=0.007, p=0.06) and control participants (p<0.001, p=0.02, respectively). These results suggest that longer *taus* of circadian rhythms may contribute to the DSWPD patients' persistent tendency to delay, their frequent failure to respond to treatment, and relapse following treatment.

Keywords: Circadian rhythm, period length, ultradian constant routine, delayed sleep, free-running

Circadian rhythms in physiological and behavioural processes are controlled by the biological body clock that cycles at a near 24-hour pace (Aschoff, 1970; Burgess & Eastman, 2008; Czeisler et al., 1999; Wever, 1979) in synchrony with the night/day cycle (Clodore et al., 1986; Duffy et al., 2001; Gradisar & Lack, 2004; Lack & Lushington, 1996). Although these endogenously generated patterns are often slightly longer than 24-hours in length, slight deviations from the 24-hour day are entrained by external light to synchronize the sleep-wake cycle, and other rhythms, to the day-night rotation of the Earth (Czeisler et al., 1989; Freedman et al., 1999). However, endogenous circadian rhythms, including the sleep/wake cycle can become desynchronized from the light/dark cycle. This desychrony can lead to chronic sleep difficulties and daytime impairments. The problems arising from this desynchrony are collectively termed Circadian Rhythm Sleep-Wake Disorders (CRSWD) of which Delayed Sleep-Wake Phase Disorder (DSWPD) is the most common (AASM, 2014).

According to previous literature and the International Classification for Sleep Disorders-3 (ICSD-3; AASM, 2014), DSWPD is described as a 2-6 h delay in the circadian and major sleep period relative to 'conventional' or socially desirable times (AASM, 2014; Dagan & Eisenstein, 1999; Regestein & Monk, 1995; Sack et al., 2007). Estimated to affect 1.1% of the general population (Lovato et al., 2013; Paine et al., 2014), prevalence is higher in subgroups of young adults and patients with insomnia where prevalence ranges from 10-16% (AASM, 2014; Lovato et al., 2013; Paine et al., 2014). Similarly, Non-24-Hour Sleep-Wake Rhythm Disorder (N24SWD) is a less common CRSWD and is readily observed in blind patients who cannot utilise light to synchronise their body with the external environment. Hence, typically patients' sleep and wake times progressively delay each day as a result of their sleep-wake cycle being longer than 24-hours (AASM, 2014).

A significant phase-delay of the circadian rhythm is regarded as the main etiological factor governing DSWPD. A continually delaying circadian rhythm is regarded as the main contributing factor to N24SWD (AASM, 2014; Sack et al., 2000; Sack et al., 2007; Uchiyama et al., 2000a). In

both conditions, attempts to adhere to more conventional sleep/wake times result in insufficient sleep that can have negative impacts on patients' physical and psychological health (AASM, 2014; Dagan & Eisenstein, 1999; Lovato et al., 2013; Regestein & Monk, 1995; Sack et al., 2000; Sack et al., 2007; Wilhelmsen-Langeland et al., 2014).

Hypothetically, appropriately timed morning light simulation should re-set the delayed circadian rhythm of DSWPD to an earlier more desirable target times. However, empirical literature to support efficacy of treatments is inconsistent and recurrently failed responses to treatment are reported (Dagan & Eisenstein, 1999; Sack et al., 2007; Saxvig et al., 2014; Wilhelmsen-Langeland et al., 2013a). As such, other mechanisms have been suggested to contribute to delays in both DSWPD and N24SWD, including a longer than normal period length or *tau* (i.e., a slower than normal circadian oscillator; Dagan & Eisenstein, 1999; Sack et al., 2007; Sack et al., 2000; Weitzman et al., 1981). For example, an individual may exhibit an endogenous *tau* that is closer to ~25h than the conventional 24h solar day. By taking significantly longer to complete one full circadian cycle, the patient's circadian system will exert pressure to delay sleep timing by an additional hour each day. Therefore, confirming longer *taus* in DSWPD could provide insight into failed chronobiological treatments.

Only two studies, employing two different experimental approaches, have previously investigated the notion of a longer circadian *tau* in DSWPD. Campbell and Murphy used the free-running protocol to investigate the *taus* in a single DSWPD patient. They found that the patient had a circadian cycle or period length (i.e., *tau*) of core temperature rhythm that was almost an hour longer than their 3 healthy control sleepers (Campbell & Murphy, 2007). In a pilot study, we further investigated this premise using an ultradian routine♦, and found that 6 DSWPD patients also had a significantly longer temperature rhythm *tau* compared to 7 normally entrained sleepers (Micic et al., 2013). A longer *tau* has also been proposed in Non-24 Hour Sleep-Wake Disorder (N24SWD) in

♦ *This is a separate sample and not embedded within the present group of participants*

which the patient has a longer than 24-hour sleep/wake cycle that has lost synchrony with the 24-hour light/dark cycle (Kitamura et al., 2013; Uchiyama et al., 2000a).

The purpose of the present study was to extend the previous findings with a larger sample and additional circadian measures. Contributing to the sparse knowledge of DSWPD aetiology may enable more durable treatments. This is the largest sample of DSWPD *taus* in the literature to date with the second largest sample of N24SWD *taus*. Additionally, it is the first study to derive *taus* of melatonin circadian rhythms in DSWPD patients. In line with previous findings, it was hypothesised that individuals with DSWPD will exhibit a significantly longer *tau* as measured by core body temperature and melatonin compared to normally entrained controls. A third, serendipitous group of N24SWD were also included in the study. It was predicted that core body temperature and melatonin *taus* would be longer still, in N24SWD compared to *taus* of DSWPD patients and controls.

3.2 Methods

3.2.1 Sample Demographic

Twenty-six (17m, 9f, age: 21.9±5.0 years) DSWPD patients were selected on the basis of ICSD-3 diagnostic criteria, as well as 18 good sleeping, normally timed controls (10m, 8f, age: 23.7±5.1 years). An additional four full-sighted patients displayed instability in their delayed sleeping pattern during the screening process (see Figure 3-1). They were diagnosed with Non-24-hour Sleep-Wake Disorder (N24SWD) according to ICSD-3 criteria and included as a separate study group (3m, 1f, age: 25.75±4.99 years). The sample was community-based, recruited via poster advertisements publicised in local newspapers, television, radio, public notice boards, educational institutions (i.e., three major Universities in South Australia), as well as online websites. Following a minimum 2-week screening process, suitable candidates were selected for an 80-hour ultradian constant routine protocol at the Flinders University Sleep Laboratory. Informed consent (Appendices A and B) was

obtained prior to commencement of interviews, questionnaires and laboratory procedures (Appendices C). The Southern Adelaide Clinical Human Research Ethics Committee granted ethics approval for the experiment. Upon completion, monetary compensation proportional to the time and contribution invested in the study was paid to participants. Participants who completed the entire study were reimbursed AUD$500.

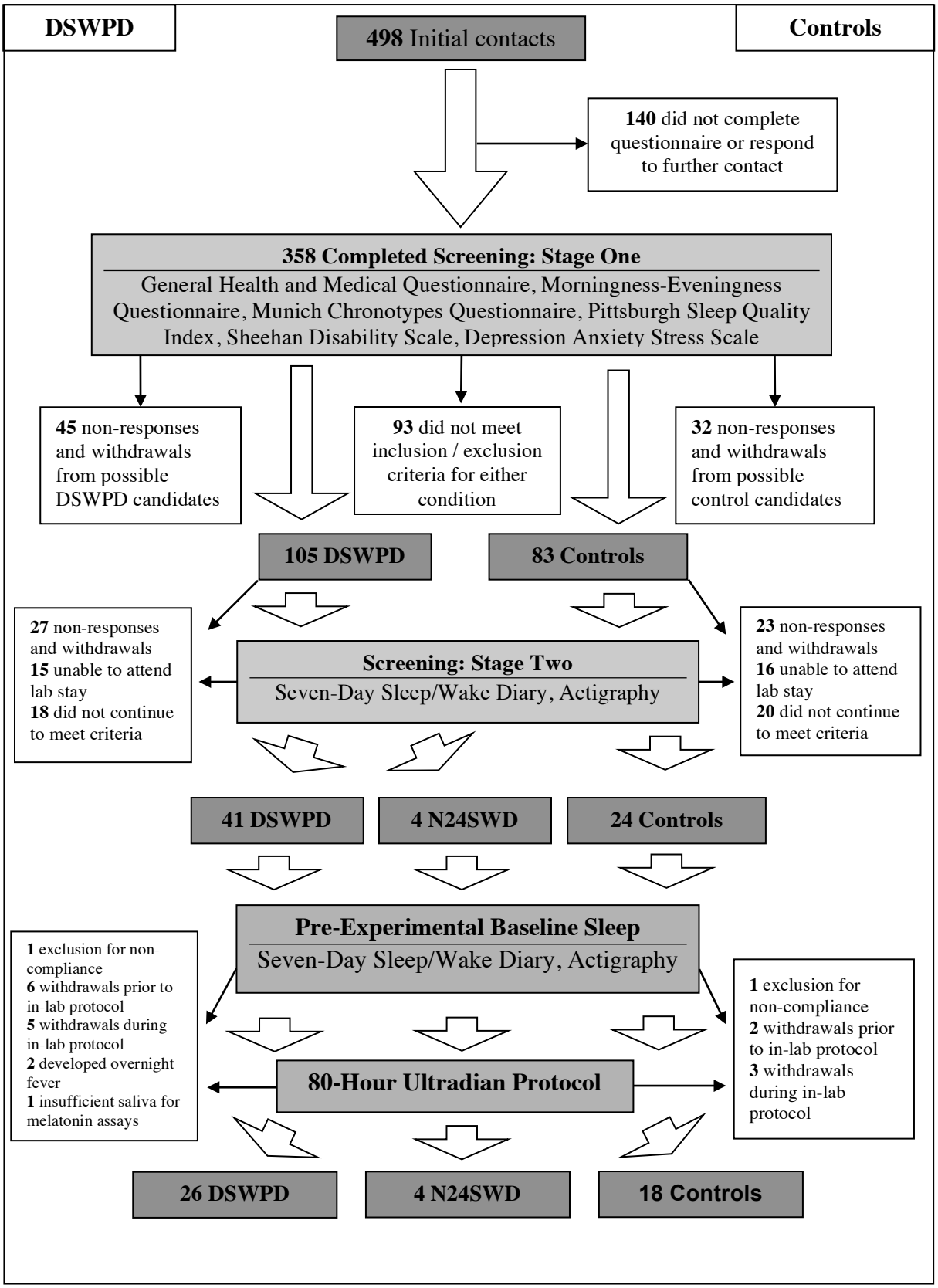

Figure 3-1. Participant flow through recruitment and involvement in the experiment prior and during the 80h Ultradian modified constant routine. N24SWD patients became apparent following first week of sleep-wake diary/actigraphy monitoring.

3.2.2 Participant Recruitment

Screening materials included a semi-structured clinical interview, a battery of psychological questionnaires, 2 weeks of sleep diaries and wrist actigraphy (Appendix D). All participants were free from medical, psychiatric and sleep disorders (except DSWPD and N24SWD). They did not take concurrent medication (including over the counter or herbal substances likely to affect sleep, alertness or circadian rhythms) without approved discontinuation prior to enrolment. They were non-smokers, not shift-workers, not pregnant or lactating, and reported consuming no illicit drug in the past 6 months. They had not traveled across ≥2 time zones in the 2 months prior to the study. Body Mass Index (BMI) was within the range of >18 and <32 kg/m^2, to screen out anorexic and obese individuals with increased risk of sleep disorders. Habitual daily caffeine consumption was <250mg and alcohol consumption was <14 standard drinks/wk. Female participants' menstrual cycles were noted and those recruited were either in the Follicular Phase of the cycle during experimentation or used hormonal control in the form of Etonogestrel implant or the contraceptive pill.

A battery of questionnaires identified those with DSWPD and normally entrained good sleepers. They consisted of the Munich Chronotype Questionnaire (MCTQ), Horne and Östberg's Morningness-Eveningness Questionnaire (MEQ), Pittsburgh Sleep Quality Index (PSQI), DSWPD-Sleep Timing Questionnaire (DWSPD-STQ)*, Sheehan Disability Scale (SDS) and Depression Anxiety Stress $Scale_{21}$ ($DASS_{21}$). Patients with DSWPD classified as evening-types (a score <42) on the MEQ. They indicated delayed sleep patterns of at least 2-hours in contrast to 'conventional' sleep times (i.e., sleep onset later than 0100h) and inability to fall asleep at a preferred earlier time per the DSWPD-STQ. They further reported daytime dysfunction and lifestyle impairments as a result of their sleep pattern (i.e., a score ≥5 on any SDS scale), but were otherwise healthy good sleepers when sleeping at their usual delayed time (i.e., ~6 on the PSQI scale with no indication of

* *developed by the authors*

co-morbidities with DSWPD). Good sleepers were selected on the basis of being healthy, good sleepers at conventional sleep times (i.e., sleep onset between 2200h and 2400h), who were neither morning nor evening types (MEQ score: 42-58), thus had a 'typical' sleep pattern and showed no daytime impairments as a result of their sleep (i.e., <5 on any SDS scale and ≤6 PSQI).

3.2.2.1 DSWPD - Sleep Timing Questionnaire

This questionnaire was developed to diagnose patients who wanted to phase-advance their sleep periods by >2-hours earlier as per the ICSD-3 criteria (AASM, 2014). Questions consisted of current sleep and wake time according to patients' perceived body clock sleep-timing as well as their preferred sleep timing according to social and daytime obligations. To classify as having DSWPD, patients were required to report a >2-hour discrepancy between their current sleep time and an earlier preferred sleep schedule. For healthy, control sleepers this discrepancy had to be <30-minutes. Five-point, Likert-type scales (0=not at all difficult; 4=extremely difficult) also assessed patients' difficulty with sleeping and waking at preferred times.

DSWPD criteria indicates the presence of recurrent difficulties falling asleep and waking up at conventional times thus DSWPD patients were required to report some difficulty with sleep onset and offset (score >1 at both ends) at their preferred times (AASM, 2014). This also ensured, control sleepers had no difficulty sleeping and awakening at necessary/conventional times.

Seven-day sleep diaries and activity monitors (AW64 Mini Mitter Actiwatch, Oregon, USA) wrist-worn activity motion monitors were used to further verify their sleep patterns. A qualified clinician (senior author, LL) confirmed participants' eligibility for the study after reviewing all previously described screening information.

3.2.3 Pre-Laboratory Procedures

Those selected were notified and invited to the Flinders University Sleep Laboratory for a familiarisation session of the laboratory environment and experimental procedures (Appendices E, F and G). Participants signed a second consent for involvement in the 80-hour laboratory session

once fully informed of protocol and procedures. One week prior to the laboratory stay, participants were asked to maintain their habitual sleeping pattern and compliance was again checked by sleep diaries and activity monitors. On the day of entry into the 80-hour laboratory session, participants were instructed to refrain from napping, undertaking strenuous exercise, and consumption of caffeine, alcohol or foods thought to impede habitual melatonin secretion (e.g., chocolate, bananas, tomatoes).

3.2.4 Design of Protocol

Data collection occurred over three years, during the Southern Hemisphere wintertime between April and August. Two to three participants were scheduled each week during this period, and required to enter the sleep lab at 15:00h Thursday for polysomnographic and core temperature recording preparation. From 17:00h participants remained in private, zeitgeber-free and temperature-controlled (20°C) bedrooms, in dim-light conditions (<10lux). The protocol comprised 80 consecutive hours of '1-hour days' ultradian modified-constant routine protocol with alternating 20-minute sleep opportunities and 40-minute of enforced wakefulness (see Figure 3-2). These 'days' were spread evenly throughout the protocol, in order to minimise accumulation of homeostatic sleep drive across the 80-hour routine. After an hour of habituation, the protocol formally commenced at 18:00h with a 20-minute sleep opportunity during which lighting was reduced to <1lux. Participants were awoken after the interval, lights were turned on to <10 lux (~8 lux measured at angle of gaze) and enforced wakefulness was timed for the remaining 40 minutes of the hour. This pattern continued until the protocol ceased at 2am on the subsequent Monday morning (i.e., for 80-hours total).

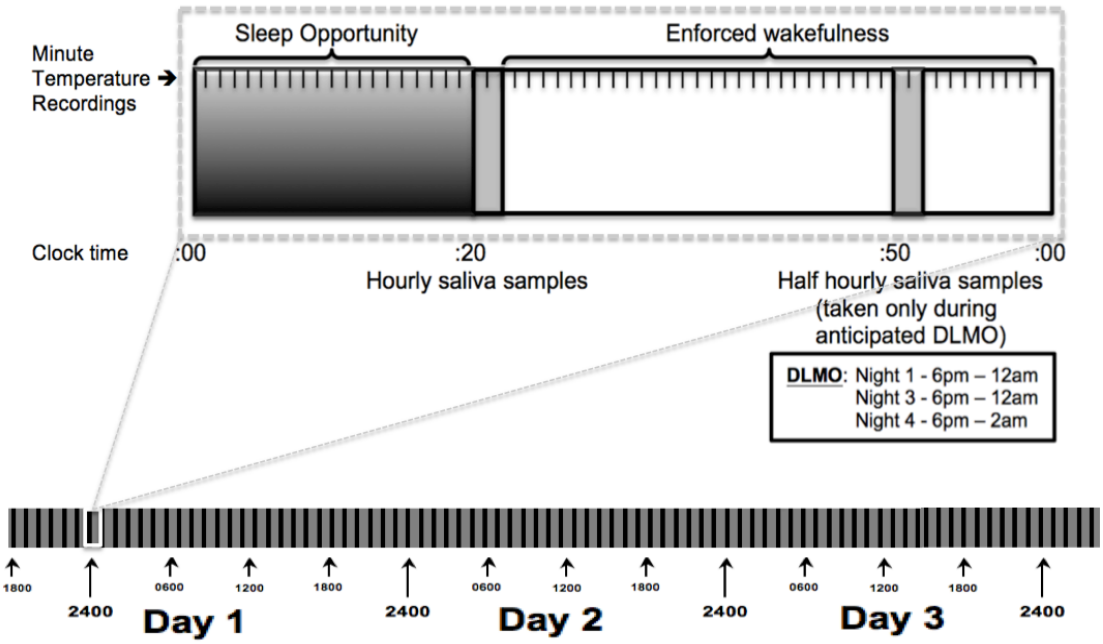

Figure 3-2. Schematic representation of the '1-hour day' modified-constant routine with 20-40 min alternating ultradian cycles.

During the enforced wakeful periods participants could engage in reading, watching films, studying, listening to music or talking with research assistants while remaining in bed at a near-supine body position (<45° angle). Care was taken to shield participants from any knowledge of time of day by removing clocks or any electronic device with time information and training research assistants to avoid revealing time of day directly or inadvertently through their behavior or disposition. Participants were permitted to leave the bed only for toilet breaks and basic hygiene (i.e., brushing teeth but without toothpaste) during which light intensity was maintained at <10lux. They were encouraged to use the commencement of enforced wakefulness periods immediately after the hourly salivary collections for these occasional activities. Participants were offered calorie-controlled snacks (820 kJoule; 200kCal) every two hours to minimise the thermogenic effects of energy intake. Snacks and accompanying 200mL of water were served at room temperature to minimize effects on core temperature. The snacks and water were served after awakening, and following salivary sampling to avoid contamination of melatonin samples.

3.2.5 Assessment of Circadian Rhythms

3.2.5.1 Salivary Melatonin.

Salivary melatonin samples with a sampling quantity of >2mL were collected during the 80-hour period using salivettes (Cat # 51.1534; Sarstedt Australia Pty. Ltd. Mawson Lakes, South Australia). Hourly samples were taken immediately upon cessation of every sleep opportunity. Half-hourly samples were also taken around the time of expected dim light melatonin onset (DLMO) to obtain a finer grained measure of DLMO. These occurred exactly half an hour following the previous sample on evenings 1 (i.e., Thursday 1850-2350), 3 (i.e., Saturday 1850-2350) and 4 (i.e., Sunday 1850- Monday 0150). Participants placed the swab in their mouth and accumulated saliva for 2 minutes while in a supine position in bed under dim light, to minimise the masking effects of physical movement and light on endogenous melatonin production.

Upon collection, saliva samples were labelled and stored frozen at -20°C and were later radio-immunoassayed for melatonin concentration (Adelaide Research Assay Facility, Robinson Research Institute, School of Paediatrics and Reproductive Health, University of Adelaide). Samples capturing individuals' full profiles of melatonin on night 1 and DLMOs on evening 4 were estimated from temperature data and sent for radioimmunoassay analysis. Those who's DLMO was not captured on evening 4 because of late phase timing had samples from night 3 also analysed. The DLMO on night 3 was used in these participants to calculate melatonin *tau*. This subsample comprised 7 participants, or 27% of the DSWPD group.

For melatonin analysis salivettes were thawed, centrifuged for 10min at 2500rpm and the swabs were removed from the casing, retaining the supernatant. To measure melatonin in the saliva, the minimum detectable dose of melatonin (i.e., analytical sensitivity) was 4.3 Pico-molar (pM) direct radioimmunoassay (RIA), using reagents from Buhlmann Laboratories (AG, Allschwil, Switzerland). The Low QC %CV was 9.2, and the high QC %CV was 15.3.

3.2.5.2 Core Body Temperature.

Participants were instructed to swallow an ingestible telemetric temperature sensor Jonah™ core temperature capsule which transmitted to a VitalSense® wireless physiological telemetric monitor (Philips Respironics, Mini Mitter Co., Inc., Bend, OR). This occurred immediately upon entry to the sleep lab (15:00h-16:00h) to maximise stabilisation of core temperature measurement from the start of the protocol at 18:00h. Temperature was recorded continuously for 80-hour in 60-second epochs. The VitalSense® consists of a data logger with a resolution of 0.01 °C, which receives radio signals every 15-seconds from the temperature-sensitive ingestible capsule. VitalSense® is a valid index of core temperature and between temperatures of 32-42°C, the Jonah™ capsules show an accuracy deviation of ±0.1°C from the actual body temperature (Byrne & Lim, 2007; McKenzie & Osgood, 2004).

Participants were instructed to maintain the VitalSense® device within 0.5 metres of their body since the capsules cannot transmit data at a distance >1 metres. The capsules pass through the gastrointestinal tract, with variable times of 12-48 hours depending on individual gastrointestinal motility. The absence of data transmission on the Vital Sense® was used as an indication of an excreted capsule from the gastrointestinal tract, and was replaced by ingesting a new capsule. During the 80 hour laboratory session, 1-3 capsules were typically ingested per participant.

3.2.6 Data Analysis and *tau* Calculation

The phase of the melatonin rhythm was calculated from the time of DLMO by linear interpolation of the melatonin concentrations. Melatonin concentrations from the protocol capturing the first and final DLMO evenings were plotted on KaleidaGraph version 4.1.3 (Synergy Software, Reading, PA). DLMO was defined as the time when melatonin concentration levels exceeded 10pM and remained elevated above the threshold for at least 2 successive data values. For individual melatonin profiles where low secretion was identified (i.e., acrophase <40pM; 23% of sample; 3 Controls; 6 DSWPD; 2 N24SWD), 25% of the acrophase value of the melatonin rhythm was used

as the threshold for that individual. Times were recorded for the first and final obtained DLMO of the protocol. Melatonin period length (Mτ) was determined for each participant by dividing the elapsed hours between first and last DLMO times by the number of cycles between these 2 DLMO times. This was commonly a denominator value of 3, or 3 fully completed circadian cycles, notwithstanding seven DSWPD participants who's DLMO was timed too late on Night 4 thus Night 3 data (i.e., denominator value of 2 full circadian cycles) was used for those individuals.

We used an absolute threshold to define DLMO because this method of detection ensured the threshold remained consistent across all individuals on all nights. It was important to keep this detection method systematic to ensure variance in estimated taus was not due to slight differences in individuals' melatonin secretion.

Recorded core temperature data from the Vital Sense® was downloaded and saved via the VitalSense® software to a Microsoft Excel 2007 software program at the end of each protocol. To obtain mean hourly core temperature values the transmitted temperatures were summed and divided by the number of recorded values per hour. During the first 5 h of capsule transit temperature-decreasing artifacts sometimes occurred following snacks and water (Byrne & Lim, 2007). This was occasionally evident in 29% of the sample (control n = 6; DSWPD n=7; N24SWD n=1) in such cases these data were excluded from the hourly mean calculations. The total data loss across the 80 hours for all participants equated to 5%. Hourly mean temperature values were analysed by a KaleidaGraph (Synergy Software, Pennsylvania, USA) least squares curve-fitting option. The curve fitting option consisted by a cosine function comprising the fundamental near-24h oscillation and its first near-12h harmonic (Brown & Czeisler, 1992; Duffy et al., 2001). The mathematical formula enables the curve to stretch or contract somewhat around a 24-hour period until the greatest variance explained was reached and the temperature *tau* (Tτ) value was generated. The program was not constrained within particular ranges in the search for periods (i.e., *taus*) since temperature data showed robust, clear trends. Curve fit solutions always had high face validity with variance

explained by the fitted curves ranging from r=0.79-98 with a mean value of r=0.90. From these best-fit curves, individual time of core temperature minimum (Tmin) and the period length (Tτ) were determined.

3.2.7 Statistical Analysis

DLMO, Mτ, Tmin, Tτ and demographic variables were entered and analysed using SPSS software version 22 (SPSS, Chicago, IL, USA) for Macintosh. Independent samples *t*-tests with two-tailed probability were used to compare group means and distributions on demographic variables and circadian phase positions. Cohen's *d* was also used to calculate effect sizes between groups. For inferential statistics, a repeated measures analysis of variance (ANOVA) was used to test the interaction and main effects of group on *taus* and effect sizes were measured using partial eta square (η^2). An analysis of covariance (ANCOVA) was used to test *tau* differences between groups by controlling for gender. Simultaneous multiple regressions and standardised Beta values further assessed sleep delay variances, as explained by circadian parameters.

3.3 Results

3.3.1 Baseline Assessment

Sleep and lifestyle characteristics between DSWPD patients and control sleepers are shown in Table 3-1 and 3-2, respectively. These data are not presented for N24SWD patients whose sleep characteristics were free-running and could not be captured by central tendency measures. Their sleep characteristics are thus illustrated in Figure 3-3. DSWPD patients had symptoms consistent with the ICSD-3 diagnosis of DSWPD as shown in Table 3-1. Table 3-2 also indicates DSWPD and N24SWD patients had significant disturbance to their lifestyle compared to controls. DSWPD presented with evening-type scores (i.e., MEQ and MCTQ) compared to the intermediate-type controls. There were no significant differences between controls and DSWPD patients on lifestyle factors such as age, BMI, caffeine, and alcohol intake. The gender ratio is representative of the

clinical population with higher prevalence of DSWPD estimated in males than females (Paine et al., 2014). All three groups obtained similar amounts of sleep during the 80-hour laboratory protocol, thus this factor would not have affected the period estimates. Throughout the 80-hour protocol, DSWPD patients obtained 722±123min of sleep, N24SWD = 776±229min and controls averaged 744±111min of sleep (F (2,45)=0.384, p=0.68).

Table 3-1

Summary of Delayed Sleep-Wake Phase Disorder (DSWPD) Diagnosis in Patients Based on ICSD-3 Criteria.

			DSWPD		Controls
Assessment Tool	**Item**	**Cut-off:**	**Group Mean ± SD**	**Cut-off:**	**Group Mean ± SD**
Diagnostic Criterion A: There is a significant delay in the phase of the major sleep episode in relation to the desired or required sleep time and wake-up time, as evidenced by a chronic or recurrent complaint by the patient or caregiver of inability to fall asleep and difficulty awakening at a desired or required clock time.					
DSWPD-STQ	Current Sleep Time	*Later than 0100:*	0217 ± 1h 18m***	*2200-0000:*	2240 ± 38m
	Preferred Sleep timing	*2200-0000:*	2259 ± 1h 6m	*2200-0000:*	2248 ± 47m
	Difference between current and preferred sleep timing	*>2 hours:*	3h 17m ± 1h 14m***	*~0.5 hours:*	-8m ± 55m
	Current Wake up Time	*Later than 1000:*	1125 ± hr 32m ***	*0600 – 0800:*	0720 ± 45m
	Preferred Wake up Time	*0600 – 0800:*	0756 ± 1h 30m	*0600 – 0800:*	0715 ± 1h 12m
	Difference between current and preferred wake time ^	*>2 hours:*	3h 19m ± 1h 47m***	*< 30 minutes:*	5m ± 1h 4m
	Difficulty Initiating Sleep at preferred time ^		2.15 ± 0.97***		1.22 ± 0.55
	Time taken to initiate sleep at preferred time		1h 28m ± 47m***		12m ± 8m
	Difficulty Awakening at preferred time ^		2.23 ± 0.86**		1.50 ± 0.51
Diagnostic Criterion B: The symptoms are present for at least three months					
DSWPD-STQ	How long has your sleeping pattern been as it currently is?		4.27 ± 4.13 yrs		N/A

Diagnostic Criterion C: When patients are allowed to choose their ad libitum schedule, the will exhibit improved sleep quality and duration for age and maintain a delayed sleep phase of the 24-hour sleep-wake pattern.[†]					
Assessment Tool	**Item**	**Cut-off**	**Group Mean ± SD**	**Cut-off**	**Group Mean ± SD**
PSQI	PSQI Score☾	*Not Specified:*	6.12 ± 3.29***	*Score of <6:*	1.78 ± 1.51
	Total Sleep Time	*7-8 hrs:*	6h 52m ± 1h 44m	*7-8 hrs*	7h 30m ± 58m
Actigraphy data confirmed by sleep-wake diary	Sleep Efficiency				
	- Overall	*>75%:*	79.62% ± 7.26	*>75%:*	84.06% ± 9.09
	- Commitment days		78.34% ± 9.50		83.86% ± 7.69
	- Free days		82.44% ± 8.54		84.42% ± 9.17
	Time in Bed				
	- Overall		8h 11m ± 50m		8h 40m ± 1h 2m
	- Commitment days	*7-8 hrs:*	7h 3m ± 1h 12m	*7-8 hrs:*	8h 14m ± 1h 7m
	- Free days		9h 13m ± 1h 7m		9h 13m ± 1h 4m
DSWPD-STQ	Total Sleep Time	*7-8 hrs:*	8h 58m ± 1h 26m	*7-8 hrs*	8h 41m ± 49m
Diagnostic Criterion D: Sleep log and, whenever possible actigraphy monitoring for at least seven days (preferably 14 days) demonstrate a delay in the timing of the habitual sleep period. Both work/school days and free days must be included with this monitoring.					
Actigraphy data confirmed by sleep-wake diary	Sleep onset				
	- Overall		0209 ± 1h 28m***		2323 ± 41m
	- Commitment days		0137 ± 1h 19m***		2308 ± 49m
	- Free days		0241 ± 1h 23m***		2329 ± 41m
	Sleep onset individual variability [#]				
	- Overall		1h 15m ± 55m**		41m ± 44m
	- Commitment days		1h 0m ± 46m**		34m ± 23m
	- Free days		53m ± 44m		48m ± 22m
	Sleep offset				
	- Overall		1035 ± 1h 13m***		0703 ± 53m
	- Commitment days		1010 ± 1h 34m***		0626 ± 41m
	- Free days		1155 ± 1h 39m***		0838 ± 45m
	Sleep offset individual variability [#]				
	- Overall		1h 40m ± 1h 2m**		38m ± 52m
	- Commitment days		1h 26m ± 1h**		39m ± 35m
	- Free days		1h 14m ± 56m		49m ± 32m

Diagnostic Criterion E: The sleep disturbance is not better explained by another current sleep disorder, medical or neurological disorder, mental disorder, medication use or substance use disorder.			
GHMQ	Do you have a sleep disorder? Non smoker Substance abuse in the past 12 months Psychiatric disorders in the past 12 months Health disorders or disabilities OTC Sleep medication Herbal substances likely to affect sleep	Necessary "No" response to all items apart from DSWPD as a response for possible delayed sleep-wake phase disorder	
	Alcohol	<14 standard alcoholic beverages per week	
	Caffeine	<250mg of caffeine per day	
$DASS_{21}$	Depression	*<15:* 16.77 ± 11.50***	*<15:* 4.78 ± 4.66
	Anxiety	*<15:* 7.92 ± 7.75**	*<15:* 3.33 ± 2.91
	Stress	*<15:* 15.23 ± 8.65**	*<15:* 7.11 ± 6.26
PSQI	No other reported sleep disturbances such as restless legs, snoring, mid-sleep awakenings, etc.		

†	Stable but delayed phase entrainment to 24-hour day overlaps in Criteria C and D (both addressed in D)
^	Range of possible scores: 0 (not at all difficult) – 4 (extremely difficult)
☾	Score of ~6 present with sleep disturbances related only to a delayed sleep pattern
#	From Sleep diary alone
*	Significant group differences indicated at <.05
**	Significant group differences indicated at <.005
***	Significant group differences indicated at <.001

Note: *Items from the battery of assessments were used as indicators of the disorder and to identify normally entrained controls. Time criteria and cut-offs for individual items were added to quantify diagnostic criterion and are indicated within the table. Assessment tools used to measure each diagnostic criterion are also indicated. Significant differences using independent samples t-tests are specified (*) between the controls and DSWPD, mean (M) and Standard Deviations (SD) are also given.*

Table 3-2

Group differences in lifestyle factors, current estimated sleep timing, sleep quality and associated impairments derived from pre-screening measures. Standard deviations are shown in parentheses and p-values indicated.

	Controls	**DSWPD**	**N24SWD**
	Lifestyle Factors		
BMI	21.86 ± 2.48	22.04 ± 2.66	24.01 ± 2.68
Caffeine intake (standard cups/day)	1.25 ± 1.19	1.27 ± 1.17	0.88 ± 0.85
Alcohol consumption (standard drinks/week)	1.06 ± 1.55	1.90 ± 3.26	0.50 ± 0.58
Weekly Commitments (number of hours)	30.50 ± 25.72	24.54 ± 12.10	6.25 ± 7.50
Weekly Commitments (number of days/week)	4.22 ± 1.80	4.38 ± 1.42	1.75 ± 2.06
MEQ score	56.67 ± 6.30	33.27 ± 5.68***	37.75 ± 16.66 †††
Mid Sleep Point [a]	3.87 ± 1.76	6.01 ± 1.88*	5.32 ± 4.49
	Sleep quality and associated impairment		
Work/school disruption [b]	1.22 ± 1.59	6.62 ± 2.64***	7.25 ± 3.40 †††
Social life disruption [b]	2.00 ± 2.40	4.73 ± 2.79*	6.5 ± 4.73 †
Family life disruption [b]	1.11 ± 1.88	5.46 ± 2.83***	7.25 ± 3.40 †††
Overall disruption [b]	4.33 ± 5.28	16.81 ± 6.03***	21.00 ± 11.49 †††
Number of days absent from commitments due to sleep pattern (subjective weekly average) [b]	0.44 ± 0.70	1.46 ± 1.84*	2.75 ± 3.10 †
Number of days of reduced productivity due to sleep pattern (subjective weekly average) [b]	0.72 ± 0.96	3.19 ± 2.14***	3.50 ± 2.52 †††
Motivation to change current sleeping pattern to preferred sleeping pattern [c]	1.39 ± 1.20	2.65 ± 1.06***	2.25 ± 1.71 †

[a] *Reported on the Munich Chronotype Questionnaire calculated using the formula* $MSF_{SC} = MSF-0.5*(SD_F-(5*SD_W+2*SD)/7)$. *MSF: Mid Sleep Factor; SD: Sleep Duration; F: Freeday; W:Work Day*
[b] *Reported on the Sheehan Disability Scale*
[c] *Reported on the DSWPD-Sleep Timing Questionnaire*
* *Significant group differences indicated at <.05 (DSWPD & control group)*
*** *Significant group differences indicated at <.001 (DSWPD & control group)*
† *Significant group differences indicated at <.05 (N24SWD, control group)*
††† *Significant group differences indicated at <.001 (N24SWD, control group)*

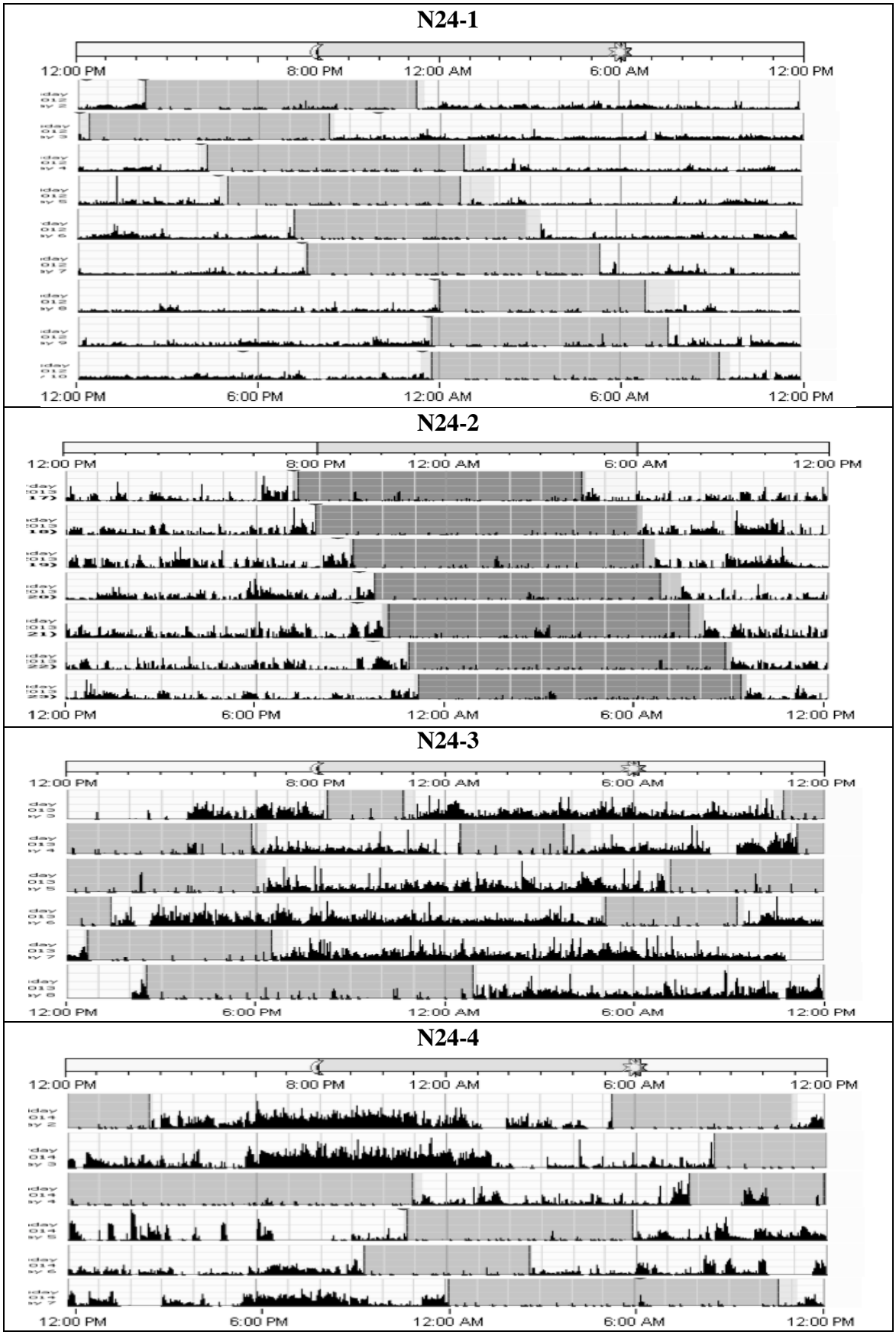

Figure 3-3. Week-long actigraphy of 4 N24SWD patients prior to commencement of the 80-hour experimental laboratory procedure. Full-shaded horizontal bars represent sleep periods. This figure

best exemplifies free-running sleep tendency in N24SWD and shows daily progression in sleep periods that are sometimes disturbed by societal commitments.

3.3.2 Circadian Phase Assessment

The timing of DLMO and Tmin on the first night of the ultradian routine was compared between controls and DSWPD using independent samples t-tests. These results confirmed significantly later timed phases of both markers, with DLMO occurring at 2312h±2h in DSWPD patients and at 2056h±1h26m in controls ($t(42)=4.04$, $p<0.001$, $d=1.27$). Likewise, Tmin occurred later in DSWPD patients (0711h±2h 10min) than controls (0420h±1h 29min), with a significant mean difference of 2h 51m ($t(42)=5.19$, $p<0.001$, $d=1.49$). Furthermore, these initial phase markers on the first night were highly correlated ($r=0.80$, $p<0.001$) with participants having later salivary melatonin onsets, also having later Tmin an average of 7h 39min±1h 26min later (see Figure 3-4). Similar associations between phase markers were found when groups were analysed separately. In DSWPD, DLMO preceded Tmin by 7h 54min±1h 32min ($r=0.73$, $p<0.001$), and in controls DLMO preceded Tmin by 7h 17min±1h 13 ($r=0.64$, $p=0.006$; see Figure 3-4).

This pattern was also consistent in N24SWD patients who displayed highly correlated phase markers (r=0.99, $p=0.006$), however this is largely due to their very disparate circadian phases on day 1 since they span the entire 24-hour period. Although the interval between DLMO and Tmin was somewhat shorter in N24SWD patients (6h 27min±59min) compared to DSWPD (7h 44min±1h 32min) and controls (7h 7min±1h 14min), these differences in phase marker timing were not statistically significant between the three groups.

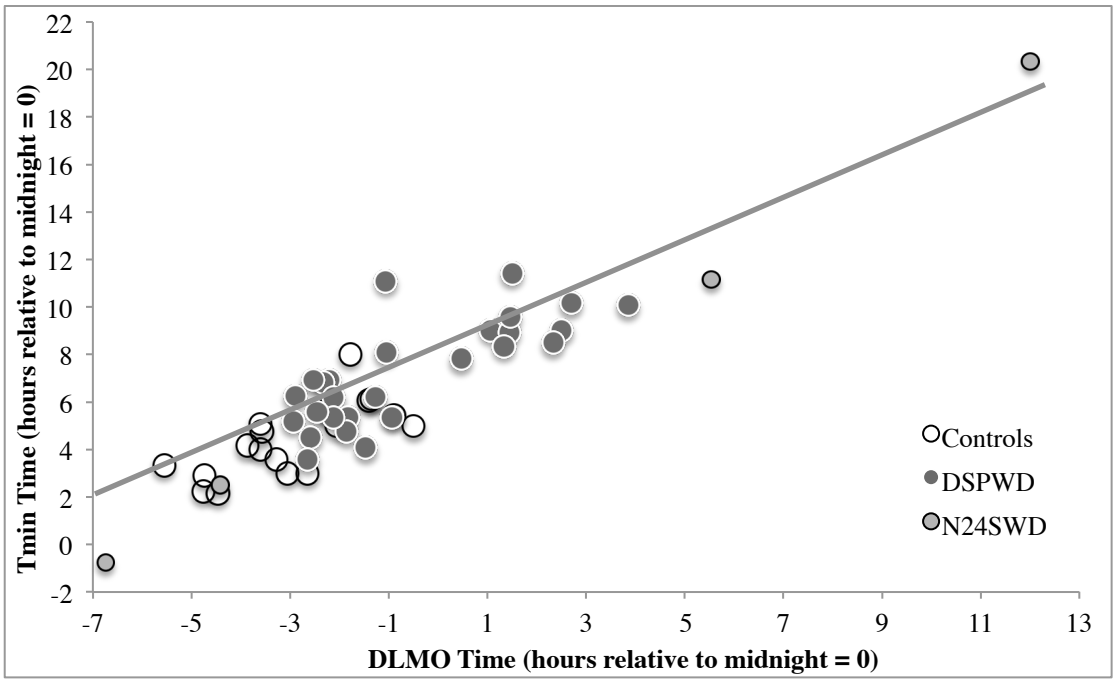

Figure 3-4. The timing of dim light melatonin onsets (DLMO) versus core temperature minimum (Tmin) of DSWPD, N24SWD patients and controls. Data were sampled from the first evening of the 80h constant routine. The timing of phase markers showed a strong, positive correlation with (r=0.91, p<0.001) and without (r=0.80, p<0.001) the data of N24SWD patients. Phase marker associations between time of Tmin and DLMO for DSWPD patients and controls combined are represented with the line of best fit.

3.3.3 Circadian *tau* Assessment

Table 3-3 represents individual *tau* estimates and Figure 3-5 presents the mean *taus* of controls, DSWPD and N24SWD patients. A (2 [*tau* marker: temperature, melatonin] x 3 [group: DSWPD, N24SWD, control]) repeated measures ANOVA revealed no overall interaction or main effect of *tau* marker but a significant main effect of group (F(45,2)=10.25, p<0.001, η^2=0.32). Post hoc tests with a Bonferonni adjustment, further reveal overall significant mean differences between *taus* of DSWPD patients and controls (*Mean Difference* [*MD*]=15min, *Standard Error* [*SE*]=5min, p=0.01), N24SWD patients and DSWPD (*MD*=23min, *SE*=9min, p=0.04), as well as N24SWD and controls (*MD*=38min, *SE*=9min, p<0.001).

Table 3-3

*Core body temperature taus (*Tτ*) and melatonin taus (*Mτ*) of each participant. Variance explained by fitted curves for temperature data and combine taus for each individual are also presented.*

DSWPD					CONTROLS				
ID	Tτ	r^a	Mτ	τ^b	ID	Tτ	r^a	Mτ	τ^b
D1	24.15	0.88	24.43	24.29	C1	24.08	0.96	23.67	23.88
D2	24.66	0.91	24.11	24.39	C2	24.45	0.85	24.64	24.55
D3	24.79	0.86	25.00	24.90	C3	24.05	0.91	24.11	24.08
D4	24.66	0.95	24.18	24.42	C4	24.04	0.94	24.42	24.23
D5	24.73	0.86	25.16	24.95	C5	24.54	0.83	24.22	24.38
D6	24.61	0.94	24.55	24.58	C6	24.53	0.92	24.61	24.57
D7	24.24	0.88	24.76	24.50	C7	24.14	0.91	23.87	24.01
D8	25.08	0.85	25.00	25.04	C8	24.27	0.86	23.83	24.05
D9	24.06	0.93	24.21	24.14	C9	24.16	0.90	24.86	24.51
D10	24.68	0.97	24.14	24.41	C10	24.34	0.95	23.65	24.00
D11	24.19	0.93	24.05	24.12	C11	24.43	0.95	24.00	24.22
D12	24.23	0.95	24.56	24.40	C12	24.48	0.90	23.61	24.05
D13	24.92	0.86	25.52	25.22	C13	23.94	0.90	23.94	23.94
D14	24.53	0.92	25.00	24.77	C14	24.5	0.95	24.39	24.45
D15	24.31	0.81	24.98	24.65	C15	24.68	0.91	24.19	24.44
D16	24.31	0.93	24.61	24.46	C16	24.68	0.92	24.66	24.67
D17	24.87	0.94	24.64	24.76	C17	24.46	0.95	24.66	24.56
D18	24.76	0.94	23.59	24.18	C18	24.63	0.98	24.54	24.59
D19	24.69	0.92	24.69	24.69					
D20	24.38	0.91	24.86	24.62					
D21	24.54	0.94	24.76	24.65	**N24SWD**				
D22	24.26	0.85	24.14	24.20	ID	Tτ	r^a	Mτ	τ^b
D23	24.63	0.94	24.90	24.77	N24-1	25.14	0.90	24.81	24.98
D24	24.82	0.95	23.99	24.41	N24-2	24.69	0.87	25.23	24.96
D25	24.79	0.79	24.53	24.66	N24-3	25.36	0.81	24.91	25.14
D26	24.75	0.92	24.29	24.52	N24-4	24.81	0.79	24.53	24.67

[a] *Variance explained by two-component (12- and 24-hour) cosine curves.*

[b] *Combined Melatonin and Core Temperature taus*

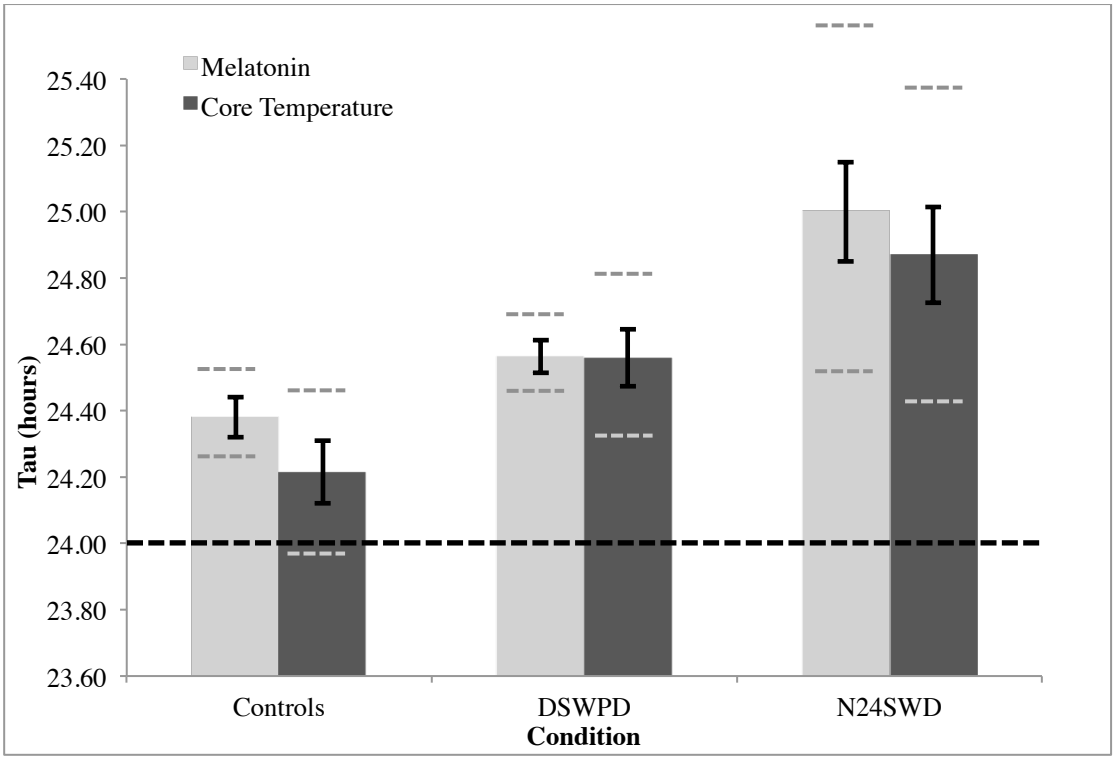

Figure 3-5. Mean circadian period lengths (*taus*) of core body temperature (Tτ: dark-coloured bars) and salivary melatonin (Mτ: light-coloured bars) in normally-entrained controls, DSWPD patients and N24SWD measured during an 80h modified-constant routine with 20-40 min alternating ultradian cycles. Black vertical bars indicate Standard Error of means, grey perforated horizontal bars indicate 95% Confidence Intervals and the black perforated horizontal bar represents the 24h timing of the Earth's daily rotations.

The main aim was to compare circadian rhythms of DSWPD and controls, therefore a (2 [*tau* marker: temperature, melatonin] x 2 [group: DSWPD, control]) repeated measures ANOVA was used to compare *taus* in DSWPD and controls. There was a significant effect of group, $F(41,1)=8.61$, $p=0.006$, $\eta^2=0.18$, demonstrating that the *taus* of core temperature and salivary melatonin in DSWPD patients (*MD*=24h 34min, *SE*=3min) were significantly longer than those of controls (*MD*=24h 17min, *SE*=4min). Independent samples *t*-tests were further used to verify the simple effects, which showed significantly longer melatonin ($p=0.03$, $d=0.70$) and core temperature ($p=0.01$, $d=0.80$) taus in DSWPD patients compared to controls. Neither a significant interaction nor main effects of *tau* marker were found. There was also a significant correlation between core body temperature and melatonin *taus* ($r=0.32$, $p=0.04$) suggesting that those patients with longer

temperature *tau* tended to have longer melatonin rhythm *tau*. Furthermore, N24SWD patients had even longer melatonin (p=0.01) and temperature *taus* (p=0.06) than both DSWPD and control participants (Mτ: p<0.001, Tτ p=0.02).

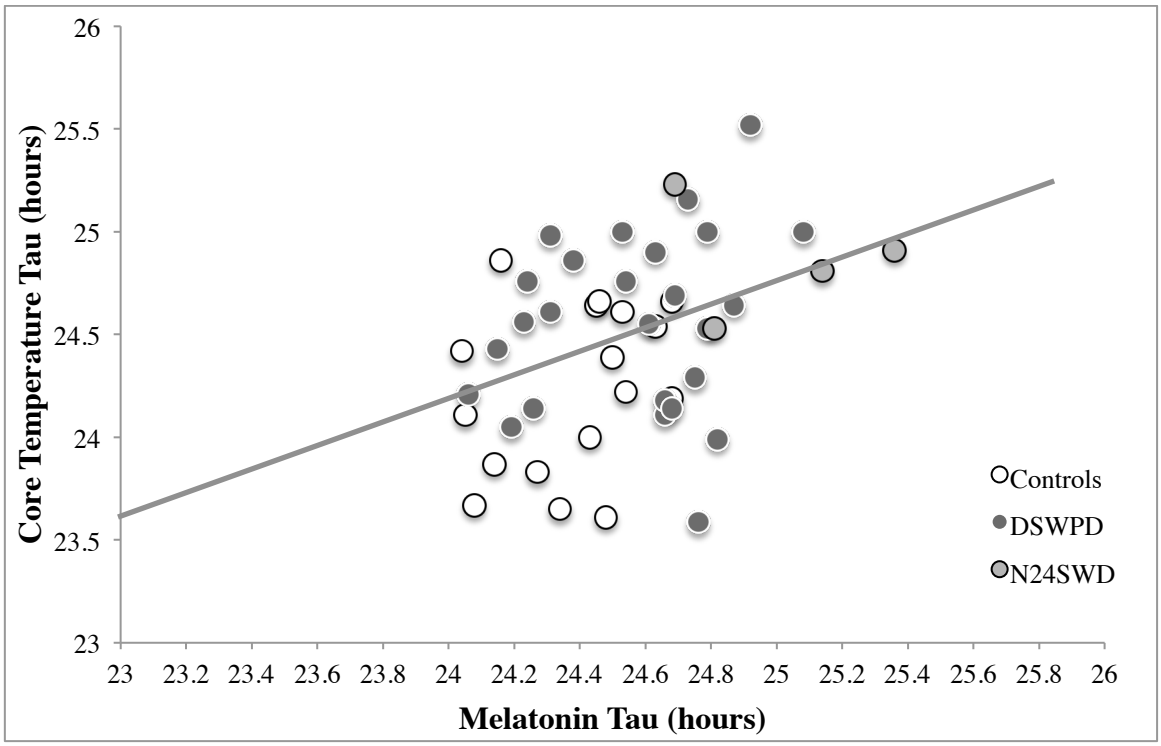

Figure 3-6. Period lengths (*taus*) of circadian rhythms as measured by salivary melatonin (x-axis) versus core body temperature (y-axis) of DSWPD, N24SWD patients and controls. Circadian taus for the overall sample were positively correlated (r=0.38, p=0.01), and remained correlated when N24SWD patients (r=0.32, p=0.04) were removed from the analysis.

Our samples included more male than female participants, particularly in the DSWPD group (65%m) compared to controls (56%m). Given the report by (Duffy et al., 2011) that females have shorter *taus* compared to males we tested the imbalance in our groups. An independent samples *t*-test revealed that males had overall longer melatonin rhythm *taus* (Mτ: 24h 35min±18min) compared to females (Mτ: 24h 25m±17min, t(46)=1.88, p=0.035 [one-tailed]; but not longer temperature *taus* (Tτ: 24h 28min±28min versus Tτ: 24h 26min±28min, t(46)=0.14, p=0.90).

Hence, two one-way between subjects analyses of co-variance (ANCOVA) were carried out to assess gender bias on *taus* between groups. These were conducted separately for Mτ and Tτ.

Checks were carried out to confirm homogeneity of regression and linear relationship between covariate and dependent variable. The between subjects factor comprised three groups: DSWPD, N24SWD patients and controls. The covariate comprised gender and this was not significantly related to the differences in Mτ, $F(1,48)=2.51$, $p=0.12$, $\eta^2=.05$. Adjusting for this covariate continued to result in in a statistically significant effect of the between-subjects factor of condition $F(2,48)=8.81$, $p=0.001$, $\eta^2=0.30$. The adjusted Mτ mean for DSWPD patients was 24h 33min±3min, N24SWD 24h 59min±8min and controls 24h 23min±4min. Similarly gender was not significantly related to the differences in Tτ, $F(2,48)=0.08$, $p=0.78$, $\eta^2=.002$.

3.3.4 Circadian Rhythms and Sleep Timing

Results suggest significant *tau* differences could contribute to delays in sleep timing. To test the contribution of a longer circadian *tau* on delayed sleep timing, a multiple regression analysis was used. Sleep timing is essentially the major concern for DSWPD patients therefore bedtime and get-up times from objective actigraphy data were used as outcome variables. Again, due to a lack of a meaningful central tendency in N24SWD measures, these patients were excluded from the analyses. Specifically, two simultaneous multiple regressions were conducted to investigate if Mτ and Tτ could predict bedtimes and get-up times in the DSWPD group. The results of the simultaneous multiple regressions are presented in Table 3-4 indicating that *taus* explained 12% of the variance in sleep onset $R^2=0.117$, $F(2,42)=2.59$, $p=0.09$ and 19% of the variance in sleep offset times $R^2=0.189$, $F(2,42)=4.442$, $p=0.02$. In particular, Mτ was the significant predictor of sleep delays but not Tτ.

Given DSWPD is caused by a delay in circadian phase, we wanted to further investigate the predictive power of both phase delays and *tau* elongation on sleep delays. The same simultaneous regressions were conducted with the addition of DLMO and Tmin as predictor variables. The results of these analyses indicate that together, longer *taus* and phase delays explain 58% of the variance in sleep onset $R^2=0.579$, $F(2,42)=12.71$, $p<0.001$ and 66% of the variance in sleep offset times $R^2=0.656$, $F(2,42)=17.62$, $p<0.001$ (Table 3-4). Thus implying that although longer *taus*

affect sleep timing, circadian phase delays (in particular Tmin timing) were better predictors of sleep delays.

Table 3-4

Prognostic power of salivary melatonin and core body temperature period lengths (taus) to estimate sleep delays as measured by bed time and get up time from actigraphy. Beta and t-values indicate the power of effect. Significant predictors are highlighted in bold.

	Bedtime			**Get up time**		
Predictors	Beta	*t*-value	*p*-value	Beta	*t*-value	*p*-value
Melatonin *tau*	**0.29**	**1.84**	**0.09***	**0.45**	**2.92**	**0.006****
Temperature *tau*	0.11	0.68	0.50	-0.06	-0.42	0.68
DLMO	0.17	0.86	0.39	-0.02	-0.12	0.91
Tmin	**0.56**	**2.92**	**0.006****	**0.67**	**3.46**	**0.001****

** Significant one-tailed difference p<.05*
*** Significant two-tailed difference p<.05*

3.4 Discussion

Using an 80-hour ultradian constant routine, the aim of the present study was to investigate salivary melatonin and core body temperature circadian rhythms in DSWPD patients compared to controls. Only two previous studies have investigated core temperature *taus* of DSWPD and controls. Campbell and Murphy (2007) studied a single DSWPD patient (Tτ: 25.38h) relative to three controls in a free-running protocol (Tτ: 24.44h), while our group (Micic et al., 2013) studied six DSWPD patients (Tτ: 24.90h) and seven controls (Tτ: 24.48h) in an ultradian routine. Both studies provide preliminary empirical evidence of longer *taus* in DSWPD. The present investigation was the first to replicate these circadian rhythm findings in the largest sample to date, of DSWPD patients compared with controls and the first to include the melatonin circadian rhythm. We found that the timing of circadian rhythms on the first night of in-lab measurement occurred significantly later in DSWPD patients relative to normal sleepers. Moreover, *taus* of DSWPD patients were significantly longer relative to controls. Therefore, outcomes are consistent with previous extensive

reports of circadian delays of DLMO and Tmin as well as longer circadian *taus* in DSWPD patients (Campbell & Murphy, 2007; Micic et al., 2013).

In addition, this is the first study to investigate *taus* of DSWPD patients with those diagnosed with N24SWD rhythms ("free running"), which is another Circadian Rhythm Sleep-Wake Disorder (CRSWD). This group was also expected to exhibit significantly longer endogenous *taus* relative to normal sleepers. The serendipitous group of 4 N24SWD patients identified during screening, exhibited *taus* that were longer still, compared to both the control and DSWPD group. Their measured DLMO delayed by an average of 1-hour every evening during the ultradian routine. Likewise, core body temperature exceeded the solar cycle by 52 minutes each day. These results are consistent with recent findings of Kitamura et al., (2013) who were the first to investigate melatonin rhythm *taus* in 6 full-sighted N24SWD rhythm patients. In a 7-day forced desynchrony protocol they found that the endogenous DLMO *taus* of N24SWD patients were significantly longer than their intermediate chronotypes who were comparable to our controls as intermediate chronotypes. Thus both studies have shown longer than normal melatonin rhythm *taus* in the N24SWD patients to comparable significance levels ($p<0.002$).

The present results suggest that longer *taus* exhibited by DSWPD and N24SWD rhythm patients under non-entrained conditions may contribute significantly to the phase-delay of their major sleep periods. In the absence of 24-hour zeitgeber exposure in our laboratory protocol, patients have a greater tendency to delay their circadian rhythms compared to normal sleeping individuals. Slower oscillations of the endogenous body clock, or Suprachiasmatic Nucleus (SCN), can potentially delay patients' peak alertness (Duffy et al., 1998) and sleepiest phases to a later time compared to normal sleepers and conventional sleep times. Despite patients' desires and occasional attempts to sleep at more conventional earlier times. Not only were the circadian taus longer in the patients than controls, but there were significant relationships within the DSWPD group between tau length and typical wake times.

Once delayed, the DSWPD patients' sleepiest phase will occur significantly later (e.g., Tmin = 0711h±2h 10min according to Table 3-1), hence posing extreme physiological difficulty for DSWPD patients to get out of bed when socially necessary at this time. Similarly, a later peak alertness period in DSWPD (e.g. 2100h-0100h) would coincide with conventional bedtimes and inhibit sleep onsets thus postponing patients' bedtime to approximately 0200h (Table 3-1). This circadian shift can explain DSWPD patients' recurrent inability to fall asleep earlier, even when sleep debt has built up over consecutive nights of insufficient sleep.

Our findings also explain patients' tendency to sleep in later on the weekends to repay their sleep debt (Taylor et al., 2008; Yang et al., 2001). Patients who have repeated nights of late bedtimes and early rise times to meet commitments will likely compensate for sleep loss on their free days. On average our DSWPD patients were falling asleep at 0137h on commitment days and 0241h on free days while controls slept at 2308h on commitment days and only ~21 minutes later on their free days (Table 3-1). Although both groups spent more time in bed on free days relative to commitment days, on their free mornings, DSWPD patients slept in 2.5h longer than controls. This extra sleep-in will avoid the morning bright light that entrains the circadian system, thus enabling the circadian system to be unmasked and uninhibited by morning light. In healthy sleepers, several studies indicate that delaying bedtimes just on the weekend results in subsequent circadian delays comparable to their estimated ~ 15 minute longer than 24h *taus* (Taylor et al., 2008; Yang et al., 2001). Therefore, patients exhibiting a significantly longer *tau* would be expected to yield comparably greater circadian delays, thus perpetuating a vicious cycle and further contributing to circadian and sleep delays.

Similarly, the near-25-hour pace at which N24SWD patients' SCNs tend to oscillate would make it nearly impossible for rhythms to synchronise to the 24-hour day. A daily pressure to phase-delay by one hour would require the N24SWD patient to effectively phase advance by that same amount each day to remain entrained. Phase response curves to singe pulses of light stimulation

would suggest that a phase advance of an hour would require a long (4-7 hours) and intense (~ 10,000 lux) light pulse (Khalsa et al., 2003; Dawson et al., 1993). Only the most arduous and strict light therapy regime is, therefore, likely to have any success in synchronizing the circadian system of N24SWD patients to the 24h clock. Such lighting conditions are unlikely to be encountered in the typical post awakening environment of a N24SWD patient.

The findings of desynchrony are particularly prominent in totally blind patients (Sack et al., 2000) whose blindness arises from ocular or retino-hypothalamic track pathology, cannot receive photic stimulation necessary to entrain to the natural 24-hour light/dark cycle (Saper et al., 2005). In essence, these blind patients' sleep patterns passively and temporally 'free-run' because circadian *taus* are unperturbed by the exogenous light/dark cycle that entrains healthy humans. In a cross over treatment study of 7 N24SWD blind patients, oral melatonin effectively entrained 6 patients whose *taus* ranged between 24.2-24.6h, however the patient with a 24.9h *tau* did not achieve the same entrainment (Sack et al., 2000). Hence, endogenous pressure from the SCN would be a prominent factor making it almost impossible for patients with significantly longer *taus* to maintain synchrony with the 24-hour light/dark cycle.

Still, it seems that the endogenous SCN output is only a single element among others that contributes to sleep misalignments. There is evidence from our and particularly Kitamura et al.'s (2013) findings that not all patients with N24SWD rhythms exhibit significantly longer *taus*. Our results indicate overlap in the length of *taus* among all three groups and approximately half of DSWPD patients exhibited *taus* that were within the 'normal' range in the present study of <24.6h (Figure 3-6). This suggests that longer *taus* can explain about 12-19% of the variance in DSWPD patients' sleep timing and other factors can also contribute to misalignments in DSWPD and N24SWD disorders. Previous research suggests that both groups of patients may also exhibit abnormal functioning of entraining mechanisms. DSWPD patients may have abnormal hypersensitivity of evening melatonin suppression, and thus be prone to greater phase delay, when

exposed to light in the evening (Aoki, Ozeki, & Yamada, 2001; Cain et al., 2014). However, a reversed abnormality in the sense of hyposensitivity to morning light would reduce its phase advancing effect and the braking effect of morning light on the stronger than normal tendency to phase delay in these patient groups. This would explain why sighted N24SWD individuals present with similar sleep disturbances to blind patients. This implicates the functioning of the retino-hypothalamic tract. A dysfunction particularly in the ganglion cells that contain melanopsin has been linked to seasonal depression (Berson, 2003) and could also explain the association between depression and circadian misalignment (Roecklein et al., 2013) also apparent in our group of patients showing higher depression scores (please see $DASS_{21}$ scores: Table 3-1). Further studies are important to specifically investigate the contribution of phase change light sensitivity in DSWPD and N24SWD patients both in the evening and morning.

A potential abnormality in pineal activation has been recently suggested arising from the melatonin secretion profiles of DSWPD patients in the present study. Compared with the normal sleeping control participants, the DSWPD patients had a reduced secretion of melatonin between the DLMO and melatonin acrophase (Micic et al., 2015). The cause of this 'blunted' initial surge of melatonin needs to be investigated. Is it a deficient signal from the SCN to the pineal or an under responsive Pineal gland? However, its effects may be to feedback less circadian advancing effect to the SCN compared to normal controls and less ability to counteract the stronger tendency to delay with their longer *taus*.

There also appears to be a clear split in the circadian phases of DSWPD patients (Figure 3-4). Fourteen are imbedded in the cluster of 'late' controls and may be classed as 'non-circadian delayed' and 12 had much later phase markers and could be classed as 'circadian delayed' DSWPD. This adds weight to the emerging literature that approximately half of those clinically diagnosed with DSWPD do not portray abnormal intrinsic delays of core temperature and melatonin rhythms (Armstrong & Rajaratnam, 2014, Rahman et al., 2009). It also reinforces the recommendation for

objective phase assessment as part of DSWPD diagnosis (Keijzer et al., 2014; Rahman et al., 2009). Clinicians may be better informed about diagnosis and efficacy of treatment by separating patients who display circadian misalignment and those who show sleep delays but with normal circadian phase. For example, the former may respond more effectively to chronobiologic therapies (Wirz-Justice et al., 2004), while the latter may require regimes that focus predominantly on cognitive behavioural therapy (CBT; Gradisar et al., 2011).

3.4.1 Clinical Recommendations

The clinical implications of these findings are to investigate treatment options that can specifically target prolonged *taus*. While these treatment options may not yet be viable, genetic studies and further identification of polymorphisms could point to future treatment (Kripke et al., 2014). With our current tools for chronobiologic intervention, the results support the rigorous and persistent use of morning bright light therapy and evening melatonin administration, to palliate the conditions. This method of treatment has been successful for treating DSWPD as well as N24SWD disorder. Combination treatments of both bright light therapy and melatonin administration show greatest efficacy in advancing patients' sleep timing (Keijzer et al., 2014; Wirz-Justice et al., 2004). It is crucial to recognise that significantly longer *taus* can explain the frequently reported long-term relapse following current form of therapy (Regestein & Monk, 1995; Saxvig et al., 2014;Weitzman et al., 1981; Wilhelmsen-Langeland et al., 2013a).

While this treatment may allow patients to wake up at a regular and conventional time, the persistent strong tendency to delay coming from an abnormally long *tau* could make them susceptible to relapse. Indeed, previous results of a randomized controlled trial with morning bright light therapy (BLT) and evening melatonin administration indicate that gradually advancing bed and rise times significantly advanced DLMO timing and improved subjective sleepiness, fatigue and cognitive performance in both treatment conditions (Saxvig et al., 2014; Wirz-Justice et al., 2004; Weitzman et al., 1981). However, only patients who received combination melatonin and

morning light therapy showed favourable outcomes in the 3-month follow up. Therefore, the combination of early evening low dose melatonin administration and morning bright light stimulation would certainly be indicated for the circadian delayed and longer *tau* DSWPD and N24SWD patients.

Nevertheless it would seem that that delayed sleep in DSWPD patients without circadian delays and *tau* elongation is not due to circadian misalignment and, therefore, chronobiologic treatments would be ineffective. This group of patients may suffer adjustment difficulties perpetuated by other factors, rather than an abnormal circadian rhythm characteristic (Dagan & Eisenstein, 1999; Regestein & Monk, 1995). Hence, for this clinical subsample, CBT aimed at increasing behavioural control over sleep/wake schedules, teaching good sleep hygiene and circadian education, and mitigating maladaptive beliefs that contribute to inappropriate sleep/wake schedules would be most indicated (Gradisar et al., 2011). In any case without a measure of circadian phase in the clinical diagnosis of DSWPD all tools in the armamentarium including chronobiologic and CBT would be indicated.

3.4.2 Limitations and Conclusions

In the past, resultant measures of circadian rhythms, particularly *taus*, have varied slightly between studies associated with methodological differences. From forced-desynchrony studies over at least one-week duration, the average *tau* of healthy individuals is estimated to be at least 24.15h long (Czeisler et al., 1999; Duffy et al., 2001; Klerman et al., 2002). In the present study using the relatively short duration (80-hour) ultradian modified constant routine methodology we were able to replicate several previous findings (Campbell & Murphy, 2007; Duffy et al., 2011; Kripke et al., 2005; Micic et al., 2013), and found *taus* in our healthy participants of 24.22-24.37h. While this methodology may yield slightly longer estimates of *tau* than the forced desynchrony methodology, we found comparably longer *taus* in the DSWPD and N24SWD patients.

It is important to understand there are several methodological considerations and limitations, which should be noted when interpreting the findings of the current study. Empirical evidence can explain why the period estimates are different between free-running and forced-desynchrony studies (Klerman et al., 1996). However, the methodology employed in the present study has not been compared with the other protocols. Hence, explanations do not exist as to why the ultradian method finds *taus* that typically range between the other two methods of assessing period lengths. Although we have relative estimates of *taus* between conditions, a possible perceived limitation is that we cannot vouch for the accuracy of methodology in measuring *taus*. Future work is important to recruit humans for ultradian routines, counterbalanced with 28-hour day forced-desynchrony studies. This would be an interesting and perhaps necessary next step to investigate how the *tau* estimates compare between the different methodologies.

Additionally while body temperature exhibits a circadian rhythm, this rhythm can be masked by factors such as posture, activity, sleep. In this highly controlled study, sleep duration during the 20-minute naps is the only factor not controlled. We know that sleep can affect core body temperature (Lack & Lushington, 1996), but these sleep opportunities do not affect individuals' Tmin timing when measured by an ultradian routine compared with a constant routine, therefore the period estimate would not be affected either. Significant correspondence between temperature and melatonin *taus* in the present study further strengthens this conclusion since melatonin is independent of sleep/wake in the dim light laboratory environment (Benloucif et al., 2005; Klerman et al., 2002).

As such, the utility of the ultradian protocol has implications from a research standpoint, making it somewhat more convenient to investigate circadian-based issues and giving merit to the ultradian technique. Outcomes suggest that overall, DSWPD patients have significantly longer *taus* of salivary melatonin and core temperature rhythms than controls, and the *taus* of N24SWD patients were longer still. As such, *taus* longer than normal are likely to contribute to sleep delays in patients

with DSWPD and N24SWD patients. At least in the DSWPD sample, further research is necessary to untangle various other aetiological factors that pose difficulties in implementing successful therapies and ensuring long-term efficacy of treatment interventions.

CHAPTER 4 BEHAVIOURAL RHYTHMS

Circadian *tau* differences in biological, behavioural and sleepiness rhythms in Delayed Sleep-Wake Phase Disorder and Non-24-Hour Sleep-Wake Rhythm Disorder patients

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Author Contributions

GM had responsibility for day-to-day administration of the project, supervision of recruitment, screening and scheduling of participants, participation in data collection, data management, analysis and write up of the manuscript. LL had primary supervisory role for the project, overall planning, supervision of the students, data analysis, and manuscript preparation. NL had supervisory role of students and was directly involved in helping to manage the project. She was also significantly involved in written publications and communications of results. MG assisted with advice, written publications, and other modes of communication of results.

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4.1 Abstract

Objective: In this study we investigated biological, sleepiness and behavioural rhythm period lengths (i.e., *taus*) of Delayed Sleep-Wake Phase Disorder (DSWPD), Non-24-Hour Sleep-Wake Rhythm Disorder (N24SWD) patients and healthy control sleepers. We also ran cross-correlation analyses between different rhythm variables to examine phase angle of entrainment. The aim was to explore if behavioural rhythms, in addition to the biological circadian rhythms contribute to misalignments of sleep timing symptomatic of DSWPD and N24SWD.

Methods: Twenty-six DSWPD participants who met diagnostic criteria (17m, 9f, age: 21.85±4.97 years) and 18 controls (10m, 8f, age: 23.72±5.10 years) participated in an 80-hour modified constant routine. Additionally, 4 full-sighted patients (3m, 1f, age: 25.75±4.99 years) were diagnosed with N24SWD and included as a discrete study group. A forced-desynchrony ultradian protocol of 1-hour 'days' in dim light, controlled conditions alternated 20-minute sleep opportunities with 40-minute enforced wakefulness. Subjective sleepiness ratings were recorded prior to every sleep opportunity and median reaction time (vigilance) was measured hourly. Amount of sleep obtained (sleep propensity) was derived from 20-minute sleep opportunities to quantify hourly objective sleepiness. Hourly core body temperature was recorded and salivary melatonin assayed to measure endogenous circadian rhythms. Rhythm data were curved using the 2-component cosine model.

Results: DSWPD and N24SWD patients had significantly longer melatonin and temperature *taus* compared to controls. There were no significant *tau* differences between groups as measured by subjective sleepiness, sleep propensity and vigilance rhythms. However, DSWPD patients showed a greater interval between maximum sleep propensity and minimum core body temperature. Their sleep propensity rhythms lagged behind core temperature rhythms by an hour more compared to controls' sleep propensity and core temperature rhythms.

Discussion: The findings provide further evidence that delayed circadian rhythms in DSWPD may result from larger phase angles between core body temperature and sleep propensity. This interval may result in later sleep timing in DSWPD patients relative to their circadian timing thus masking their light exposure during a time that is critical to phase-advancing the circadian system.

The International Classification for Sleep Disorders-3 (ICSD-3; AASM, 2014) manual categorises Delayed Sleep-Wake Phase Disorder (DSWPD) and Non-24-hour Sleep-Wake Disorder (N24SWD) N24SWD as circadian-based disorders. DSWPD patients exhibit circadian rhythms that are timed approximately 3 or more hours later than normal (Oren, Turner, & Wehr, 1995; Ozaki, Uchiyama, Shirakawa, & Okawa, 1996). Since circadian parameters and sleep are closely associated, patients have great difficulty initiating sleep at a more conventional earlier time. Circadian rhythms in N24SWD patients cannot be synchronised to the 24-h light-dark cycle thus resulting in free running sleep patterns that are significantly longer than the 24-h period (AASM, 2014). Empirical literature indicates that Circadian Rhythm Sleep-Wake Disorders (CRSWD) originate from multiple factors (Dagan et al., 1996; Okawa & Uchiyama, 2007). The aim of the present study was to investigate if subjective sleepiness, sleep propensity and vigilance rhythms contribute to DSWPD and N24SWD.

In addition to circadian rhythm delays, abnormal relationships between the timing of circadian rhythms and sleep/wake cycles have also been hypothesised to underwrite the pathology of DSWPD and N24SWD. The relationship between the timing of the biological clock and the timing of the sleep/wake cycle and (thus exposure to the light/dark cycle) is called the phase angle of entrainment (PAE). Previous studies report alterations between the PAE of controls with those of DSWPD and N24SWD patients. There is evidence that DSWPD patients have longer PAE interval between their circadian rhythm timing (e.g., temperature nadir) and sleep offset (Campbell & Murphy, 2007; Ozaki et al. 1996; Uchiyama et al. 1992; Uchiyama et al. 2000a; Watanabe et al. 2003). The PAE in N24SWD patients appears to be even longer (Uchiyama et al., 2000b). Choosing to sleep at a relatively late PAE extends a longer period of eyes closed darkness following the temperature nadir and blocks retinal light stimulation during the most effective phase advancing period of the circadian system (Khalsa et al., 2003). Hence the late timing of DSWPD and N24SWD patients' sleep periods relative to their biological clock could further exacerbate their circadian and sleep/wake rhythm delay.

More recent ambulatory findings in DSWPD patients have not supported this notion and suggest that the PAE does not differ between DSWPD and controls (Chang et al., 2009; Wyatt, Stepanski, & Kirkby, 2006). When patients were instructed to maintain bed- and rise-times according to their habitual sleep, they slept at the same PAE as controls. However, much of the PAE research in DSWPD does not control for the masking effects of timing of the sleep/wake cycle and the environmental changes, such as the social context or external lifestyle pressures.

Similar to DSWPD, evening-type individuals exhibit ~2-3 hour differences in their circadian timing relative to morning-chronotypes (Emens et al., 2009; Mongrain et al., 2004; Mongrain, Carrier, & Dumont, 2006). However, one study shows that the phase of minimum subjective sleepiness in evening-types was timed 9 hours later relative to morning-types (Lack et al., 2009). The evening-types' maximum subjective sleepiness phase was timed 7 hours later compared to morning-types', thus suggesting that the delay in sleepiness rhythms could be due to longer subjective sleepiness period lengths. Subjective and objective sleepiness rhythms are an important factor in the choice of sleep time and their role for determining individuals' sleep patterns should be investigated (Gradisar & Lack, 2004). Hence, the longer subjective sleepiness period could result in delays of individuals' sleep timing. This would result in greater exposure to evening light and mask the phase-advancing effects of morning light, thus delaying individuals' endogenous circadian rhythms. Given DSWPD patients exhibit extreme evening-type preference, these findings could help to elucidate factors that lead to DSWPD, specifically the PAE. In particular, it would be suggested that DSWPD patients sleep at significantly late phases of their circadian cycle than normally entrained sleepers.

In an effort to tease apart confounding factors related to the PAE literature, we used a time-free environment to investigate subjective sleepiness, sleep propensity (i.e., objective sleepiness or how much sleep is obtained) and vigilance rhythms (i.e., median reaction time) in DSWPD, N24SWD patients and controls. By removing external factors that contribute to circadian and sleep

timing, as well as controlling homeostatic sleep pressure, we aimed to investigate circadian sleepiness and vigilance rhythms in DSWPD, N24SWD patients and controls relative to their biological rhythms.

We aimed to investigate if Lack et al., (2009) findings could be extended to the clinical sample of DSWPD and N24SWD patients. Thus, it was hypothesised that DSWPD and N24SWD patients will show significantly later maxima of alertness as measured by behavioural rhythms (i.e., subjective, objective and vigilance) compared to their circadian maxima of biological rhythms (i.e., melatonin and core temperature). In addition to their longer circadian biological *taus*, DSWPD and N24SWD patients would be predicted to have significantly longer behavioural *taus* compared to their endogenous circadian *taus*. These sleepiness and behavioural *taus* will be longer compared to controls.

4.2 Methods

4.2.1 Participants

Eighteen healthy control sleepers (age 23.72±5.10 years, 10m, 8f) and 26 participants who met the ICSD-3 criteria for DSWPD (age *M*=21.85±4.97, 17m, 9f) participated in a modified constant routine in the Flinders University Sleep and Circadian Research Laboratory. During the screening process, 4 full-sighted patients (3m, 1f, age: 25.75±4.99 years) were diagnosed with N24SWD and included as an additional third study group. There were no significant differences in age $F(2,47)=1.46$, $p=0.24$, or gender, $X^2\,(2, N=48)=0.73$, $p=0.69$, between groups. The Southern Adelaide Flinders Clinical Human Research Ethics Committee granted ethics approval for the experiment. Monetary compensation of A$500 was paid to participants who completed the study in its entirety. Participants were recruited via poster advertisements displayed on public notice boards, and educational institutions. Informed consent was obtained and a battery of screening measures

was used to verify participants' eligibility as normal sleepers, having DSWPD or N24SWD. Semi-structured clinical interviews confirmed all participants were physically and medically healthy.

4.2.2 Inclusion/Exclusion Criteria

In order to meet criteria as DSWPD patients, candidates had to report evening-type scores on the Morningness-Eveningness Questionnaire (MEQ), a minimum of 2-hour discrepancy between their preferred and current sleep pattern, sleep onset that was later than 1am but quality of sleep that was otherwise sound according to the Pittsburgh Sleep Quality Index (PSQI) when sleeping at their habitual sleep pattern. Furthermore, they had to report significant daytime impairment on the Sheehan Disability Scale (SDS) that was associated with the delay in their sleeping pattern. Control sleepers were individuals who displayed normal entrainment to a 24-hour day and thus scored intermediate scores on the MEQ and showed no preference to adjust their sleeping patterns earlier or later than 30 mins from their current sleep time. They reported good sleep quality (<6 PSQI) and had no daytime impairments related to their sleep. Sleeping patterns of both groups were monitored using a week-long subjective sleep/wake diary. This was accompanied by a Mini Mitter Actiwatch (Philips Respironics, Pensacola, FL) and Actiware 5 software to confirm diary data and ensure participants met sleep requirements.

Exclusion criteria included having co-morbid sleep disorders, using drugs of abuse, or concurrent medication likely to affect sleep/alertness, circadian rhythms or melatonin without approved discontinuation prior to enrolment (including over the counter medicines or herbal substances). Further exclusion criteria included smoking >1 cigarette/day and on average, consuming >250 mg per day habitual caffeine, and/or >14 standard alcoholic drinks per week or being outside the extended normal Body Mass Index (BMI) range of <18 and >32kg/m^2. Exclusion occurred if participants had a history of psychiatric disorders or substance abuse in the past 12 months, were pregnant/lactating, travelled >2 time zones in past 2 months or were involved in night shift work in past 2 months (night shift defined as a work schedule that includes at least 6 h of work

between 10pm and 8am). All female participants recruited for the study were either in the Follicular Phase of the cycle during experimentation or used a form of hormonal control (i.e., Etonogestrel implant or the contraceptive pill).

4.2.3 Measures

4.2.3.1 Core Body Temperature (T).

Core body temperature was measured every minute during the constant routine using Jonah® ingestible core body temperature capsules (Philips/Respironics, California). VitalSense® monitors (Philips/Respironics, California) that recorded and stored capsule data were used during the experiment and recordings were later downloaded and saved to Microsoft Excel® Version 14.3.8 for Mac 2011. The Jonah capsule passed through the gastrointestinal tract without affecting bodily functions and four temperature readings per minute are transmitted at 15-second intervals to capture more precise core temperature values. These devices have been shown to transmit accurate readings (e.g., ±0.1°C) and validation studies demonstrate the VitalSense® and Jonah® capsule to be a valid index for measuring core body temperature. Capsules were ingested 2-hours prior to protocol commencement to allow stabilisation of the capsule in the gut. If the capsule was expelled from the gastrointestinal tract, participants were asked to immediately ingest another. One capsule was typically ingested during the 30-hour protocol and in rare cases two capsules were required. To ensure signals were recorded, the monitor remained in close proximity to the participant, and was placed on their bed, within 50cm of their body. Hourly measurements were determined by averaging minute recordings.

4.2.3.2 Salivary Melatonin.

Saliva samples were collected hourly immediately upon awakening from each 20min sleep opportunity using Salivettes®, (Cat # 51.1534; Sarstedt Australia Pty. Ltd. Mawson Lakes, South Australia). Samples were also taken at half-hourly intervals during estimated times of DLMO on

the first and final evening of the ultradian protocol. They were also sampled on the second to last evening for participants whose DLMO was estimated to occur past 2am on the final evening (i.e., after the cessation of the experiment). Samples were labelled and immediately frozen at -20°C after collection. The frozen samples were later analysed by the Robinson Research Institute, School of Paediatrics and Reproductive Health, University of Adelaide. They were thawed, centrifuged and reagents (Buhlmann Laboratories AG, Allschwil, Switzerland; Voultsios et al., 1997) were added to measure melatonin in the saliva. Sensitive of the direct radioimmunoassays (RIA) were <4.3pM and the intra assay co-efficient of variation was always <10%. Inter assay co-efficient of variation was 15% at 100pM.

4.2.3.3 Sleep Propensity (SP).

Sleep propensity (or objective sleepiness) is the inverse measure of sleep onset latency, or how long participants slept during each sleep opportunity. Therefore, higher sleep propensity values indicate more objective sleepiness/less alertness. It was assessed at hourly intervals, during the 20-minute sleep opportunity using the Compumedics enhance Somte portable recorders (Compumedics, Melbourne, Australia). Prior to commencement of the constant routine, participants were fitted with electroencephalogram (EEG), electrooculography (EOG), and electromyography (EMG) electrodes attached to a portable Compumedics Somte (Compumedics, Melbourne, Australia). In accordance with conventional 10-20 system, Polysomnography (PSG) data were recorded by placing two electrode pairs at C4+A1 and O1+A2 sites on the scalp to record EEG and appropriately placed electrode pairs (plus reference) at the EOG and EMG sites. Electrode impedances were monitored throughout the 80h constant routine and kept at <8K Ohms. A trained sleep technician determined onset of sleep using conventional criteria (Rechtschaffen & Kales, 1968), and was blind to condition allocation. Polysomnography (PSG) 3.0 software (Compumedics, Melbourne, Australia), was used to measure sleep propensity as the amount of sleep obtained in each 20 minute sleep opportunity, irrespective of the stage of sleep. Sleep onset was defined as three consecutive epochs

of any stage of sleep (typically Stage 1). Once sleep onset occurred it persisted for the remainder of the 20 minute sleep opportunity. This helped to dissipate homeostatic sleep drive and avoid accumulation of excessive drive across the 80 hour laboratory session.

4.2.3.4 Subjective Sleepiness (SS).

Prior to each measure of sleep propensity, participants were asked to indicate their perceived level of sleepiness using the Stanford Sleepiness Scale (SSS; Hoddes et al., 1972; Maclean et al., 1992). Scores on the SSS vary between 1 and 7, with 1 corresponding to *feeling active and vital, alert, wide awake* and 7 corresponding to *almost in reverie, sleep onset soon, lost struggle to remain awake*. Therefore, higher SSS scores depicted higher sleepiness/lower alertness.

4.2.3.5 Psychomotor Vigilance Task (PVT).

Psychomotor vigilance was assessed using a 5-minute Palm version of the task (Dinges & Powell, 1985; Loh et al., 2004). Participants were instructed to press the response button as quickly as possible when a target stimulus appeared on the PVT screen. Participants used the thumb of their dominant hand to respond to a visual stimulus (i.e., by pressing a pushbutton as soon as the stimulus appeared). They were instructed that speed and accuracy performance were equally important on all tasks. The inter-stimulus intervals varied between 2-10s with the duration of a single Palm PVT session lasting for 5 minutes irrespective of the number of completed trials. Response Time (RT), in milliseconds (ms), was calculated from the appearance of a stimulus until the participant's response. Outcome measure of "vigilance" was measured by inversing RT, such that lower RT indicated greater alertness/vigilance and higher RT indicated lower alertness/vigilance. The PVT has a negligible learning curve and within one testing session, participants typically reach asymptotic responding capability (Doran, Van Dongen & Dinges, 2001). The 5-minute PVT has been established as a reliable measure that is sensitive to circadian modulation of neurobehavioral functions (Loh et al., 2004; Wyatt et al., 1997).

4.2.4 Constant Routine

After a rigorous screening process, participants underwent an 80-hour constant ultradian routine with 1-hour 'days' consisting of 20-minute sleep opportunities alternating with 40-minutes of enforced wakefulness (Figure 4-1). Participants resided in a time-free, controlled environment and were required to remain at bed rest in dimly lit (<10 lux) conditions. Social interaction was limited to trained research assistants who were available at all times throughout the protocol. Otherwise, during enforced wakefulness, participants remained in a near-supine position and undertook quiet activities in their private bedroom (e.g., reading, watching DVDs, playing games, listening to music). They consumed 200 kCal equi-caloric snacks at 2-hour intervals with 200mL water and meals were served 15-minutes prior to administration of the PVT Task.

Protocols were conducted between May and August in 2012-2014 (i.e., Southern Hemisphere winter time). Participants were familiarised with the protocol and tasks prior to commencement of the experiments, then the protocol formally commenced at 1800h on Thursdays. The VitalSense device was activated with core temperature data recorded at minute intervals. Every 'hour-day' commenced on the hour, with a sleep opportunity that ended 20-minutes past the hour. Participants were asked to rate their subjective sleepiness, then lights were turned off to <1lux during sleep opportunities and turned back on to <10 lux during enforced wakefulness. During the 20min sleep opportunity sleep was recorded onto a flash card that was then labelled (participant ID, sleep opportunity number), saved and backed-up after each recording. The saliva samples for melatonin assays were taken immediately upon the cessation of a sleep opportunity and 10-min before the next sleep opportunity during times of anticipated DLMO. The PVT task was presented to participants 40-minutes into the hour, or 20-minutes into enforced wakefulness to allow possible effects of sleep inertia to dissipate. In addition to being free of knowledge of time, participants were also blind to time intervals between the testing sessions and the time remaining to the cessation of experiment.

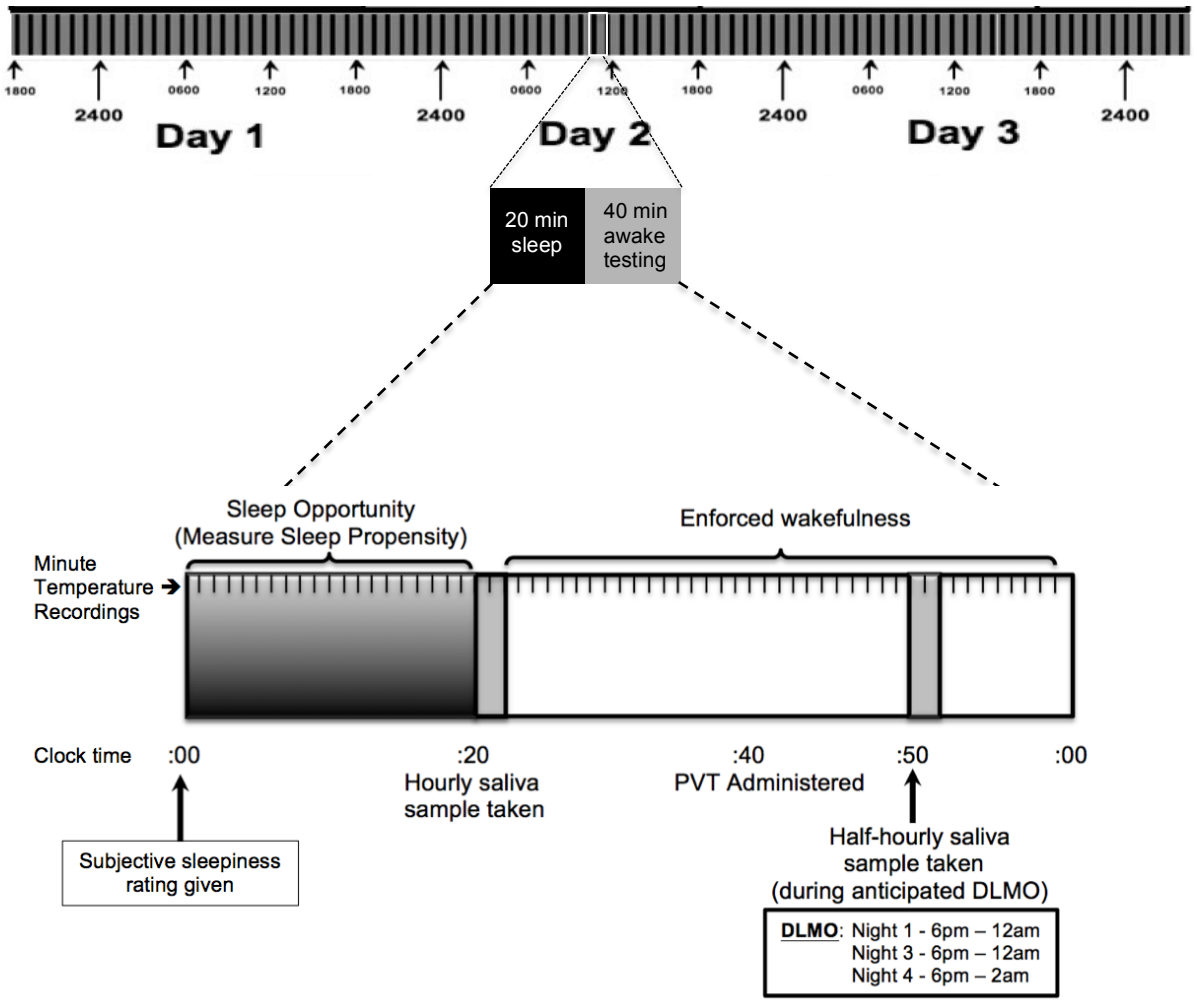

Figure 4-1. Schematic representation of the 80-hour ultradian constant routine.

Note: Protocols formally commenced on Thursdays, 6pm, and concluded on Mondays, 2am. Participants were kept in temporal isolation and dim-light (<10 lux) for the entirety of the protocol. At the very top of the figure, black vertical bars indicate sleep opportunities of 20 minutes in darkness. Grey vertical bars indicate 40-minute intervals of wakefulness in very dim illumination. This timing is shown in the enlarged box beneath "Day 2" and details of the alternating "1-hour" day protocol are presented below the box. Ingestible core temperature capsules measured core body temperature, at minute intervals. Sleep opportunities were given every hour and commenced precisely on the hour. Participants gave their subjective sleepiness (SS) ratings using the Stanford Sleepiness Scale immediately prior to the sleep opportunity, polysomnography (PSG) recorder was turned on and lights were turned out (<1lux). Sleep propensity (SP) was measured as the number of minutes participants slept during each sleep opportunity. At precisely 20 minutes past the hour, lights were turned on (<10lux), participants were awoken and remained awake for the remaining 40

minutes. Salivary melatonin was sampled hourly, immediately upon awakening and at half-hourly intervals during expected dim light melatonin onset (DLMO) times. This second, half-hourly sample was taken 30 minutes into the wakeful interval. The Psychomotor Vigilance Task (PVT) was ministered 20 minutes into the wakefulness interval.

4.2.5 Data Analysis

The resultant 80-h core body temperature (T), subjective sleepiness (SS), sleep propensity (SP) and vigilance (V) values were plotted using KaleidaGraph® version 4.1.3 for Mac. Circadian rhythms of core temperature were established using two component (24-hour and 12-hour components) cosine curves that best accounted for the plotted values (Brown & Czeisler, 1992). Uniform methodology was used to generate two-component cosine curves for established 80-point values of sleep propensity (SP), subjective sleepiness (SS), and vigilance (V) rhythms. Circadian *taus* (τ) were derived from the cosine formula and by careful visual inspection of each individual curve of best fit, acrophase (Max) and nadir (Min) times were identified for T, SP, SS, V. These were compared using SPSS Statistics software to assess time associations between maximum objective and subjective sleepiness, and lowest vigilance.

Assayed salivary melatonin values were also charted using KaleidaGraph® software. Dim Light Melatonin Onsets (DLMO) were estimated using an absolute threshold of 10pM on the first and final evening of the ultradian routine. The resultant DLMO values on the last evening of the routine were subtracted from the first evening. Outcome values were divided by the number of lapsed cycles between the two DLMO evenings to derive the melatonin circadian *tau* length (Mτ).

4.3 Results

Table 4-1 reveals the preliminary analyses conducted to assess the timing of DSWPD patients' sleeping patterns one week prior to entry to the laboratory conditions and the timings of their minimum and maximum alertness periods during the 80-hour constant ultradian routine. These were compared to healthy control sleepers. A series of independent samples *t*-tests confirm that the

timing of DSWPD patients' sleeping patterns and circadian rhythm measures were significantly delayed by 2-3 hours compared to controls. Greatest between group differences were reported for sleep timing on free days, as well as DLMO, maximum sleep propensity, minimum vigilance and maximum vigilance timings. Due to the free-running nature of N24SWD patient's sleeping patterns, central tendency data would not be meaningful and thus is not presented for this clinical subgroup.

Table 4-1

Comparison of mean sleep times on free days, work days (days of commitments) and combined for DSWPD patients relative to controls. Minimum and maximum points of alertness measured by core body temperature, melatonin, subjective sleepiness, sleep propensity and vigilance during the first 24-hour phase markers of the ultradian routine are also indicated.

Circadian Parameter	**Controls**	**DSWPD**	Mean Diff. ± SE	Cohen's *d*[a]	*t*-value	*p*-value
		Habitual Mid-Sleep Timing[c]				
Mid-Sleep Average	0343 ±37m	0610 ±1h27m	2.44 ± 0.32	2.09	7.54	<.001
Mid-Sleep Free Days	0404 ± 32m	0718 ± 1h 26m	3.23 ± 0.31	5.91	10.50	<.001
Mid-Sleep Work Days	0317 ± 32m	0506 ± 1h 7m	1.82 ± 0.25	1.96	7.15	<.001
		Core Body Temperature (T)				
T Minimum	0455 ± 1h56m	0700 ± 2h24m	2.09 ± 0.68	0.94	3.06	.004
T Maximum	1820 ± 2h26m	2018 ± 2h25m	1.95 ± 0.74	0.81	2.63	.012
		Melatonin (DLMO)				
DLMOff[b]	0817 ± 1h07m	1041 ± 3h00m	2.41 ± 0.76	1.00	4.04	<.001
DLMO[b]	2056 ± 1h26m	2312 ± 1h58m	2.27 ± 0.56	1.27	3.16	.003
		Subjective Sleepiness (SS)				
SS Maximum[b]	0611 ± 2h25m	0846 ± 2h44m	2.13 ± 0.84	0.78	2.53	.015
SS Minimum[b]	1716 ± 3h20m	2026 ± 4h13m	3.17 ± 1.14	0.82	2.66	.011
		Sleep Propensity (SP)				
SP Maximum	0552 ± 1h20m	0846 ± 2h30m	2.90 ± 0.73	1.11	4.00	.001
SP Minimum	2045 ± 1h46m	2247 ± 2h01m	2.35 ± 0.63	0.82	3.71	.001
		Vigilance (V)				
Minimum[b]	0553 ± 2h20m	0854 ± 2h09m	2.98 ± 0.69	1.27	4.43	<.001
Maximum[b]	1838 ± 3h32m	2158 ± 3h49m	3.50 ± 1.13	0.90	3.09	.005

[a] *Cohen's d Size of effect: d>.20 = small; d>.50=medium; d>.80 = large.*

[b] *Mean clocktime ± standard deviation (SD) in hours & minutes (hhmm±hmm)*

[c] *Actigraphy data confirmed by sleep-wake diaries*

The main aims of the study were to explore circadian *tau* differences between DSWPD patients, N24SWD patients and controls. A 3 by 5 repeated measures analysis of variance (ANOVA) was used to investigate between- and within-groups *taus* of the circadian rhythms core body temperature, melatonin, subjective sleepiness, sleep propensity and vigilance. There were significant main effects of both groups ($F(43,4)=2.95$, $p=0.03$, $\eta^2=0.23$) and different rhythms ($F(43,4)=3.89$, $p=0.023$, $\eta^2=0.17$) but no significant overall interaction effect. Follow-up post-hoc independent *t*-tests presented in Figure 4-2 indicate that DSWPD patients in general exhibited longer *taus* relative to controls. N24SWD patients portrayed significantly longer *taus* of core body temperature and melatonin compared to both controls and DSWPD patients.

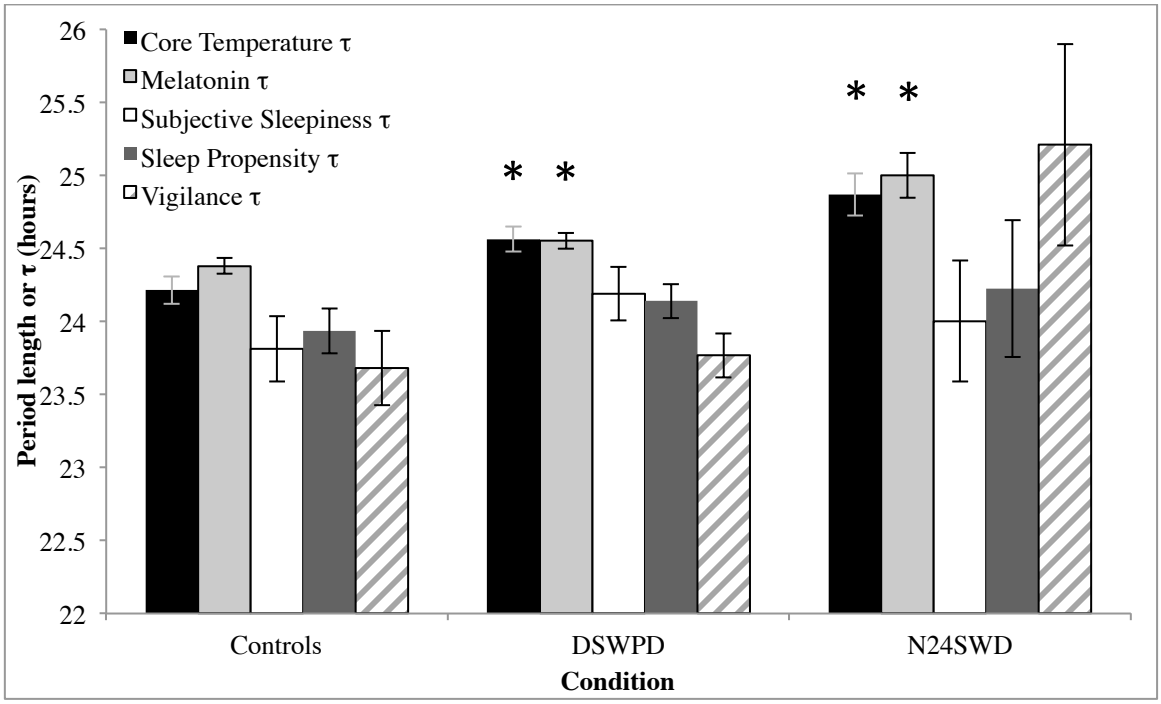

Figure 4-2. Group means in rhythm lengths (*taus* or τ) of core body temperature τ, melatonin τ, subjective sleepiness τ, sleep propensity τ and vigilance τ during the 80-hour ultradian routine. * = $p < 0.05$ difference relative to controls.

Phase lag tests between groups for overall circadian phase markers of biological, sleepiness and behavioural rhythms are presented in Table 4-2. As a preliminary test, the differences in identified temperature circadian nadirs and behavioural phases (i.e., vigilance minimum and

sleepiness/ sleep propensity maximums) were used to test the assumption that there could be phase angle differences between core temperature and behavioural sleepiness in DSWPD patients relative to controls. The average of the 3 circadian cycles during the 80-hour ultradian routine were used to analyse the average rhythm phase differences. These reveal significant phase lag averages across the three circadian cycles between temperature nadir and sleep propensity in DSWPD patients compared to controls.

Table 4-2

Between group mean differences ± standard deviations in rhythm phase differences in core body temperature, subjective sleepiness & behavioural rhythms throughout the 80-hour ultradian constant routine.

	Mean ± SD (hours)			Cohen's d^a		
	Controls	**DSWPD**	**N24SWD**	**DSWPD – Controls**	**N24SWD – Controls**	**DSWPD – N24SWD**
Tmin-SSMax	1.03 ± 1.98	1.29 ± 2.40	0.13 ± 1.56	0.12	0.46	0.50
Tmin-SPMax	0.72 ± 1.42	1.99 ± 2.20	0.95 ± 2.24	**0.66**	0.15	0.47
Tmin-VMin	0.33 ± 2.01	1.31 ± 2.43	0.32 ± 0.99	0.47	0.01	0.86

Significant difference indicted in bold font; p< 0.05

[a] *Cohen's d Size of effect: d>.20 = small; d>.50=medium; d>.80 = large.*

Next, cross correlations were calculated within each individual to test associations between biological and behavioural rhythms. Correlation coefficients were calculated from all 80h data without lagging to investigate spontaneous associations between rhythms for DSWPD, and N24SWD patients relative to controls. Due to the lack of the complete 80h data points for melatonin rhythms, this variable was excluded from analyses. The results of a One-Way ANOVA are presented in Table 4-3 that show significant differences in associations between temperature and sleepiness rhythms of N24SWD patients compared to both DSWPD patients and controls. There were no significant differences in the associations of rhythms between the DSWPD and control groups. For these two groups, the strongest correlations were between core body temperature and subjective sleepiness as well as subjective sleepiness and sleep propensity. However, there were significant differences between rhythm associations in N24SWD patients relative to DSWPD and

controls. Specifically, N24SWD patients portrayed significant differences in their SS-Temp associations and SP-Temp associations relative to controls.

Table 4-3

Rhythm associations between core body temperature, subjective sleepiness, sleep propensity and vigilance from the 80h ultradian routine. Correlation coefficients were calculated without lagging data to investigate spontaneous associations between rhythms for DSWPD, and N24SWD patients relative to controls.

	Means ± Standard Deviation			Cohen's *d* comparisons		
Rhythm Associations	**Controls**	**DSWPD**	**N24SWD**	**DSWPD-Controls**	**N24SWD - DSWPD**	**N24SWD - Controls**
Timing of SS relative to Temp	-0.58 ± 0.10	-0.55 ± 0.19	-0.33 ± 0.06	0.21 n.s.	**1.25** *p*= 0.03	**2.59** *p*< 0.01
Timing of SP relative to Temp	-0.55 ± 0.12	-0.50 ± 0.23	-0.40 ± 0.18	0.29 n.s.	0.45 n.s.	**1.17** *p*= 0.05
Timing of V relative to Temp	0.48 ± 0.14	0.45 ± 0.23	0.30 ± 0.21	0.17 n.s.	0.66 n.s.	0.96 *p*= 0.07
Timing of SP relative to SS	0.57 ± 0.14	0.58 ± 0.16	0.41 ± 0.23	0.07 n.s.	0.99 *p*= 0.09	1.05 *p*= 0.08
Timing of V relative to SS	-0.47 ± 0.20	-0.48 ± 0.14	-0.27 ± 0.25	0.06 n.s.	**1.30** *p*= 0.02	0.97 n.s.
Timing of V relative to SP	-0.48 ± 0.10	-0.51 ± 0.11	-0.42 ± 0.22	0.29 n.s.	0.68 n.s.	.51 n.s.

Significant difference indicted in bold font

Note: Increasing values of the SS and SP variables indicate greater physiological sleepiness. Lower values of the V and Temp measure indicate greater physiological sleepiness.

The striking and obvious finding in Table 4-3 is that the simultaneous (0 lagged) associations between the different circadian rhythms over the 80-hour protocol are similar between controls and DSWPD. However, the associations in the N24SWD group are all lower than in the other two groups. Results suggest that for N24SWD there appears a looser association between their different rhythms compared to DSPWD and controls.

Cross-correlation curves were then lagged to obtain the greatest correlation coefficients between all rhythms and calculate the relative phase-lag between behavioural and biological rhythms. This analysis is depicted in Table 4-4, showing that the interval with the greatest cross correlation between core temperature and feelings of subjective sleepiness in DSWPD patients and controls lagged by 0.39 and 0.96h respectively and were not significantly different. However,

N24SWD patients' subjective sleepiness rhythms preceded core temperature rhythms by 2.5h during the constant ultradian routine which was significantly different from the other two groups. Furthermore, controls' sleep propensity followed their core temperature rhythms by 0.94h while the timing of DSWPD patients' sleep propensity was 2.19h after their core temperature timing, a significantly greater delay than in the control group.

There were also significant differences in the timing of N24SWD patients' vigilance versus sleep propensity rhythms relative to DSWPD patients and controls. In DSWPD patients and controls vigilance preceded sleep propensity by approximately 0.5h while in the N24SWD patients vigilance occurred around 20min after the rhythm of sleep propensity. As would be expected, cross correlations with optimal phase lag produced higher correlations than without phase lag. Again, the N24SWD patients indicated lower cross correlations than the other two groups, indicating less synchrony between rhythms for the N24SWD group than the other two.

Table 4-4

Rhythm associations between core body temperature, subjective sleepiness, sleep propensity and vigilance from the 80h ultradian routine. Rhythm data was phase-lagged to obtain greatest cross-correlation coefficients for each participant. The table shows the group means of these maximised phase lags. Derived phase-lag differences between groups and highest associations between rhythms between for DSWPD, and N24SWD patients relative to controls.

	Means ± Standard Deviation			Mean Difference ± Standard Error Cohen's *d*		
Rhythm Associations	**Controls**	**DSWPD**	**N24SWD**	**DSWPD-Controls**	**N24SWD - Controls**	**N24SWD - DSWPD**
Timing of SS relative to Temp	0.39 ± 1.29h *r*=-0.62 ± 0.12	0.96 ± 2.25h *r*=-0.63 ± 0.11	-2.50 ± 2.89h *r*= -0.48 ± 0.11	0.57 ± 0.61h *d*= 0.30	**2.89 ± 1.11h** ***d*= 1.77**	**3.46 ± 1.07h** ***d*= 1.49**
Timing of SP relative to Temp	0.94 ± 1.43h *r*=-0.63 ± 0.09	2.19 ± 2.23h *r*=-0.64 ± .011	0.50 ± 2.08h *r*=-0.51 ± 0.09	**1.14 ± 0.61h** ***d*= 0.64**	0.56 ± 1.09h *d*= 0.35	1.69 ± 1.06h *d*= 0.76
Timing of V relative to Temp	0.00 ± 1.33h *r*=0.51 ± 0.14	0.54 ± 2.27h *r*=0.53 ± 0.13	-0.33 ± 2.52h *r*=0.44 ± 0.08	-0.54 ± 0.60h *d*= 0.28	-0.33 ± 1.23h *d*= 0.21	-0.87 ± 1.20h *d*= 0.38
Timing of SP relative to SS	0.44 ± 1.25h *r*=0.59 ± .11	0.04 ± 0.72h *r*=0.60 ± 0.13	0.75 ± 2.22h *r*=0.50 ± 0.09	0.41 ± 0.34h *d*= 0.41	0.71 ± 0.59h *d*= 0.22	0.31 ± 0.61h *d*= 0.71
Timing of V relative to SS	-0.44 ± 1.04h *r*=-0.55 ± 0.18	-0.31 ± 1.01h *r*=-0.56 ± .12	-0.33 ± 2.08h *r*=-0.36 ± 0.23	-0.14 ± 0.34h *d*= 0.13	-0.03 ± 0.67h *d*=0.09	0.11 ± 0.68h *d*=0.02
Timing of V relative to SP	-0.50 ± 1.04h *r*=-0.46 ± -0.52	-0.62 ± 0.85h *r*=-0.54 ± 0.09	0.33 ± 0.58h *r*=-0.46 ± 0.18	-0.12 ± 0.28h *d*= 0.13	0.83 ± 0.57h *d*= 0.84	**0.94 ± 0.56h** ***d*= 1.15**

Note. Significant differences in *r*-values between N24SWD patients and controls for Temperature – Subjective Sleepiness rhythm (p=.04) and Temperature – Sleep Propensity associations (p=0.02).
Increasing values of the SS and SP variables indicate greater physiological sleepiness. Lower values of the V and Temp measure indicate greater physiological sleepiness.

Since there were differences in *taus* between groups for the rhythm variables, the cross correlations across all 80 hours may be reduced by differential phase change between different rhythms arising from the different taus. Therefore, the same analyses were conducted during only the initial 40h of the ultradian routine to control for period length. Hourly melatonin data points from the first nocturnal period (i.e., start of ultradian routine to individual Dim-Light Melatonin Offset [DLMOff]) were also included in this analysis. This investigation enabled direct comparisons of phase angles for participants, without the effect of different rhythm lengths on the second and third night of the protocol. The results further supported the finding that DSWPD patients had a significantly greater interval between the timing of the core temperature rhythm and

sleep propensity rhythm compared to controls (please see Supplementary Table 4-6; Appendix H) that was the same trend as shown in Table 4-2 and 4-4.

The cross-correlation analyses indicate that melatonin and temperature rhythms were highly correlated in all three groups (r=0.72-0.83) with the temperature nadir occurring about an hour (r=0.75-1.8) after the salivary melatonin acrophase. Subjective sleepiness was highly correlated with both biological rhythms and sleep propensity, while vigilance best correlated with sleep propensity. Irrespective of their significantly longer biological *taus*, DSWPD patients had significantly greater lags in phase angles between Tmin and sleep propensity compared to controls on the first night of the ultradian routine. This trend appears during the two subsequent nights of the ultradian routine as illustrated in Figure 4-3 showing a generally more phase delayed sleep propensity rhythm relative to circadian temperature rhythm of the DSWPD group compared to the control group.

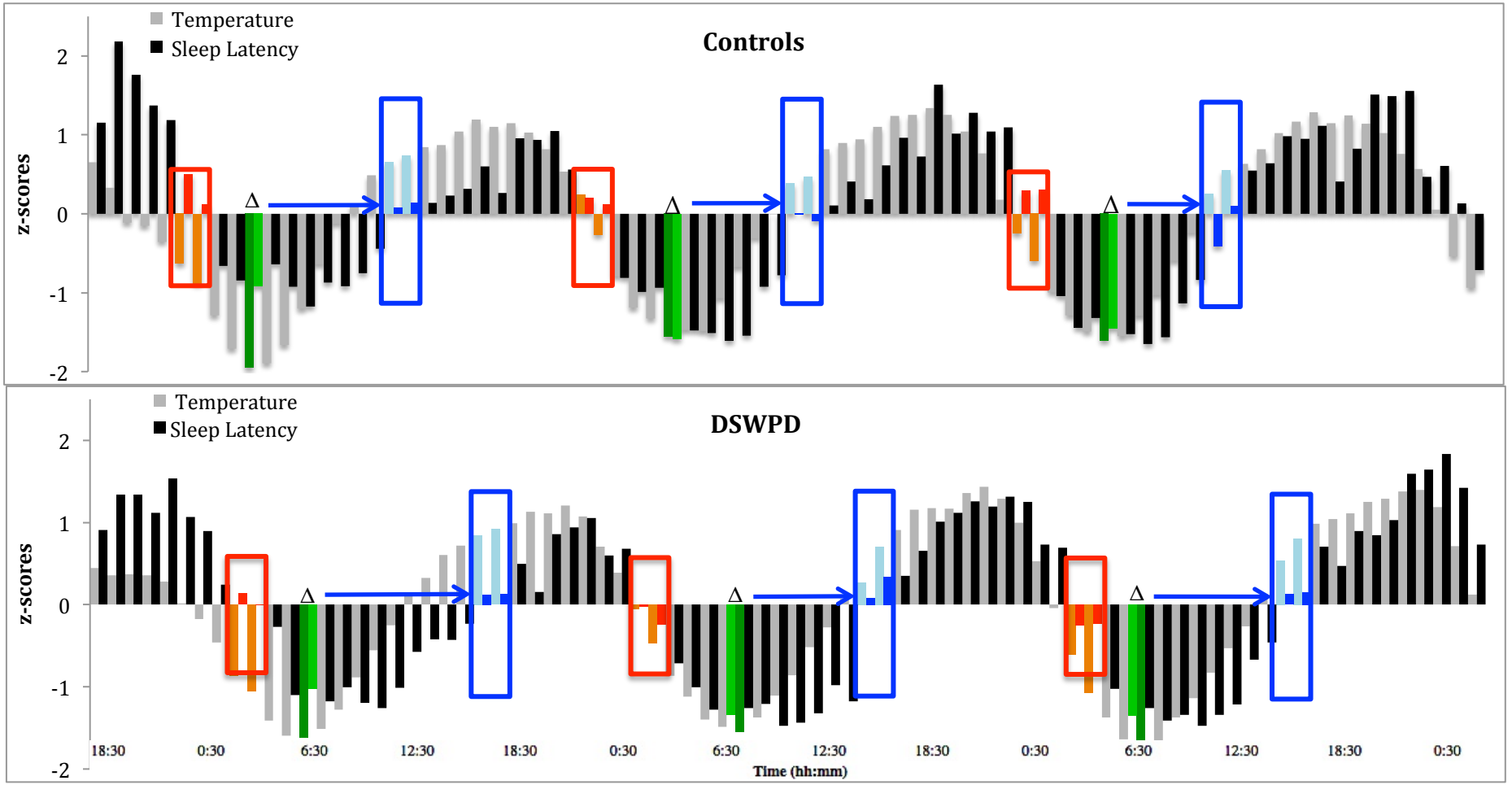

Figure 4-3. Mean 80-hour core temperature and sleep propensity data for DSWPD patients (bottom), and controls (top).
Note: Data was derived during the ultradian constant routine and converted to Z-scores to enable direct comparisons between the rhythms. 'Sleep Propensity' variable (i.e., amount slept) was converted to 'Sleep Latency' (i.e., time taken to fall asleep) to correspond with the core temperature rhythm. Sleep latency is presented with black vertical bars, core body temperature is presented with grey vertical bars. Red shorter rectangles indicate the descent toward the circadian nadir from the Wake Maintenance Zone (WMZ) and the larger blue rectangles the ascent from greatest sleep need toward maximum sleep latency/alertness (i.e., the approximate Wake-up Zone; WUZ). Tmin is depicted with green vertical bars and the Δ-symbol and the horizontal arrows indicate the relative phase angle between the timing of Tmin and ascent to maximum sleep latency/alertness. It can be seen in general (particularly in the first and third cycle) that the periods of rapid increase of sleep propensity (red boxes) and the re-emergence of alertness (blue boxes) are relatively (to temperature minima) phase delayed in the DSWPD group compared to the control group.

Visual inspection of the figure indicates difference in core temperature and sleep propensity phase angle of entrainment between DSWPD and controls during the first circadian cycle. In particular, there appeared a larger phase angle difference between core body temperature nadir and the approximate "wake-up zone" (WUZ). This occurs around the time of the circadian mesor, during the ascent from Tmin and the sleep period, typically between maximum sleep propensity and the early awakening Wake Maintenance Zone (WMZ) (i.e., minimum sleep propensity). To attempt to capture the approximate timing of this period, phase timings of the sleep propensity mesor were identified by visual inspection of the two-component cosine curve of best fit. This analysis was performed separately for each individual and their circadian cycle by separately curving 27-hours of data in each circadian cycle. The timings of each individuals' derived Tmins were subtracted from their sleep propensity mesor (SP mesor) in each cycle. This analysis, presented in Table 4-5, enabled measurement of individuals' phase angle of entrainment between Tmin and the approximate sleep propensity WUZs.

Table 4-5

Phase angle of entrainment between Core Body Temperature nadir (Tmin) and Sleep Propensity mesor (SPmesor) during three circadian cycles in an ultradian routine.

Tmin - SP mesor PAE	**Controls** M ± SD	**DSWPD** M ± SD	MD ± SE Cohen's *d*
Circadian Cycle 1	8.11 ± 2.64	10.34±2.62	2.23 ± 0.81 *d* = 0.85*
Circadian Cycle 2	8.33±2.41	8.33 ± 2.62	0.00 ± 0.91 *d* = 0.00
Circadian Cycle 3	6.61±2.20	8.36 ± 2.44	1.75 ± 0.75 *d* = 0.74*

* $p < 0.05$

The results of these analyses revealed that this Tmin-SPmesor interval was generally larger in DSWPD patients relative to controls. Particularly during the first cycle, this was a significant phase angle difference of 2.23±0.81 hours $t(42)=2.77$), $p= 0.008$. The difference in the third cycle was

also significant. Furthermore, Figure 4-4 indicates that the interval between Tmin-SPmesor on the first evening of the constant routine was significantly correlated with melatonin *tau* (r=0.31, p=0.04, n=48) and core body temperature *tau* (r=0.32, p=0.03, n=48).

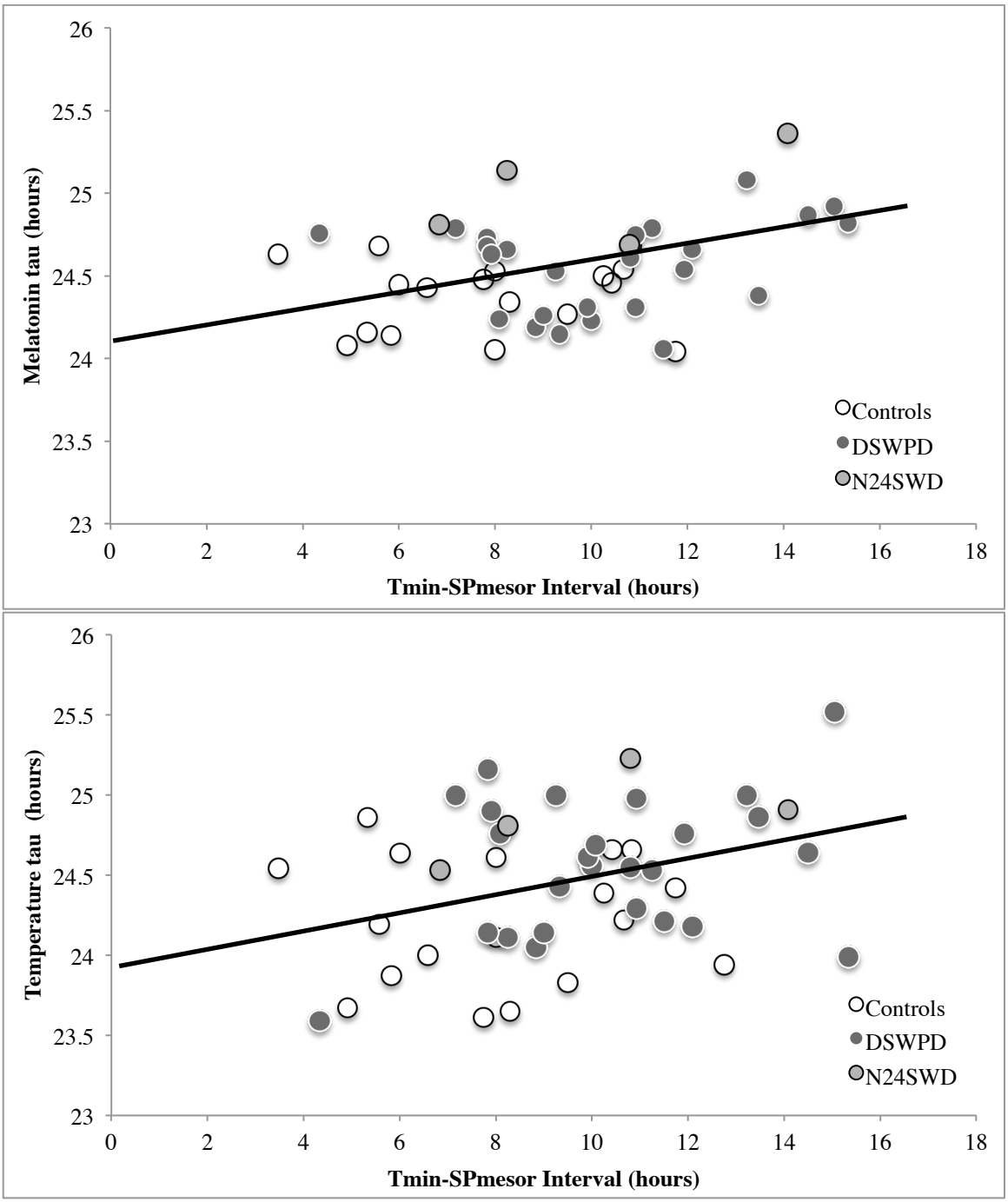

Figure 4-4. Core body temperature minimum (Tmin) to Sleep Propensity mesor (SP mesor) interval during the first circadian cycle of the ultradian constant routine correlated with melatonin (upper figure) and temperature *taus* (lower figure).

In a final analysis, Pearson's correlation coefficients were generated to assess associations between biological and behavioural rhythm phase markers in the first 24 hours of the constant routine with participants' typical sleep timing (i.e., during the baseline week of sleep/wake ambulatory monitoring immediately prior to the laboratory session). Table 4-5 confirms that the phase markers of all biological and behavioural rhythms were significantly associated with mid-sleep on free days and workdays in the week prior to the start of the constant routine. As expected, phase markers were more strongly associated with mid-sleep on free days (i.e., when participants could sleep at their preferred times) compared to work days. In general there were stronger correlation coefficients with patients' sleep times in the week prior to the constant routine compared to biological rhythms.

Table 4-6

Relationships between mid-sleep timing and phase markers of core body temperature (Tmin), melatonin (DLMO), subjective sleepiness (SS Max), sleep propensity (SP Max) and vigilance (V Min).

	Mid-Sleep Average	Mid-Sleep Free Days	Mid-Sleep Work Days
		DSWPD	
Tmin	***r* = 0.68** $p < 0.001$	***r* =0.79** $p < 0.001$	***r* = 0.39** $p = 0.04$
DLMO	***r* =0.49** $p < 0.001$	***r* =0.42** $p = 0.03$	$r = 0.32$ $p = 0.12$
SS Max	***r* =0.65** $p < 0.001$	***r* = 0.49** $p = 0.01$	***r* = 0.48** $p = 0.01$
SP Max	***r* =0.72** $p = 0.001$	***r* = 0.57** $p < 0.01$	***r* = 0.44** $p = 0.02$
V Min	***r* =0.71** $p < 0.001$	***r* = 0.49** $p = 0.01$	***r* = 0.44** $p = 0.02$
		Controls	
Tmin	$r = 0.38$ $p = 0.15$	$r = 0.31$ $p = 0.22$	***r* = 0.43** $p = 0.09$
DLMO	$r = 0.22$ $p = 0.39$	$r = -0.28$ $p = 0.29$	$r = 0.05$ $p = 0.86$
SS Max	***r* = 0.60** $p = 0.01$	***r* = 0.70** $p = 0.001$	***r* = 0.25** $p = 0.33$
SP Max	***r* = 0.71** $p = 0.001$	***r* = 0.73** $p = 0.001$	***r* = 0.55** $p = 0.02$
V Min	***r* =0.49** $p = 0.04$	***r* = 0.59** $p = 0.01$	$r = 0.19$ $p = 0.46$

Significant difference (p<0.05) indicated in bold font

4.4 Discussion

In this modified ultradian constant routine we aimed to thoroughly examine circadian rhythms of core body temperature, melatonin, subjective sleepiness, sleep propensity and vigilance in DSWPD, N24SWD patients and controls. This investigation was necessary to further elucidate how behavioural choices in sleep time, thus the phase angle of entrainment (PAE) differences in the two CRSWD may contribute to their sleep disturbances. In general, N24SWD patients showed the longest overall circadian *taus*, followed by DSWPD patients. Controls exhibited *taus* that were closest to the 24h light/dark cycle. DSWPD and N24SWD patients have significantly longer core temperature and melatonin *taus* relative to controls. However, contrary to the proposed hypotheses,

there were no significant between-group differences in *taus* of behavioural rhythms as measured by subjective sleepiness, objective sleepiness and vigilance.

DSWPD patients did exhibited greater phase lag of sleep propensity rhythms relative to the core temperature rhythm. The sleep propensity rhythm lagged by 1.14h later than controls across the 80-hour ultradian protocol. In particular, they showed a phase angle difference between core body temperature nadir and minimum sleep propensity during the first circadian cycle upon entry to the constant routine. Hence it seems that alterations in phase angle differences of sleep propensity and core body temperature in DSWPD could also contribute to patients' stronger tendency to phase delay their sleep period.

It has been established that the circadian period length (*tau*) cycle at a period length that is just slight over 24-hours in good sleepers (Czeisler, 1981). Hence, it is unsurprising that controls in the present study also showed biological *taus* greater than a conventional day. Since all groups showed Mτ and Tτ lengths significantly longer than 24-hours, it may be reasoned that this difference between the conventional day and circadian rhythm would cause delays even in controls' sleep timing. However, the control participants with circadian *taus* >24-hours remain synchronised and in phase with the 24-hour world presumably with little trouble. This may be entirely the result of them getting sufficient exposure to entraining effects such as exposure to light in the mornings that would 'put the brakes on' their tendency to phase-delay (Duffy, Rimmer, & Czeisler, 2001).

In addition to longer biological *taus*, if sleep propensity rhythms of DSWPD patients were delayed relative to core temperature rhythms, it would further contribute to DSWPD patients' difficultly to remain entrained. Exposure to light at the correct time in the morning has been shown to entrain circadian rhythms and sleep/wake cycles of people with DSWPD (Watanabe, Kajimura, Kato, Sekimoto, & Takahashi, 1999; Zeitzer, Dijk, Kronauer, Brown, & Czeisler, 2000). Overall longer biological *taus* in DSWPD patients will prolong feelings of alertness and energy in the evenings. This is indicated by not only their typical evening preference on the Morningness-

Eveningness Questionnaire (Abe et al., 2011; Horne, & Ostberg, 1976; Mongrain, Carrier, & Dumont, 2006) but also the delayed timing of sleep propensity rhythms of DSWPD patients in the present study, by more than an hour more compared to controls. The delay in sleep propensity rhythms suggests that patients with DSWPD have a greater tendency to fall back asleep at a time they should awaken, thus masking the beneficial effect of light exposure in the morning. Hence, when they eventually awake, they obtain exposure to light after the time that would prove most effective in advancing circadian rhythms (Watanabe et al., 1999; Zeitzer et al., 2000). Coupled with an endogenous preference for chronically late bedtimes and a tendency to sleep-in in the mornings, circadian rhythms of DSWPD patients will tend to drift later, or phase delay, resulting in sleep difficulty and a delayed sleep/wake pattern.

Objective sleepiness is an important factor in determining sleep time based on both psychological and physiological states. Unlike subjective sleepiness that refers to how sleepy or alert an individual is feeling, objective sleepiness is a measure of a person's ability to fall asleep. There is evidence to suggest that DSWPD patients feel heightened arousal in the late evening and may defer their relative bedtime for various reasons (Dagan & Eistenstein, 1999; Gradisar et al., 2011; Richardson, Gradisar & Barbero, 2015, Wilhlemsen et al., 2012). It is plausible that patients are reluctant to cut short a generally positive experience in the evening or experience negative cognitions that preclude them from sleeping thus deferring their bedtime, particularly on free-days. The choice of a much later bedtime and wake-up time would result in the delay of morning light exposure. Delayed choice of bedtime leads to later awakenings and further delays of the circadian rhythm (Burgess & Eastman, 2006; Taylor, Wright, & Lack, 2008; Yang & Spielman, 2001).

Our findings provide evidence for this notion and suggest that DSWPD patients obtained less sleep prior to their temperature nadir compared to controls and maintain a stronger sleep propensity need after their temperature nadir. Habitual sleep data on Table 4-1 indicates that DSWPD patients awaken at their maximum sleep propensity on work-days and sleep-in well past this period on free-

days. Since their maximum need for sleep co-occurs with their required wake-up time, it explains patients' frequent reports of chronic difficulty awakening in the morning to meet daytime commitments (Lack & Wright, 2007; Wyatt, 2004). It also explains the tendency for DSWPD patients to over-sleep on free-days. Although the longer sleeps are recuperative to catch up on the sleep lost throughout the workweek (Crowley & Carskadon, 2010; Taylor, Wright, & Lack, 2008), it also enables patients to sleep-in past the period of their maximum sleep need. This masks exposure to light at the optimal time for the circadian system to phase-advance and remain entrained (Khalsa et al., 2003; Lewy, Ahmen, Jackson & Sack, 1992; Watanabe et al., 1999; Zeitzer et al., 2000). Hence coupled with an endogenous preference for chronically late bedtimes and a tendency to stay up late in the evenings, DSWPD patients' circadian rhythms will drift later, resulting in sleep difficulty and a delayed sleep/wake pattern.

In the present study, phase markers of all measured rhythms were significantly correlated with individuals' sleep timing, and particularly on 'free-days' when individuals could self-select their sleep and wake times. This suggests that although *taus* contribute to the sleep delays in DSWPD patients, behavioural and sleepiness factors play a role in patients' tendency to delay their sleeping patterns. Our results further question assumptions that DSWPD is purely a circadian-based disorder and give support to literature advocating phase angle differences between DSWPD and controls (Ozaki et al., 1996; Shibui, Uchiyama, Okawa, 1999; Uchiyama et al., 2000a).

The results could also be relevant to the psychosomatic development of the sleep disturbance (Dagan & Eistenstein, 1999; Gradisar et al., 2011; Richardson, Gradisar & Barbero, 2015, Wilhlemsen et al., 2012). For example, a delay in the accumulation of greater sleep propensity per circadian phase in the evenings, could lead to DSWPD patients' experiences of difficulty initiating sleep than controls at comparable circadian phases. These experiences of sleep onset difficulties would discourage them from going to bed at a comparable circadian phase and contribute to their behavioural decision to stay up later. Patients could develop resultant sleep onset insomnia from

repeated attempts to sleep at times when sleep propensity has not sufficiently increased (Lack & Wright, 2012).

Circadian rhythm timing is subject to the effect of light (Duffy & Wright, 2005; Wright et al., 2013). Therefore choices in bed- and rise-times that largely determine individuals' exposure to visual stimulation, could contribute to circadian misalignment in both DSWPD and N24SWD patients. It is important to note that the subjective and objective sleepiness rhythms are not the same as the self selected monophasic sleep period in the approximate 24-hour sleep/wake cycle. They are simply the rhythms of sleepiness with sleep distributed across 24 sleep opportunities each day. As Dijk & Schantz (2005) note, the sole contribution of neither the circadian oscillator nor the homeostatic drive can predict individuals' sleep and performance - only the interaction of both. Therefore the controlled, dimly lit, confined-nature of our ultradian study limits our ability to interpret how behavioural and external factors (e.g., social influences, choice of bedtime) affect circadian timing. In naturalistic environments, we predict that these external environmental factors would only exacerbate the significant sleep propensity and core temperature phase-lags.

Perhaps Campbell and Murphy's (2007) free-running study better reflects the consequence of self-selected sleep on circadian *taus*. Free-running studies permit patients to choose their bed- and wake-times at their discretion while remaining in typically 30-50lux environments. This light intensity can have circadian effects (Crowley et al., 2015). Therefore, if DSWPD patients have heightened night-time arousal (Dagan & Eisenstein, 1999; Gradisar et al., 2011) they could be inclined to choose later bedtimes. This would further postpone the exposure to light and exert pressure on circadian rhythms to delay in relation to the self-selected sleep period. In turn, the outcome measure of *taus* will be longer but partly a result of daily delays induced by delayed light exposure. This may account for the longer measured *taus* for the control and DSWPD participants in the Campbell and Murphy (2007) study compared to the respective *taus* for those groups in our study. Participants in our study experienced very dim and constant light exposure, therefore cyclic

changes in phase are most likely due to the SCN's natural drift and no other factor. Perhaps the Campbell & Murphy (2007) results reflect how the combination of longer circadian *tau* plus relatively phase-delayed sleep propensity rhythm contributes to DSWPD sleep delays in more natural environments.

Similarly, these findings could explain inconsistencies in literature pertaining to PAE in DSWPD. Ozaki et al., (1996) proposed that DSWPD patients have significantly longer intervals between circadian phase (e.g., DLMO/Tmin) and sleep timing (i.e., sleep offset; Shibui, Uchiyama, Okawa, 1999; Uchiyama et al., 2000a). However, empirical literature is divided with evidence also showing similar phase angles of entrainment between controls and DSWPD patients (Wyatt et al., 2006; Chang et al., 2009). The prolonged phase angle in DSWPD relative to controls, particularly with sleep offset, could be the result of longer sleep durations (Ozaki et al., 1996), which in itself is also not a consistent finding (Saxvig et al., 2013). However, it could also relate to methodology used to assess circadian markers and PAE.

In studies that do not find phase-angle differences (Chang et al., 2009; Wyatt et al., 2006), measurements of DLMO are taken in conventional dimly lit environments following ambulatory monitoring of habitual sleep, thus the lighting is controlled during phase assessment. However, investigations that reveal a phase-angle difference consist of simultaneous habitual sleep and circadian phase monitoring at home for ~2 weeks, or phase assessments in laboratory settings after a night of sleep deprivation (Shibui, Uchiyama, & Okawa, 1999; Uchiyama et al., 2000b; Ozaki et al., 1996). Given the present study also indicates phase angle differences between sleep propensity and core temperature, certainly disparities in methodology may explain the inconsistent findings.

Furthermore, in the present study, the findings of DSWPD patients do not extend to N24SWD patients who did not show significant differences in phase angles of entrainment in behavioural rhythms relative to core temperature. The N24SWD patients showed longer vigilance rhythms in addition to their longer biological *taus* and, interestingly, their subjective sleepiness rhythms

preceded all other rhythms during the constant routine. Hence, our results indicate that in time-free environments, significantly longer core temperature *taus* of N24SWD patients oscillate at a different pace to the near-24 hour behavioural rhythms. This does not support the scarce circadian-based literature on N24SWD that suggests patients have a significantly shorter PAE interval of sleep onset to temperature nadir, as well as a significantly longer PAE interval between temperature nadir and sleep offset (Uchiyama et al., 2000a; Uchiyama et al., 2002).

The finding in Table 4-3 indicates lower associations in the N24SWD group compared to the DSWPD and control groups' significant associations in rhythms. This suggests a weaker connection or some degree of dissociation between rhythms in the N24SWD group that allows them greater latitude to free run their sleep/wake cycle. Perhaps, the free-running sleep/wake cycles of these patients can be likened to permanent jetlag and constant changes to 'time-zones' such that endogenous rhythms cannot stabilise to any given time and become entrained. Individuals who travel across multiple time zones can entrain to their new environment within a few days using light as a predominant zeitgeber. However, it appears that the peripheral clocks take longer to become entrained and will adapt at different rates (Yamazaki et al., 2000) causing some dissociation between various rhythms. Different adaptation rates of the peripheral clocks can affect individuals' mood and well-being (Wirz-Justice, 2006). Therefore, if the SCN in N24SWD patients struggles to synchronise with the natural light/dark cycle due to instability in their sleep/wake cycles and circadian rhythms, they may have ongoing endogenous circadian rhythm de-synchronisation in their peripheral clocks.

By measuring core body temperature, subjective sleepiness and sleep propensity in healthy individuals, Gradisar and Lack (2004) found that core temperature minimum preceded maximum subjective sleepiness that was then followed by maximum objective sleepiness. Hence, when core body temperature begins to decrease, individuals start to feel sleepy and initiate sleep soon after the onset of those sleepy feelings. These results suggest that although DSWPD patients exhibit longer

period lengths and differences in phases, the three rhythms are comparably synchronised in both groups. However, in the 80-h constant routine, the N24SWD biological rhythms of core temperature appear to decouple and take on different phases relative to the sleepiness and behavioural rhythms.

In early studies of human *tau* in good sleepers (Czeisler et al., 1999; Kripke et al., 2005) and during periods of extended isolation from zeitgebers, the sleep/wake cycle and the endogenous biological phase markers start to decouple. Endogenous circadian rhythms continue oscillating every 24-25 hours, whilst the sleep/wake cycle decouples and initiates a longer rhythm of 28- to 36-hours or shorter rhythm (18-20 hours), depending on individual differences (Gander et al., 1984; Strogatz, Kronauer, & Czeisler, 1987). Although this decoupling is disputed by some researchers, these findings suggest that it is possible that biological circadian rhythms and sleep/wake cycles can dissociate and perhaps oscillate at their own pace.

4.4.1 Conclusions

Undoubtedly the relationships between the biological basis, behavioural tendencies and environmental factors in CRSWD are complex. The results of the present study may begin to bridge the gap in literature between DSWPD being identified as an exclusively physiological phenomenon and one that has external perpetuating bases. Furthermore, results in N24SWD patients suggest that the dis-entrainment of individuals' habitual sleep-wake cycles could lead to some internal de-synchrony of biological, sleepiness and behavioural rhythms. Perhaps it is due to these misalignments in sleep and circadian timing that behavioural strategies enforcing strict bed and rise times with appropriate light administration show greatest benefits to consolidating and advancing, delayed sleeping patterns (Saxvig et al., 2013; Sharkey et al., 2011).

CHAPTER 5 MELATONIN PROFILES

Nocturnal melatonin profiles in patients with Delayed Sleep Phase Disorder and control sleepers

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Author Contributions

GM organized and carried out experiments, contributed to data analysis, wrote the manuscript and assisted with the primary study design. LL and NL contributed to recruitment, organization of all experiments, data analysis, and drafted the manuscript. SF and HB also drafted the final paper, and along with LL, designed the overall investigation. MG contributed to data analysis, assisted with the primary study design and drafting the manuscript. DK contributed to assaying melatonin samples, assisted with data analysis and drafting the manuscript.

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5.1 Abstract

A significant delay in the timing of endogenous circadian rhythms has been associated with Delayed Sleep-Wake Phase Disorder (DSWPD). More recently other mechanisms have also been proposed to account for this disorder. To further explore the aetiology of DSWPD, the present study compared nocturnal melatonin profiles of 26 DSWPD patients (18m, 8f, age: 21.73±4.98 years) and 17 normally-timed good sleepers (10m, 7f, age: 23.82±5.23 years) in a time-free, dim-light (<10 lux), laboratory environment. A 30-hour modified constant routine with alternating 20-minute sleep opportunities and 40-minutes of enforced wakefulness was used to measure the endogenous melatonin circadian rhythm. Salivary melatonin was sampled half-hourly between 1820h-0020h and then hourly from 0120h until 1620h. DSWPD patients had significantly later timed melatonin profiles that were delayed by approximately 3 hours compared to normal sleepers, and there were no notable differences in the relative duration of secretion between groups. However, melatonin secretion between dim light melatonin onset (DLMO) and acrophase was less prominent in DSWPD patients compared to good sleepers who showed a more acute initial surge of melatonin following the DLMO. Although the regulatory role of melatonin is unknown, abnormal melatonin profiles have been linked to psychiatric and neurological disorders (e.g., major depression, obsessive compulsive disorder, Parkinson's disease). These results therefore suggest that in addition to a delayed endogenous circadian rhythm, a diminished initial surge of melatonin secretion following DLMO may contribute to the aetiology of DSWPD.

5.2 Introduction

A delay in circadian rhythm timing is currently assumed to be a significant contributing factor for the aetiology of Delayed Sleep-Wake Phase Disorder (DSWPD). By measuring physiological phase markers, such as core body temperature and melatonin, past empirical studies have identified a 2-6 hour delay in the timing of circadian rhythms of patients with DSWPD compared to those who habitually sleep at conventional times (Oren, Turner, & Wehr, 1995; Ozaki et al., 1996; Saxvig et al., 2013; Shibui, Uchiyama, & Okawa, 1999; Uchiyama et al., 2000a). These phase markers have been shown to correspond with the sleep/wake pattern, where early sleepers have early-timed phase markers and late sleepers have later-timed circadian phases (Duffy, Rimmer, & Czeisler, 2001; Mongrain et al., 2004). Other pathophysiological changes observed in DSWPD include an abnormally slower circadian oscillator as indicated by a longer period length (Campbell & Murphy, 2007; Micic et al., 2013; Regestein & Monk, 1995; Weitzman et al., 1981) and an abnormal phase response curve to light, including a decreased ability to phase advance from light following the sleep period (Czeisler et al., 1981; Ozaki et al., 1996) and/or an increased phase delay effect to evening light (Aoki, Ozeki, & Yamada, 2001).

Although animal, and a few human studies indicate an influence of melatonin secretion on physiological, immunological, medical and psychiatric disorders, the regulatory role of this hormone is not conclusively established (Bolitho et al., 2014; Karasek & Winczyk, 2006). Delays in melatonin secretion are associated with delays in sleep patterns of DSWPD patients and exogenous melatonin can adjust the timing of circadian clocks (Burgess et al., 2010; Lewy et al., 1992; Lewy et al., 2001). In terms of sleep disorders, effects of overall amounts of nocturnal melatonin secretion and sleep have been researched in sleep disturbances such as insomnia, but not in Circadian Rhythm Sleep-Wake Disorders (CRSWD; Mahlberg, Tilmann, Salewski, & Kunz, 2006; Myers & Badia, 1995; Zeitzer et al., 1999). Sleep disturbances and low levels of melatonin are highly prevalent in the elderly (Haimov et al., 1994; Zisapel, 2005). However, it is unclear if lower melatonin levels in

elderly patients with sleep disorders are observed simply due to a spurious relationship with age (i.e., melatonin decreases with age and sleep disturbance is more prevalent in elderly; Mahlberg et al., 2006; Zeitzer et al., 1999). Nevertheless, reductions in melatonin secretion have also been found in young adulthood (i.e., 20-30 years of age), during a period in the lifespan where DSWPD is most prevalent (Kennaway et al., 1999). These findings suggest melatonin secretion factors other than just the timing may affect sleep parameters and lead to sleep disorders. For example, absolute melatonin secretion and the nocturnal profile of secretion may also be linked to sleep. However, this has not been comprehensively researched in DSWPD patients and was the aim of the present study.

Melatonin has been investigated in DSWPD patients across the sleep period and significant delays in melatonin phase timing were correlated with the sleep/wake patterns of DSWPD patients compared to controls (Shibui, Uchiyama, & Okawa, 1999). The midpoint of melatonin secretion, as well as Dim Light Melatonin Onset (DLMO) and Dim Light Melatonin Offset (DLM^{Off}) times were delayed in DSWPD patients, but the duration of secretion did not differ compared to controls. In a more recent finding, DSWPD patients appear to have a slower rate of increase in saliva melatonin levels compared to controls, in addition to the later DLMO (Rahman, Kayumov, Tchmoutina, & Shapiro, 2009). However, since the objective in this study was investigated from partial DLMO sampling (i.e., 19:00 h – 03:00 h) data were not available to determine whether there was an extended period of melatonin secretion following acrophase of melatonin concentration. Furthermore, the apparent slower rate of increase in mean melatonin curves could have arisen by greater variance of DLMO times in the DSWPD sample. Therefore, it would be important to further investigate the rate of increase to acrophase, timing of acrophase, and rate of decrease from acrophase. The present study examined differences in overall melatonin secretion profiles between adults diagnosed with DSWPD and conventionally timed good sleepers. This should contribute to the limited knowledge currently available about the aetiology of the disorders and, in turn, may aid

in developing a diagnostic profile for the circadian rhythm disorders and improve treatment effectiveness.

5.3 Materials and Methods

5.3.1 Participants

Forty-three participants were selected for a laboratory-based study at the Flinders University Sleep Laboratory. Samples comprised 26 patients diagnosed with DSWPD (18m, 8f, age: 21.73±4.98 years), and 17 normally-timed good sleepers (10m, 7f, age: 23.82±5.23 years). There were no significant differences in age, $t(41)=1.32$, $p=0.19$, or gender, $X^2\,(1, N=43) = 1.167$, $p=0.28$, between the groups. The selection process occurred in two phases. Phase one screening consisted of a questionnaires battery assessing lifestyle, current sleeping patterns and sleeping preferences. Phase two screening confirmed sleeping patterns using a prospective 7-day sleep diary and actigraphy monitoring. These measures were used to assess DSWPD criteria according to the ICSD-3 (AASM, 2014). To confirm eligibility, responses and logs were further analyzed by the senior author (LL) who has more than 20 years of clinical and research experience with Circadian Rhythm Sleep-Wake Disorders (CRSWD).

5.3.2 Phase One Screening

An initial sample of 497 interested candidates responded to poster advertisements posted at major university campuses in Adelaide, as well as online websites and through word of mouth. Informed consent was obtained from participants prior to their involvement in both the screening and laboratory phases. Ethics approval for the experiment was granted by the Southern Adelaide Clinical Human Research Ethics Committee. All respondents completed screening phase one to determine their eligibility as either a normal sleeper or having DSWPD. The test battery was administered as an online survey (SurveyMonkey.com) and consisted of a semi-structured General Health and Medical Questionnaire interview, Munich Chronotypes Questionanaire (MCTQ;

Roenneberg, Wirz-Justice, & Merrow, 2003; Roenneberg et al., 2004; Zavada et al., 2005), Horne and Östberg's Morningness-Eveningness Questionnaire (MEQ; Horne & Ostberg, 1976; Zavada et al., 2005), Pittsburgh Sleep Quality Index (PSQI; Buysee et al., 1989), DSWPD-Sleep Timing Questionnaire (DSWPD-STQ) developed by the authors, Sheehan Disability Scale (SDS; Arbuckle et al., 2009; Leon et al., 1997) and Depression Anxiety Stress $Scale_{21}$ ($DASS_{21}$) (Antony, Bieling, Cox, Enns, & Swinson, 1998; Brown, Chorpita, Korotitsch, & Barlow, 1997b; Lovibond & Lovibond, 1995a; Lovibond & Lovibond, 1995b).

5.3.2.1 Inclusion/Exclusion Criteria.

To meet criteria for DSWPD, respondents had to score <42 on the MEQ (i.e., be evening-types; Rahman et al., 2009), and report daytime dysfunction and lifestyle impairments as a result of their sleeping pattern on the SDS. DSWPD patients were required to report scores ≥4 (range 0-10) on any given SDS subscale, as this is the cut-off indicative of significant impairment and dysfunction. The overall score and individual responses from the PSQI were used to screen for other sleep disturbances. Otherwise, in line with ICSD-3 criteria, those with DSWPD were healthy good sleepers when sleeping at their habitual delayed time (AASM, 2014). Participants had to indicate delayed sleep patterns of at least 2 hours in contrast to 'conventional' sleep times (i.e., sleep onset later than 0200h), and who were unable to fall asleep at a preferred earlier time according to the DSWPD-STQ (AASM, 2014).

The SDS was used to select normally-timed good sleepers and identify the absence of any impairment in functioning at work/school, social and family life as a result of their sleep pattern. Normal sleepers indicated little to no sign of distress or daytime dysfunction associated with their sleep patterns (scores ≤2 on all three SDS items); a PSQI score <6 (Buysse et al., 1989); conventional sleep times (i.e., sleep onset = 2200h-2400h); and neither morning nor evening type (MEQ score between 42-58). A qualified clinician (LL) confirmed the suitability of good sleepers.

5.3.2.2 DSWPD – Sleep Timing Questionnaire (DSWPD-STQ).

The DSWPD-STQ was developed to assess subjective feelings regarding symptoms of DSWPD and evaluate circadian misalignment. The authors aimed to tap into patients' subjective impressions of their current sleep pattern by asking potential patients to: *"Please indicate the time when your "body clock" currently allows you to fall asleep most easily", "How long do you think it would take you to fall asleep at this time?", "How difficult would you find it to fall asleep at this time?", "Please indicate the time when your "body clock" currently allows you to wake up most easily?", "How difficult do you find it to wake up at this time?"*. To obtain an impression of circadian misalignment, respondents were then asked to disregard their "body clock" and only consider daytime obligations and social commitments, for questions including: *"Please indicate the time when you would PREFER to fall asleep most easily?", "With your CURRENT sleeping pattern in mind, how long do you think it would take you to fall asleep at this PREFERRED time?", "With your CURRENT sleeping pattern in mind, how difficult do you think it would be to fall asleep at this PREFERRED time?"*. A single measure of motivation was also assessed by asking patients to *"please indicate how motivated you are to change the timing of your CURRENT sleeping pattern to your PREFERRED?"*. Questions regarding difficulty to fall asleep/wake-up were measured on a 5-point Likert-type scale 0="Not at all" 4="Extremely".

Participants were not on medication (i.e., sleep/OTC medication, herbal remedies likely to affect sleep, alertness, melatonin and circadian rhythms). Body Mass Index (BMI) was not outside normal range of <18 and >32 kg/m^2. Participants smoked <1 cigarette/day, consumed an average of <250mg caffeine/day and averaged <14 standard drinks/week of alcohol consumption. Exclusion criteria also included history of substance abuse in the last 6 months, pregnancy or lactation and transmeridian travel (≥2 time zones) in the last 2 months. Suitable candidates indicated no psychiatric disorders in the past 12 months and night shift work in the last 2 months. Female participants were either in the follicular phase of their menstrual cycle during in-lab data collection,

or used a form of hormonal control (i.e., Etonogestrel implant or the contraceptive pill). Upon completion of the study, participants received monetary compensation proportional to the time spent in the study and AUD$500 for full involvement.

5.3.3 Phase Two Screening

During a week of screening and an additional week before the laboratory phase, participants kept a sleep/wake diary and wore a Mini Mitter Actiwatch, AW64 (Philips Respironics, Pensacola, FL) activity motion monitor. Actiware 5 software was used to download and process recorded data that were used to assess diligence in keeping the sleep diary. Recorded actigraphy data were collected in 30-second epochs and analysed both manually, to insert the 'rest' interval, then with the actiware-sleep scoring algorithm. The starting point of the 'rest' interval was set at the time of reduced activity, by visually inspecting actigraphy data in conjunction with the sleep diary. The ending point was set at the time of increased activity where it coincided with reports on the subjective sleep diary. Measures obtained from actigraphy analyses included bedtime, get-up time, time in bed, total sleep time, onset latency, sleep efficiency and wake after sleep onset. These measurements were further used to confirm the sleep pattern of potential candidates and monitor compliance with a regular sleep/wake schedule before participation in the laboratory session.

5.3.4 Outcome Measures

5.3.4.1 Melatonin Radioimmunoassay (RIA).

Saliva was collected using Salivettes®, (Cat # 51.1534; Sarstedt Australia Pty. Ltd. Mawson Lakes, South Australia) while the subjects lay in a supine position in bed, in dim light (<10 lux) to minimise the masking effects of physical movement and light on endogenous melatonin production. Food and water were consumed only after saliva collection to reduce contamination or dilution of the sample. Participants were instructed to place the swab in their mouth and accumulate saliva for 2 minutes. After collection, samples were stored frozen at -20°C. For analysis, samples were

thawed and centrifuged for 10 minutes at 2500 rpm, the swabs removed from the casing and the supernatant retained. A sensitive (4.3 pM) direct radioimmunoassay (RIA) using reagents from Buhlmann Laboratories AG, Allschwil, Switzerland (Voultsios, Kennaway, & Dawson, 1997) was used to measure melatonin in the saliva. Intra assay co-efficient of variation was always < 10%. Inter assay co-efficient of variation was 15% at 100pM.

5.3.5 Experimental Procedure

This study used the first 30 hours of a larger 80-hour modified-constant routine study and involved saliva collection between 18:20 h and 16:20 h the following day. Samples were collected half hourly between 18:00 h – 00:00 h and hourly thereafter. Laboratory sessions occurred between April and August of 2012 to 2014 (i.e., Southern Hemisphere winter time). The entire 80 h experiment commenced at 18:00 h, Thursday afternoons and ceased at 02:00 h on Monday mornings. This schedule was standardised across every experimental session. All participants attended the Flinders University Sleep Laboratory for a 1-hour meeting, 1 week prior to their scheduled laboratory session to familiarise themselves with the environment they would occupy during the experimental session and the procedures of the protocol (e.g., their rooms, snacking, salivettes and saliva collection).

Participants were instructed to maintain their typical sleep pattern during the seven days prior to their assigned laboratory session. Twenty-four hours ahead of the study, participants were asked to refrain from strenuous exercise, napping or consumption of alcoholic or caffeinated beverages. Compliance to these instructions was monitored by a 7-day sleep/wake diary and actigraphy, provided at the familiarisation meeting. On the day of admission to the laboratory, participants were instructed to have their last meal at 13:00 h and attend before 16:00 h. Consumption of cheese, bananas, chocolate, and tomatoes prior to commencement of and during the laboratory session was not allowed since their intake has been said to perturb spontaneous release of salivary melatonin (Peuhkur, Sohvola, & Korpela, 2012; Voultsios, Kennaway, & Dawson, 1997).

Upon arrival, participants were familiarised and set up for the experimental procedures, shown to their separate bedrooms at 1730h and permitted to habituate to their new environment but were instructed to remain awake. Two or three participants typically resided in the sleep laboratory for any one laboratory session. The room remained in almost complete darkness (i.e., <1 lux) during sleep opportunities and was dimly lit at a maximum of 10 lux at the participant's head position during enforced wakefulness. A constant room temperature of 20ºC was maintained across the protocol.

5.3.5.1 Laboratory Sessions

The protocol formally commenced with a 20-minute sleep opportunity at 1800h. Each hour, these 20-minute sleep opportunities alternated with 40 minutes of enforced wakefulness until the conclusion of the experiment, to simulate sequential 1-hour 'days'. During their laboratory stay, participants followed 1-hour ultradian routine whilst residing in a sound attenuated room, free from zeitgebers (i.e., no time indicators such as windows, mobile phones, radios, TV and clocks). Whilst in their room, participants were required to remain in bed and maintain a near supine position, with head and shoulders sufficiently elevated to enable ease of reading, writing etc. This postural position was also maintained during sleep opportunities. Toilet breaks were the only exception when participants were allowed to leave the bed and use normal toilet facilities. Basic hygiene activities like toilet opportunities and brushing teeth (with water only) were kept to a minimum and only permitted when necessary. These occurred near the end of enforced wakefulness intervals, once saliva samples had been collected. During the 40-minute wakeful intervals between sleep opportunities, participants were allowed to engage in sedentary activities such as reading, studying, listening to music, watching DVDs and casual conversations with researchers, to remain awake and occupy free time.

This environment eliminated the influence of time cues on feelings of sleepiness and alertness. Participants were blind to the duration of sleep and wakefulness periods to further ensure

their states of sleepiness remained unaffected by external factors or estimates of clock time. Saliva samples were collected immediately after sleep opportunities (i.e., at 20-minutes past the hour) and again 50-minutes past the hour, between 1620h-0020h.

Every second hour, participants were given an opportunity to drink 200mL of water and consumed a small equi-caloric meal. Snack opportunities occurred 30 minutes past the hour and 10 minutes after initial saliva sample collection.

5.3.6 Statistical Analysis

Since salivary samples were assayed half-hourly between 18:20 h and 00:20 h, and hourly thereafter, intermediate half-hourly data points were missing in the latter part of the melatonin curve. As such, individual missing values were added by mathematical linear interpolation between the previous and subsequent hourly values. The same method was then implemented to identify individual DLMO and DLM^{Off} times. DLMO/DLM^{Off} was determined by linear interpolation where the corresponding X co-ordinate ('time') matched a Y co-ordinate of 10pM (Voultsios, Kennaway, & Dawson, 1997) or 25% of maximum overall concentration for those individuals ($N = 4$) with low absolute levels (e.g., < 40pM for peak melatonin value). Timings of overall melatonin profiles were then adjusted and aligned to onset times for both normal sleepers and those with DSWPD. Data are presented as the mean and standard error (*M±SE*) in all figures. In tables, means and standard deviations (*M±SD*), as well as mean differences with complementary standard errors (*ME±SE*) between groups are reported. Where appropriate, a repeated measures ANOVA was used and between group differences compared using *t*-tests. Cohen's *d* values (Cohen, 1988) are also provided to indicate size of effects.

5.4 Results

Table 5-1 shows the sleep characteristics including subjective and objective habitual sleep parameters, sleep quality, sleep preferences and daytime impairments related to the sleeping pattern

of the DSWPD subjects and normal sleepers. Scores on these variables were obtained during phase one screening of the study. This information confirms the validity of the selection process with significant sleep pattern delays observed in the DSWPD patients. The table also illustrates a significant lifestyle disruption in the DSWPD patients as well as an earlier desired sleep period.

Table 5-2

Group differences between DSWPD patients and normal sleepers in current estimated sleep timing, sleep quality and associated impairments derived from pre-screening measures. Differences in habitual sleep timing based on subjective reports on the sleep/wake diary are indicated which were further confirmed by actigraphy monitors. Means and p-values are presented, with standard deviations displayed in parentheses.

	DSWPD	**Controls**	Mean Diff. ± SE	*Cohens' d* [f]	*p*
		Recorded sleep timing [a]			
Sleep Onset	0209 ± 1h 28m	2318 ± 37m	3h 12m ± 19m **	2.36	< .001
Sleep Latency	54m ± 4m	18m ± 8m	37m ± 9m **	1.07	< .001
Wake up time	1016 ± 1h 35m	0759 ± 52m	2h 34m ± 23m **	1.69	< .001
Total Sleep time	6h 48m ± 50m	7h 05m ± 2h 9m	1h 10m ± 27m*	0.18	.55
		Self-estimated sleep timing [b]			
Bed time	0138 ± 1h 6m	2247 ± 47m	2h 55m ± 19m **	2.95	< .001
Sleep latency	53m ± 52m	16m ± 9m	37m ± 7m *	0.90	.001
Get up time	1009 ± 1h 47m	0653 ± 1h 1m	3h 10m ± 26m **	2.08	< .001
Total sleep time	6h 54m ± 1h 43m	7h 33m ± 84m	39m ± 25m	0.43	.130
Preferred sleep time [c]	2253 ± 1h 2m	2253 ± 44m	44m ± 18m	0.01	.980
Preferred wake up time [c]	0801 ± 1h 30m	0719 ± 1h 12m	42m ± 26m	0.49	.116
MEQ score	33.09 ± 5.76	57.06 ± 6.26	23.9 ± 1.86 **	4.43	< .001
MSF_{SC} [d]	0547 ± 1h 48m	0342 ± 1h 28m	2h 5m ± 34m **	1.25	< .001
		Sleep quality and associated impairment			
PSQI score	6.15 ± 3.32	1.76 ± 1.56	4.39 ± 0.75 **	1.59	< .001
Work/school disruption [e]	6.77 ± 2.47	1.29 ± 1.61	5.48 ± 0.68 **	2.52	< .001
Social life disruption [e]	4.73 ± 2.79	2.12 ± 2.42	2.61 ± 0.83 *	0.99	.003
Family life disruption [e]	5.31 ± 2.80	1.18 ± 1.91	4.13 ± 0.72 **	1.66	< .001
Overall disruption [e]	16.81 ± 6.03	4.59 ± 5.33	12.22 ± 1.80 **	2.07	< .001
Days absent from commitments due to sleep pattern (weekly average) [e]	1.42 ± 1.84	0.47 ± 0.72	0.95 ± 0.40 *	0.91	.023
Days of reduced productivity due to sleep pattern (weekly average) [e]	3.08 ± 2.12	0.71 ± 0.99	2.37 ± 0.48 **	1.34	< .001
Motivation to change current sleeping pattern [c]	2.732 ± 0.92	1.47 ± 1.18	1.26 ± 0.32 **	1.22	< .001

* *Significant group differences indicated at <.05; **Significant group differences indicated at <.001*

[a] *Objective data from sleep/wake diary and actigraphy*

[b] *Retrospective estimate given on the PSQI*

[c] *Reported on the STQ*

[d] *MSFsc = MSF - 0.5*(SD_F - (5*SD_W + 2*SD_F)/7); MSF: mid sleep on free days; SD_F: sleep duration on free days; SD_W :sleep duration on work days*

[e] *Reported on the SDS*

[f] *Size of effect: d>.20 = small; d>.50=medium; d>.80 = large).*

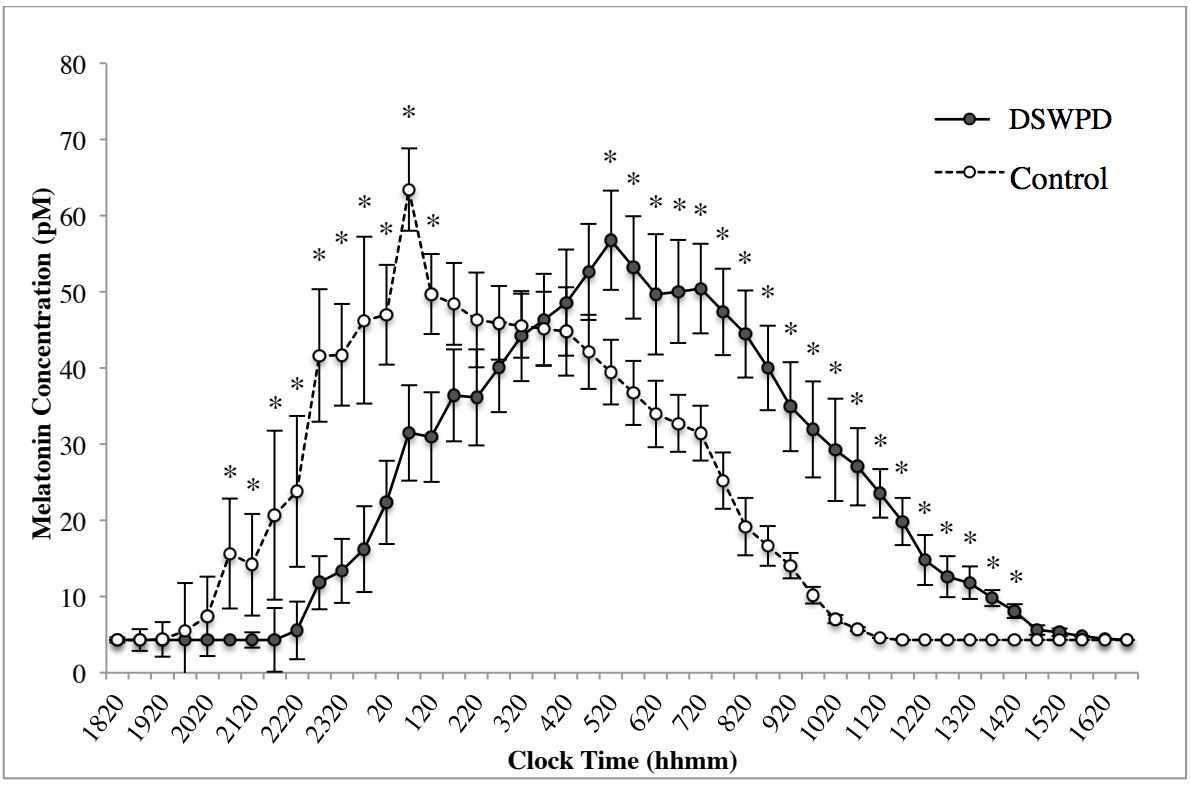

Figure 5-1. DSWPD and control group differences in melatonin secretion at half-hourly intervals during the first 22 hours of data collection. Significant differences (p<0.05, 2-tailed) in melatonin secretion between groups are indicated by * and error bars denote Standard Error values.

1.1.1 Melatonin Profiles

Melatonin levels were less than the detection threshold (4.3pM) in DSWPD and controls prior to 1820h and after 1520h the next day (please refer to supplementary documentation for individual melatonin profiles of DSWPD and controls; Appendix I). The mean onset of melatonin secretion (DLMO) in the normal sleepers occurred at 20:54 h ± 01:27 h, while for DSWPD patients it was delayed by approximately 3 hours (23:59 h ± 02:03 h; p< 0.001, Figure 5-1). In line with previous studies using healthy sleeping participants (Burgess et al., 2003; Burgess & Eastman., 2005), DLMO preceded sleep onset by 02:38 h ± 01:39 h in controls and 03:26h ± 01:45h in DSWPD patients. Worthy of note, shorter sleep onset-DLMO intervals were typically observed in controls than patients but this was not a significant difference (p=0.15).

Differences of overall melatonin secretion at half hourly clock time intervals were compared between groups using independent samples *t*-tests[3]. Figure 5-1 illustrates significant differences in melatonin amounts during the rising phases and descending phases of the profiles. These differences between the groups are most likely due simply to the delay of the DSWPD curve with respect to the normal sleepers. Visual inspection of the profiles suggested that the rate of melatonin increase in the DSWPD subjects was less than the controls. However, this reduced slope could be due to greater variability of the timing of the DLMOs in the DSWPD group and thus the mean curve being less representative of individual profiles in the DSWPD group.

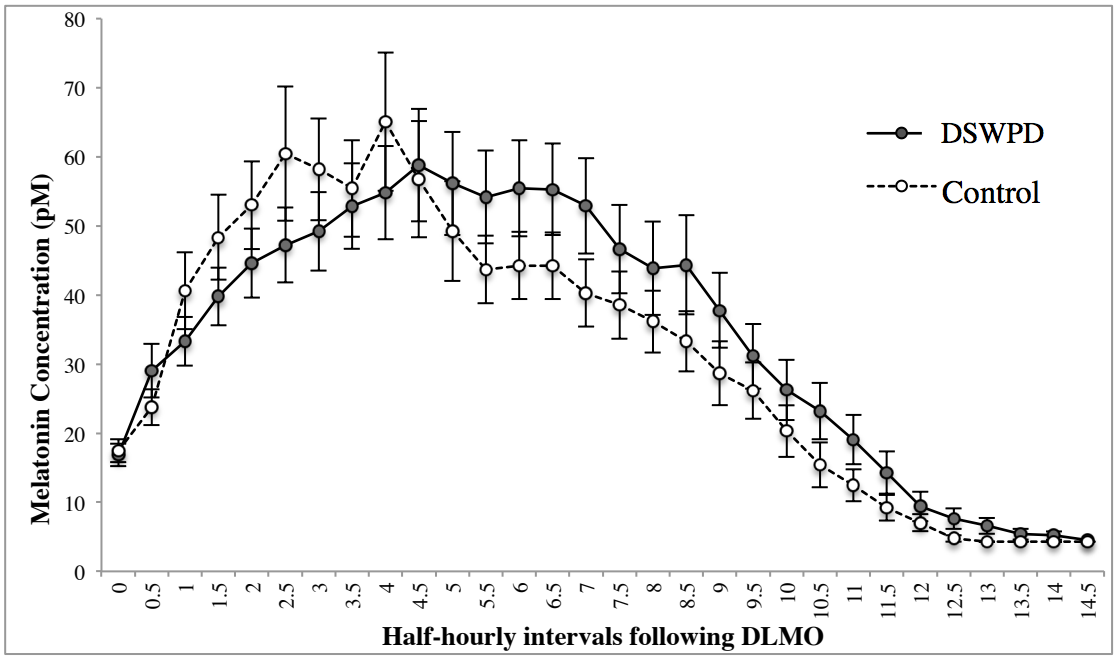

Figure 5-2. Mean melatonin secretion from DLMO, between good sleepers and DSWPD. Profiles are aligned relative to individual times of dim light melatonin onset, error bars denote Standard Error values.

To control for the circadian phase difference between groups and individuals, the profiles were therefore re-plotted relative to the DLMO for each group as shown in Figure 5-2. Since the

[3] A mixed between-within groups ANOVA would have been ideal to assess melatonin secretion over time, and between groups. However due to a large number of levels accounting for the 'time' factor, insufficient residual degrees of freedom restricted the use of this analysis.

variability in total melatonin production may also affect results, these mean curves of absolute melatonin concentration were re-plotted controlling for total melatonin production. The area-under-curve (AUC) was calculated for each individual and the following formula used to establish percentages *(half hourly concentration value/AUC)*100*. These percentages were then averaged for each group (See Figure 5-3). Differences between groups at each time point were again compared with independent samples *t*-tests. After this correction for total melatonin production, the melatonin profiles of DSWPD and control participants differed 1.5-5 hours following DLMO. Bonferonni corrections were not used in this analysis since differences between profiles cluster only in a particular section of the nocturnal profile with consecutive time points during this section serving effectively as replication studies.

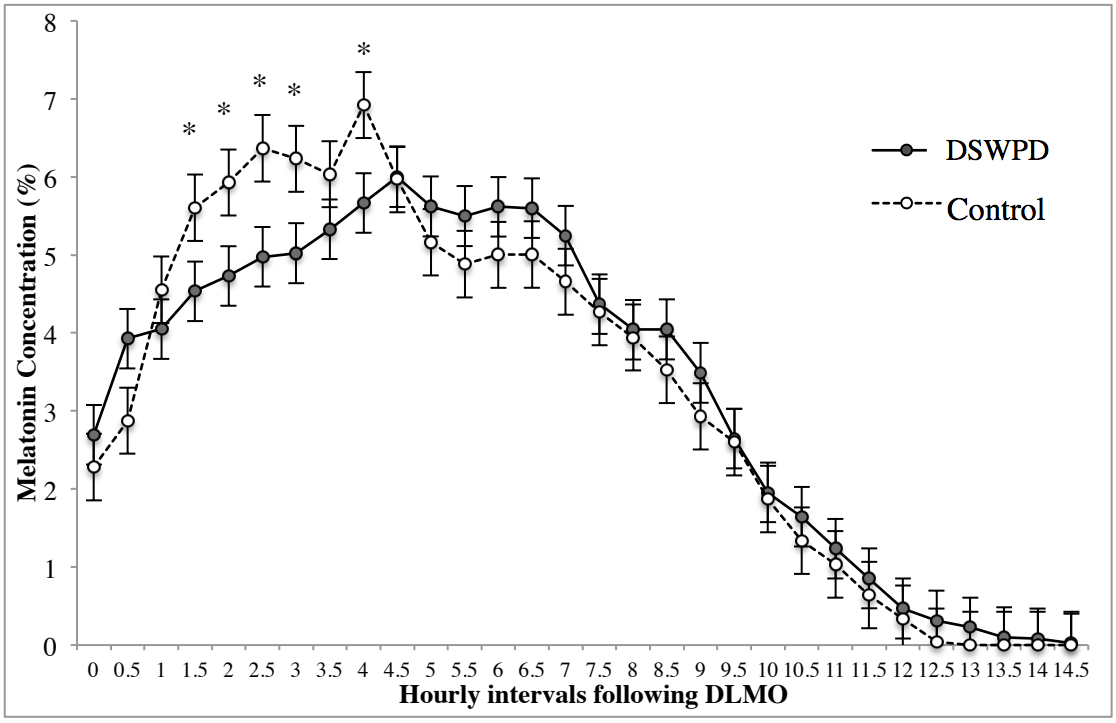

Figure 5-3. Melatonin secretion relative to overall amount of melatonin produced by individual participants. Percentages were calculated to control for variability in total melatonin secretion (half hourly concentration value/AUC)*100. Profiles are aligned at half-hourly intervals from times of dim light melatonin onset. Significant differences (p<0.05, 2-tailed) in percentage of melatonin secretion between good sleepers and DSWPD are indicated by * and error bars denote Standard Error values. The melatonin profiles differed, between groups 1.5-5 hours following DLMO

More global differences in melatonin profiles between DSWPD and normal sleepers were compared using a series of independent samples *t*-tests. The profile characteristics that were compared are presented in Table 5-2. Duration was calculated between DLMO and DLM^{Off}. Although there was a tendency for DSWPD to show a relatively later peak of melatonin secretion, they secreted similar concentrations of melatonin before and after the acrophase of the profile while normal sleepers secreted more melatonin in the first half of the night. Since the between-group differences occurred predominantly in the upslope of the melatonin profile, a measure of slope was also calculated by dividing the AUC during this time by the number of hours lapsed between individuals' DLMO and peak (pM/hour). Results suggest a trend for DSWPD to have a slower rate of melatonin increase per hour, which, although statistically non-significant, showed a medium-large effect size according to Cohen's *d* benchmarks. The AUC between DLMO and 5^{th} hour was also tested between groups since predominant differences are found during this period. This difference showed significantly less melatonin secretion of the DSWPD group in the first 5 hours following DLMO.

Table 5-2

Group differences in melatonin profiles of those with Delayed Sleep-Wake Phase Disorder and good sleepers. Means, standard deviations and p-values from independent samples t-tests are presented. Due to the low sample size, effect sizes (Cohen's d) are provided to demonstrate effects when significance is not obtained.

	DSWPD	**Controls**	*d*[a]	*p*
Sleep onset-DLMO duration (*hours*)	3.43 ± 1.75	2.63 ± 1.65	.47	.149
Area under curve (AUC) (*pM*)	994 ± 605	916 ± 435	.143	.648
Overall duration (*hours*)	11.8 ± 1.7	11.4 ± 1.2	.262	.462
Onset-to-peak duration (*hours*)	5.5 ± 2.14	4.98 ± 3.3	.195	.531
Peak-to-offset duration (*hours*)	6.3 ± 2.2	7.3 ± 2.1	.46	.126
Onset-to-peak duration (%)[b]	48.5 ± 19.4	37.3 ± 17.8	.60	.056*
Rate of melatonin increase *(pM/hours)*[c]	9.4 ± 5.8	13.0 ± 8.5	.52	.106
Initial 5 hours AUC (*pM*)[d]	42.8 ± 22.3	47.9 ± 23.2	.23	.461
Initial 5 hours AUC (%)[e]	46.9 ± 10.2	52.8 ± 7.08	.78	.048**
Onset-to-peak AUC proportion (%)[f]	52.5 ± 20.2	66.4 ± 20.5	.55	.089*
Peak-to-offset AUC proportion (%)[g]	55.3 ± 20.8	41.6 ± 19.6	.54	.092*

* *significant one-tailed difference*

** *significant two-tailed difference*

[a] *Size of effect: d>.20 = small; d>.50=medium; d>.80 = large.*

[b] *Percentage calculated as: (onset-to-peak duration/overall duration)*100*

[c] *Derived from onset-to-peak AUC / onset to peak duration (pM/hours)*

[d] *Calculated from DLMO until the 10th half hourly interval (i.e., 5 hours post DLMO)*

[e] *Percentage calculated as: (AUC from onset-to-5-hrs/overall duration)*100*

[f] *Percentage calculated as: (onset-to-peak AUC/overall AUC)*100*

[g] *Percentage calculated as: (peak-to-offset AUC/overall AUC)*100*

5.5 Discussion

This investigation explored differences in overnight melatonin profiles between adults with DSWPD and normal sleepers, in order to contribute further to the currently limited understanding of the DSWPD aetiology. Apparent in a previous study (Rahman et al., 2009) we expected to find a slower increase of melatonin to acrophase and an overall longer duration of melatonin secretion in those with DSWPD. In our study, there was a significant delay in the melatonin secretion profiles of the DSWPD patients compared to normal sleepers. Patients also showed a slower increase of melatonin from DLMO to acrophase as evidenced by less absolute and proportional melatonin in the early part of the melatonin profile (1-5 hours following DLMO). Derived profiles depict the

robust nature of melatonin production in the early stages of the evening for normal sleepers compared to those with DSWPD.

It has been established that the normal circadian period length (*tau*) is approximately 24.2h long, (i.e., 0.2h longer than a 24h solar day; Burgess & Eastman, 2008; Czeisler et al., 1999; Micic et al., 2013). Therefore, most individuals would have a mild tendency to phase delay if entraining cues are absent. The melatonin profile we have observed in the normal sleepers may favour the stability of their circadian rhythm. Previous empirical studies suggest that doses of exogenous melatonin have phase-shifting effects when administration is timed appropriately relative to the melatonin Phase Response Curve (Burgess et al., 2010; Lewy et al., 1992). When administered 1-9 hours before DLMO or at approximately from 12:00 h to 20:00 h, exogenous melatonin can have phase-advancing effects. There is also recent evidence that the peak phase advance in the phase response curve is later for lower doses of melatonin and approaches the timing of the DLMO (Burgess et al., 2010). An extrapolation of this trend would suggest that even increases in physiologic concentrations of melatonin immediately following DLMO may have a small phase advancing effect. Therefore, an early robust endogenous melatonin surge may facilitate a small daily advance in the circadian timing system and help to counter an average tendency to phase delay by 0.2 hours each day. Patients with DSWPD with a diminished initial surge of melatonin may not have this 'protective mechanism', leading to a tendency toward phase delay. This could also increase the difficulty with phase advancing and remaining stabilized to an earlier sleep time, even after treatment using evidence-based therapies (i.e., chronotherapy, melatonin, and bright light therapy; Alvarez et al., 1992).

Uchiyama et al., (2000b) challenged the notion that DSWPD was merely a circadian pacemaker timing disorder. Similar to the present study, they employed a 10/20-minute sleep/wake ultradian protocol in an attempt to explain patients' inability to reset the sleep phase. Following 24 hours of sleep deprivation, they determined sleep phase by sampling hourly plasma melatonin and

measured relative half-hourly sleep propensity via polysomnograph recordings during 10-minute sleep opportunities. While sleep deprived controls were able to compensate for sleep loss at most phases of the circadian pacemaker, DSWPD patients were unable to sleep until a significantly later time in their melatonin profiles. Uchiyama et al., (2000b) concluded that DSWPD patients had a poor ability to compensate for prior sleep loss, which could imply that the delay in DSWPD sleeping patterns is due to a deficit in the accumulation of homeostatic sleep drive across time thus contributing to delays of bedtimes, later light exposure, and stronger tendency to phase delay. A blunted initial melatonin secretion profile may also be contributing to the apparent inability to initiate sleep early.

The findings of the present study give further support to mechanisms other than circadian rhythm delays for the aetiology of DSWPD. To the authors' knowledge, three separate studies have thus far assessed melatonin between DSWPD and normal controls to enable profile comparisons (Rahman et al., 2009; Shibui, Uchiyama, & Okawa, 1999; Uchiyama et al., 2000b). However, none have directly assessed relative amounts of melatonin secretion between groups at circadian phase aligned time points. By visual examination, it appears that the presented figures illustrating melatonin curve differences across groups, in all three studies, show a similar blunted initial melatonin release for DSWPD patients in comparison with the profiles of good sleepers. Nonetheless, since their aims were different to those of the present study, none ran specific analyses to assess whether these differences in profile shapes were statistically significant. If these observations are consistent with the significant results of the present investigation it may explain the poor compensatory mechanism in sleep propensity of DSWPD suggested by Uchiyama et al., (2000b). Hypothetically, the lack of robustness in the DSWPD melatonin profiles during the first half of the curve may reduce feelings of sleepiness and sleep propensity in DSWPD. This in turn may delay bedtime, and artefactually increase phase delaying light before bedtime and delay exposure to light in the morning. Therefore, the less robust early melatonin secretion pattern in

DSWPD may not only reduce protection against the tendency to phase delay, but lead to greater phase delay from the phase delaying effects of later evening and early morning light exposure.

Likewise, previous studies have alluded to discrepancies in behavioural tendencies that may be delaying sleeping patterns of those with DSWPD. Evening types have been found to have a significantly delayed circadian rhythm of subjective sleepiness compared to morning-type sleepers. A study by Lack et al., (2009) reported that evening types had a delayed endogenous circadian rhythm occurring 2-3 hours later compared to morning types. However, the subjective sleepiness rhythm was delayed by seven hours in evening-types as opposed to morning types. Lack and associates reasoned that, since subjective sleepiness rhythms were more delayed than circadian rhythm differences in people with DSWPD, circadian rhythms simply follow lifestyle choices driven by subjective sleepiness and subsequent differential exposure to time cues such as dark-light cycles. Taking into account the results of the present study it would be interesting to see if evening types (considered to be susceptible to the development of DSWPD) also have a diminished secretion of melatonin in the hours immediately following melatonin onset.

There is compelling empirical evidence to support the efficacy of exogenous melatonin in successfully advancing sleep time in patients with DSWPD (Lewy et al., 1999). The results of the present study may further advocate this form of intervention to correct patients' melatonin profiles and sleep pattern by augmenting melatonin amounts in the early part of the evening and sleep period. Melatonin amounts of approximately 1-5mg have been found most effective for the treatment of DSWPD and it is recommended that doses should be kept as low as possible and as early as the patient can tolerate (van Geijlswijk, Korzilius, & Smits, 2010). Furthermore, these authors encourage clinicians to measure circadian timing to determine the most effective phase for administration of exogenous melatonin. Although the current diagnosis of DSWPD relies mostly upon self-report sleep diaries, this study further highlights the need for objective measures (i.e.,

actigraphy and/or salivary DLMO; Rahman et al., 2009; Zee, 2010) to diagnose DSWPD and facilitate the most efficacious treatment.

Outcomes of the present study suggest that the mechanism behind DSWPD may be multifaceted and an anomaly in patients' melatonin profiles may also be contributing to a delay in their circadian system and sleeping patterns. Results suggest that DSWPD patients, in comparison with normal controls, show a significant depression in melatonin secretion during the first half of the profile, followed by a melatonin decline not unlike the good sleepers' after the acrophase. It would seem that a lack of this initial surge of melatonin secretion in the early part of the DSWPD night could be making patients vulnerable to circadian delay. These results therefore provide further support for the treatment of DSWPD with orally administered melatonin.

CHAPTER 6 PERSONALITY PROFILES

Personality differences in Delayed Sleep-Wake Phase Disorder and Non-24-Hour Sleep-Wake Rhythm Disorder patients relative to healthy sleepers.

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Author Contributions
GM had responsibility for day-to-day administration of the project, supervision of recruitment, screening and scheduling of participants, participation in data collection, data management, analysis and write up of the manuscript. LL had primary supervisory role for the project, overall planning, supervision of the students, data analysis, and manuscript preparation. NL had supervisory role of students and was directly involved in helping to manage the project. She was also significantly involved in written publications and communications of results. MG assisted with advice, written publications, and other modes of communication of results.

Micic, G., Lovato, N., Gradisar, M., Lack, L. (submitted 28/11/2015). Personality differences in Delayed Sleep-Wake Phase Disorder and Non-24-Hour Sleep-Wake Rhythm Disorder patients relative to healthy sleepers. *Sleep Medicine*

6.1 Abstract

Delayed Sleep-Wake Phase Disorder (DSWPD) is associated with a delayed timing of circadian rhythms and this delay is suggested to be the basis of the disorder. However, this has been questioned due to frequent relapses following treatment based on this aetiology. Recent studies have emerged suggesting personality factors may contribute to sleeping patterns in DSWPD, thus adding to its causes. The aim of this study was to further investigate circadian and personality factors in DSWPD as well as Non-24-Hour Sleep-Wake Rhythm Disorder (N24SWD) patients relative to control sleepers. This sample comprised 16 DSWPD (age=21.1±2.8, 10m, 7f), and 3 N24SWD patients (age=24.0±4.4, 2m, 1f). Controls were 7 males and 7 females (age=23.4±5.9). Prior to commencement of an 80-hr modified constant routine, eligible participants' sleeping patterns were monitored for a week and they completed a personality questionnaire (NEO-PI-R). An ultradian routine with alternating 20-min sleep opportunities and 40-min of enforced wakefulness was used to measure the timing of endogenous circadian temperature and melatonin rhythms. Compared to controls, DSWPD patients reported higher neuroticism, significantly lower extraversion, conscientiousness, and agreeableness. Similarly, N24SWD patients showed significant differences in conscientiousness, extraversion and agreeableness personality dimensions, but unlike DSWPD, neuroticism was not statistically different to controls. Within the DSWPD group personality factors, particularly conscientiousness, were associated with phase timings of circadian rhythms as well as sleep measures and lifestyle factors. These findings suggest that CRSWD may not only stem from circadian abnormalities but personality factors may also drive lifestyle choices, including sleep timing.

Keywords: circadian rhythm sleep-wake disorders, delayed sleep**,** light, zeitgebers, entrainment

6.2 Introduction

Delayed Sleep-Wake Phase Disorder (DSWPD) is defined as an abnormally late sleep period (e.g., 3am – 12pm) compared to conventional or socially desirable times (AASM, 2014; APA, 2013; Weitzman et al., 1981). The delay in patients' major sleep period is presumably caused by a delay in patients' circadian rhythms (Okawa & Uchiyama, 2007; Sack et al., 2007). It is particularly prevalent in the adolescent and young adult population (Gradisar et al., 2001; Gradisar, Smits, & Bjorvatn, 2014) and typically associated with significant morbidity (AASM, 2014; Alvarez et al., 1992; APA, 2013; Barion & Zee, 2007).

Non-24-Hour Sleep-Wake Rhythm Disorder (N24SWD) is characterised by successively delayed sleeping patterns analogous to those observed in 'free-running' experiments where patients are isolated from time cues (Hayakawa et al., 2005; Uchiyama & Lockley, 2009). During phases when the circadian system is misaligned relative to the natural light/dark cycle, N24SWD patients experience night-time insomnia and daytime sleepiness.

In both disorders, significant difficulty is associated with stabilizing the sleep period to a socially-conventional time and attempts to do so often fail (Alvarez et al., 1992; Barion & Zee, 2007; Dagan & Eistenstein, 1999; Dagan, 2002; Sack et al., 2007; Kitamura et al., 2013; Regestein & Pavlova, 1995). Although they are classified as distinctive disorders, biological investigations and clinical evidence imply that DSWPD and N24SWD pathology could share a common basis (Okawa & Uchiyama, 2007). Both disorders are believed to be of a physiological genesis involving delayed circadian rhythms and other rhythm abnormalities that predispose these patients to delay in their circadian rhythms. However, there are also significant indications of abnormal lifestyle and psychosocial factors that may (1) cause; (2) be a result of or; (3) exacerbate the conditions, thus making them difficult to treat (Dagan et al., 1996; Dagan., 2002; Shirayama et al., 2003; Takahashi, Hohjoh, & Matsuura, 2000; Wilhelmsen-Langeland et al., 2013a).

Incidence of personality disorders or other psychiatric ailments in circadian rhythm sleep-wake disorder (CRSWD) patients could influence social cues in lifestyles (McArthur et al., 1996). Indeed empirical literature suggests that personality aspects and psychosocial conditions are related to and can influence sleep timing (Higashitani et al., 1992), and be a cause of CRSWD although the relationships are complex (Dagan, 2002). Peers, parents, teachers, doctors, etc. may perceive patients as lazy and unmotivated because they appear sluggish as a result of insufficient sleep. Consequently, over time patients may adopt similar views and beliefs about themselves and succumb to corresponding expectations about their attitudes, behaviours and habits (Dagan, 2002). This proposal is in line with Wilhelmsen-Langeland et al., (2012) who found DSWPD patients wavered between rejecting the blame for their habits, and at other times affirming that they are lazy.

Personality traits underwrite attitudes, habits and emotive tendencies that impact individuals' lifestyles. They are likely to influence behaviours such as daytime activities, organizational ability, and choice of sleep times. Much like light (Duffy & Wright, 2005), social cues can contribute to either entraining or perpetuating circadian misalignment as secondary zeitgebers or entraining cues (Ehlers, Frank & Kupfer, 1988; Grandin, Alloy & Abramson, 2006). For example, choices in mealtimes, caffeine intake, social interactions and relationships, chores and responsibilities, and social expectations can dictate bedtime and rise times. In turn, the time a person wakes up and is exposed to light, or goes to sleep and inhibits light stimuli entrains circadian timing (Duffy & Wright, 2005). It is important to examine psychosocial and personality factors in the aetiology of CRSWD because the exact processes underlying circadian disruption and the interaction with personalities and psychiatric vulnerability are poorly understood.

One holistic approach to assessing human psychosocial domains entails the use of the Five-Factor Model (Costa & McCrae, 1992a). This model is the most widely used taxonomy of personality, encompassing the five personality dimensions of extraversion, neuroticism, openness, agreeableness and conscientiousness that account for all personality dimensions without overlap

(Costa & McCrae, 1992b; Wiggins & Trobst, 1997). The NEO-PI-R is a validated measure of these dimensions and has been successfully applied in counseling, clinical psychology, psychiatry, behavioural medicine and health psychology (McCrae & John, 1992). The first and only study to assess the five personality factors in DSWPD patients showed elevated scores on neuroticism, yet low on conscientiousness and extraversion compared to healthy controls (Wilhelmsen-Langeland et al. 2013b). Studies investigating diurnal preferences (chronotypes eveningness/morningness) and personality similarly reveal positive associations between early bedtimes, conscientiousness and agreeableness, while later bedtimes relate to less extraversion and greater neuroticism (Adan et al. 2012; Randler, 2008; Tsaousis, 2010). These findings are consistent and seem applicable to DSWPD patients who typically exhibit evening-type preferences.

Personality data are not available for N24SWD patients, yet empirical literature suggests that the prevalence rates of psychological disorders, such as depression and personality disorders, are elevated in this sample (McArthur et al., 1996) as it is in DSWPD (Alvarez et al., 1992; Dagan et al., 1996; Shirayama et al., 2003; Takahashi et al., 2000). In individuals with N24SWD who are blind, the disorder is likely physiological and caused by the ineffectiveness of entraining stimuli such as light (Okawa & Uchiyama, 2007). N24SWD in sighted patients has also been attributed to circadian abnormalities but the contribution of psychiatric effects on both sighted and non-sighted patients' sleeping patterns cannot be discounted (Kitamura et al., 2013). Hayakawa et al. (2005) suggest that 28% of sighted N24SWD patients report psychiatric complaints, thus highlighting the importance of assessing differences in psychosocial and personality dimensions in N24SWD patients.

This paper has important a priori strengths to investigate the psychological profile of N24SWD patients. These patients tend to be rare in both literature and clinical settings. However, they do turn up in clinics and practitioners could benefit from a better understanding of its aetiology. In addition, this is the first study to associate circadian phase and period length (*tau*) with

the big five personality factors in normal sleeping individuals as well as DSWPD and N24SWD patients. Outcomes of the investigation may support the use of supplementary therapies for better treatment outcomes in patients.

The objective of this study was to examine differences in personality factors between DSWPD, N24SWD patients and normal sleepers. Furthermore, we aimed to investigate if personality traits are associated with lifestyles, psychosocial factors and sleep characteristics. This will help to inform CRSWD diagnosis and augment more effective long-term treatments programs. In line with the findings of Wilhelmsen-Langeland et al., (2013b), it is hypothesised that DSWPD patients in the present study will indicate significantly higher scores on neuroticism, as well as significantly lower conscientiousness and extraversion compared to healthy control sleepers. As N24SWD stems from a circadian misalignment, it is predicted that N24SWD patients will also report significantly higher neuroticism and lower conscientiousness and extraversion relative to controls.

6.3 Methods

Comprehensive details of the methodology applied in this study have been previously published (Micic et al., 2015a) and will be briefly summarized here.

6.3.1 Participants

Thirty-three patients and healthy sleepers participated in this study. They were selected from the community via advertisements placed on public noticeboards and South Australia's three major Universities. There were 16 DSWPD comprising 10 males (62.5%) and 6 females (37.5%) aged 18-29 yrs. Controls consisted of 7 males (50%) and 7 females (50%) aged 18-37 yrs. An additional third study group consisting of 3 full-sighted patients were diagnosed with N24SWD, 2 of whom were male and 1 female. The mean age in years was 21.1±2.8 for DSWPD patients, 24.0±4.4 for

N24SWD patients and 23.4±5.9 for controls. Neither age, $F(2,33)=1.27$, $p=0.30$, nor gender, X^2 (2, $N=34)=0.11$, $p=0.82$, varied significantly across groups.

Based on the International Classification of Sleep Disorders (3rd Ed.) thorough screening procedures, and inclusion/exclusion criteria were implemented to ensure suitability of all participants to designated conditions (see Micic et al., 2015a). Ethics approval was granted by the Southern Adelaide Clinical Human Research Ethics Committee and informed consent was obtained prior to administration of questionnaires and testing provisions. Eligible candidates participated in an 80-hr modified constant routine at the Flinders University Sleep Laboratory, South Australia. The protocol was designed to simulate 1-hr 'days', alternating 20-min sleep opportunities with 40-min enforced wakefulness. Upon completion of the entire study participants were financially compensated for their time with AUD$500.

6.3.2 NEO–Personality Inventory–Revised

The NEO PI-R, (Costa & McCrae, 1992a) is one of the most commonly used assessments of "the big five" personality factors and consists of 240 disposition statement items. Specifically, "Form S" is designed for self-report with statements of each item rated on a 5-point scale, from *strongly disagree* to *strongly agree*. Scores are totalled to generate the five factors of general personality functioning: Neuroticism/Emotional Instability, Extraversion, Openness to Experience, Agreeableness, and Conscientiousness. Each dimension gauges 6 subcomponents of the personality trait through 8 items dedicated to each subcomponent. All 30 subcomponents can be seen in Table 6-2. The NEO-Personality Inventory-Revised (NEO-PI-R) has good concurrent/construct validity and test-retest reliability with high coefficient alphas for Neuroticism ($\alpha=.92$), Extraversion ($\alpha=.89$), Openness ($\alpha=.87$), Agreeableness ($\alpha=.86$), Conscientiousness ($\alpha=.90$; Costa & McCrae, 1992a).

6.3.3 Additional Testing Apparatus

Data collected from the screening measures and constant routine were also included in the subsequent analyses. Reported during the screening phase were responses to the General Health and Medical Questionnaire (GHMQ), Delayed Sleep-Wake Phase Disorder-Sleep Timing Questionnaire (DSWPD-STQ), Pittsburgh Sleep Quality Index (PSQI), Munich Chronotypes Questionnaire (MCTQ), Morningness-Eveningness Questionnaire (MEQ), Sheehan Disability Questionnaires (SDS) and Depression Anxiety Stress Scale-21, short form ($DASS_{21}$). We included data recorded on the 7-day sleep/wake diary plus actigraphy prior to commencement of the laboratory procedure. This information was further analysed separately for days free of commitments (i.e., enabling spontaneous sleep/wake times) as well as commitment days (i.e., enforced sleep/wake schedules). Data from the constant routine were also incorporated in the present analyses including Dim light melatonin onset (DLMO) time, Melatonin rhythm length period or *tau* (Mτ), Temperature minimum time (Tmin), Temperature rhythm *tau* (Tτ).

6.3.4 Protocol Procedures

After initial screening, meetings with individual participants were arranged 1 week prior to experimentation. During the meeting, participants were familiarized with the protocol, testing apparatus and experimental procedures. All questions were answered in-depth and a second information pack was given to participants. This package contained information regarding parameters of living within the confines of the laboratory, a second consent form, sleep-wake diaries/actigraphy and the NEO-PI-R. Participants were requested to read the information carefully and complete/sign all documents prior to the next meeting (i.e., the commencement of the protocol). The personality inventory was implemented as a secondary aim of the study and in the second and third years of a 3-year data collection period. As such 69% of participants from the original sample (Micic et al., 2015a) completed the questionnaire. NEO-PI-R was scored by individuals who were blind to the condition of participants. Participants then completed 1 week of sleep-wake monitoring

via sleep diaries and actigraphy prior to laboratory experiment to ensure habitual sleeping patterns were maintained before commencement of the protocol. Following this thorough screening, eligible participants underwent an 80-hr ultradian modified constant routine at the Flinders University Sleep Laboratory of South Australia. Please refer to Micic et al., (2015a) reference for comprehensive details regarding the constant routine procedures and the on-line supplementary materials.

6.4 Results

6.4.1 Personality Differences

Differences in personality profiles between the three groups were analysed using a multivariate analysis of variance (MANOVA). Results of the MANOVA for the main effect of group (DSWPD, N24SWD, control) confirmed a significant main effect between groups for general personality dimensions $F(10,54) = 2.29$, $p = .025$; Wilks' Lambda = .50; partial $\eta^2 = .298$.

The next analysis was applied to the five separate personality factors in the NEO-PI-R. Since DSWPD and N24SWD are classed as distinct sleep disorders, two more MANOVAs were run to establish specific personality differences between DSWPD patients and controls, then N24SWD patients and controls. The results of the analyses indicated a significant main effect between DSWPD and controls on personality dimensions overall ($F(5,25) = 2.63$, $p = .048$; Wilks' Lambda = .66; partial $\eta^2 = .344$), as well as N24SWD patients and controls ($F(5,11) = 3.89$, $p = .028$; Wilks' Lambda = .36; partial $\eta^2 = .638$). Table 6-1 presents F- and p-values and partial η^2 for the group differences on the five personality factors. Compared to controls DSWPD patients were significantly higher on Neuroticism and lower on Extraversion, Agreeableness and Conscientiousness dimensions. Similarly N24SWD patients were lower on the Extraversion, Agreeableness and Conscientiousness general personality dimensions, albeit not Neuroticism and Openness.

Table 6-1

Personality Trait Dimensions Between Delayed Sleep-Wake Phase Disorder (DSWPD), Non-24-Hour Sleep-Wake Rhythm Disorder (N24SWD) and Controls.

	F-value	*p*-value	partial η^2
NEO-PI-R Dimensions		**DSWPD, N24SWD, controls**	
Neuroticism	2.65	.09	.15
Extraversion	4.19	.02	.21
Openness	1.10	.35	.07
Agreeableness	7.00	<.01	.31
Conscientiousness	3.08	.06	.17
		DSWPD, controls	
Neuroticism	4.89	.035	.14
Extraversion	3.66	.06*	.11
Openness	2.07	.16	.07
Agreeableness	7.23	.01	.20
Conscientiousness	4.57	.04	.14
		N24SWD, controls	
Neuroticism	0.00	.99	.00
Extraversion	7.71	.01	.34
Openness	0.76	.40	.05
Agreeableness	14.24	<.01	.49
Conscientiousness	2.60	.03	.27

** one-tailed significant difference; p<0.05*

Note. Values Shown in Bold Indicate Statistically Significant Differences Between Groups.

Means and standard deviations for NEO-PI-R trait dimensions and subcomponents (*t*-test scores) are presented in Table 6-2. Since DSWPD and N24SWD are two autonomous sleep disorders, patient groups were individually compared to controls using separate independent samples *t*-tests[4]. As per the results of the MANOVA, significant group differences were found between DSWPD and N24SWD patients compared to controls on all general personality dimensions apart from Openness.

[4] One-Way Analyses of Co-Variance (ANCOVAs) were also conducted for DSWPD relative to controls and N24SWD compared to controls. After performing initial checks to ensure normality assumptions as well as collinearity, homogeneity of variances / regression slopes were not violated, age and gender were controlled to account for possible interactions with personality differences. However as groups were selected to ensure participants did not vary on age/gender, overall, the ANCOVA did not yield a different trend of results. Furthermore, presenting participants' actual responses rather than adjusted means would be more informative. These are actual participant values and provide standard deviation values (rather than standard error) enabling calculation of effect sizes. Thus, the outcomes of the *t*-tests are reported instead of ANCOVA.

N24SWD and controls did not differ on the Neuroticism dimension while DSWPD participants obtained significantly higher scores on this dimension.

However, Neuroticism scores for N24SWD patients need to be taken cautiously, as one participant in the N24SWD group was an extreme outlier (Neuroticism total = 49) from the other two (totals of 101 and 93) so the mean value for this small and negatively skewed group (80) may not be representative. The median value of 93 is much closer to the DSWPD mean than the control mean.

6.4.2 Neuroticism

Specifically for Neuroticism, DSWPD reported greater anxiety, depression and vulnerability compared to controls. While N24SWD reported significantly lower Impulsiveness scores compared to controls. The impulsiveness and self-consciousness factors were also notably lower in N24SWD compared to DSWPD.

6.4.3 Extraversion

Both DSWPD and N24SWD reported lower positive emotions relative to controls. N24SWD also indicated significantly lower scores on warmth, gregariousness, and excitement seeking compared to controls. Excitement seeking was notably lower in N24SWD compared to DSWPD, such that DSWPD patients reported the highest excitement seeking scores of all three groups and N24SWD patients indicated the lowest scores.

6.4.4 Openness

Overall, the groups' personality factors did not differ on the openness dimension. However, relative to controls, both DSWPD and N24SWD reported significantly lower scores on the aesthetic and feelings categories. N24SWD patients also reported significantly higher scores on ideas relative to controls and DSWPD.

6.4.5 Agreeableness

Trust was significantly lower in N24SWD relative to DSWPD, which was in turn lower than trust scores reported by controls. DSWPD reported lower straightforwardness and tendermindedness scores relative to controls. Tendermindedness and modesty were lower still in N24SWD compared to both DSWPD and controls.

6.4.6 Conscientiousness

DSWPD and N24SWD patients did not significantly differ on the conscientiousness dimension to each other. Both groups of patients indicated significantly lower Conscientiousness scores to controls, particularly on the categories of competence, achievement striking and self-discipline.

Table 6-2

Group Differences on the NEO–Personality Inventory–Revised (NEO-PI-R).

	Means ± Standard Deviation			Significance & size of effect: Cohen's d [a]		
NEO-PI-R Dimensions	**Controls**	**DSWPD**	**N24SWD**	DSWPD-Control	N24SWD-Control	DSWPD-N24SWD
Neuroticism	**81.1 ± 23.8**	**100.1 ± 23.8**	**81.0 ± 28.0**	0.80**	0.01	0.79
N1 - Anxiety	14.9 ± 4.8	18.1 ± 5.3	17.0 ± 9.6	0.63	0.37	0.19
N2 - Angry Hostility	14.4 ± 6.4	16.4 ± 5.2	15.0 ± 6.6	0.35	0.09	0.26
N3 - Depression	14.4 ± 5.2	17.7 ± 5.6	17.0 ± 3.5	0.61	0.52	0.13
N4 - Self Consciousness	14.1 ± 5.2	16.8 ± 5.1	10.3 ± 3.1	0.52	0.76	1.32**
N5 - Impulsiveness	17.4 ± 5.6	17.4 ± 5.3	11.3 ± 4.04	0.00	1.13*	1.18*
N6 - Vulnerability	12.0 ± 4.9	14.2 ± 4.6	10.3 ± 8.3	0.46	0.31	0.76
Extraversion	116.1 ± 16.2	104.1 ± 18.4	85.7 ± 23.1	0.69*	1.76**	0.97
E1 - Warmth	21.4 ± 3.8	19.5 ± 5.5	15.0 ± 7.5	0.39	1.43**	0.78
E2 - Gregariousness	17.5 ± 3.9	15.6 ± 6.0	10.0 ± 2.6	0.37	2.00**	0.98
E3 - Assertiveness	16.8 ± 4.7	15.4 ± 3.8	18.7 ± 3.8	0.33	0.41	0.87
E4 - Activity	18.4 ± 4.2	16.7 ± 4.1	16.7 ± 6.1	0.41	0.38	0.00
E5 - Excitement Seeking	17.8 ± 4.3	19.5 ± 4.9	11.0 ± 5.0	0.37	1.56**	1.73**
E6 - Positive Emotions	21.5 ± 4.1	17.0 ± 4.6	14.3 ± 3.8	1.03**	1.77**	0.60
Openness	126.9 ± 12.3	118.1 ± 20.1	120.3 ± 8.6	0.52	0.56	0.11
O1 - Fantasy	20.2 ± 4.2	19.2 ± 4.7	21.3 ± 3.2	0.27	0.27	0.46
O2 - Aesthetic	24.1 ± 3.4	18.9 ± 7.5	17.7 ± 7.5	0.86**	1.53**	0.16
O3 - Feelings	21.9 ± 3.9	18.3 ± 5.6	16.3 ± 3.2	0.73**	1.47**	0.37
O4 - Actions	15.7 ± 4.6	15.8 ± 4.4	12.7 ± 2.1	0.02	0.69	0.74
O5 - Ideas	21.7 ± 2.9	23.0 ± 5.3	27.0 ± 4.6	0.30	1.67**	0.77
O6 - Values	21.9 ± 4.2	21.7 ± 4.8	25.3 ± 3.8	0.05	0.82	0.77
Agreeableness	117.9 ± 14.4	101.2 ± 19.1	81.0 ± 20.7	0.97**	2.40**	1.05
A1 - Trust	18.0 ± 5.1	14.8 ± 4.8	9.0 ± 10.4	0.65*	1.48**	1.02
A2 - Straightforwardness	20.4 ± 3.6	15.2 ± 5.3	17.0 ± 5.3	1.13**	0.88	0.34
A3 - Altruism	22.1 ± 5.6	20.6 ± 3.3	18.7 ± 5.0	0.33	0.62	0.54
A4 - Compliance	17.4 ± 6.0	15.9 ± 5.9	11.0 ± 5.3	0.25	1.08	0.84
A5 - Modesty	18.9 ± 4.5	17.1 ± 5.4	13.7 ± 0.6	0.36	1.24**	0.67*
A6 - Tendermindedness	21.0 ± 3.9	17.9 ± 4.2	11.7 ± 4.2	0.76**	2.36**	1.48**
Conscientiousness	123.4 ± 17.7	105.5 ± 27.0	96.7 ± 18.5	0.77**	1.50**	0.34
C1 - Competence	22.0 ± 3.1	18.9 ± 4.9	15.3 ± 4.0	0.74**	2.07**	0.75
C2 - Order	17.3 ± 6.9	17.1 ± 5.7	18.0 ± 4.4	0.03	0.11	0.16
C3 - Dutifulness	23.2 ± 4.4	20.5 ± 5.5	19.3 ± 3.8	0.54	0.90	0.22
C4 - Achievement Striving	20.6 ± 4.3	17.2 ± 5.2	13.3 ± 4.2	0.71*	1.70**	0.76
C5 - Self Discipline	20.9 ± 4.8	15.3 ± 6.6	10.7 ± 4.9	0.96**	2.12**	0.72
C6 - Deliberation	17.4 ± 4.9	17.2 ± 5.2	20.0 ± 3.6	0.04	0.55	0.55

*** Significant differences between DSWPD & Controls (p<0.05, two-tailed); * Significant differences between DSWPD & Controls (p<0.05, one-tailed)*

^^ Significant differences between N24SWD & Controls (p<0.05, two-tailed); ^ Significant differences between N24SWD & Controls (p<0.05, one-tailed)

Significant differences between DSWPD & N24SWD (p<0.05, two-tailed); # Significant differences between DSWPD & N24SWD (p<0.05, one-tailed)

[a] Cohen's d Size of effect: d>.20 = small; d>.50=medium; d>.80 = large.

6.4.7 Lifestyle, Circadian and Sleep Variables

Group means for lifestyle factors reported during the screening phase of recruitment, objective sleep/wake data and the relative psychosocial impairments are reported in Micic et al. (2015a). Although the present study recruited a sub-sample from this original group, participants were selected based on lifestyle and sleeping patterns thus the trends remained the same. Please see Appendix J: Supplementary Material for descriptive tables specific to lifestyles, sleep and circadian timing of this subgroup.

Statistically, the samples did not differ on BMI, caffeine/alcohol consumption, amount of commitments, total sleep time, preferred sleep time, nor the number of days their sleeping patterns precluded them from meeting obligations. Compared to controls, DSWPD participants reported significantly lower MEQ scores, higher PSQI and later MSF^{SC}. They also reported significantly later bed and rise times, as well as substantial difficulty falling asleep and awakening at their preferred earlier times. The delay in sleeping patterns was further coupled with disruptions to all aspects of reported lifestyle factors and significant reductions in productivity. DSWPD patients showed elevated scores for depression, anxiety, and stress and were more motivated to change their current sleeping pattern. N24SWD patients significantly differed on their diurnal preference and sleep quality compared to controls. They had later sleep patterns with greater variability in their sleep timing and significant difficulty with sleeping at a preferred time. Patients also reported significant lifestyle disruption, particularly in the work/school and family life domains, along with reduced daytime productivity and reduced motivation to change their sleeping pattern. Their scores for depression and stress were elevated but not statistically significant compared to controls due to the large variability of scores in the N24SWD sample.

6.4.8 Circadian and Sleep Variables

DSWPD patients had significantly later timed circadian markers compared to controls. Large standard deviations around the mean of N24SWD patients' circadian markers were congruent with

successive delays observed in their habitual sleeping pattern prior to the laboratory session. Overall, all groups differed according to their sleep-wake timing but not in the overall duration or quality of sleep. This was true despite the fact that when groups were split for free- and work-days, DSWPD patients spent less time in bed and obtained significantly less sleep on work days compared to controls. Additionally, patients with DSWPD had significantly longer circadian period lengths (*taus*) compared to controls and *taus* of N24SWD patients were even longer. Longer *taus* are a probable cause of their difficulty in maintaining synchrony of their sleep times with conventionally required sleep times.

6.4.9 Personality, Sleep and Psychosocial Dimensions

Pearson's correlations were used to inspect relationships between measures taken in-lab, screening variables and the personality inventory. Analyses were performed separately for DSWPD patients to derive associations in circadian, sleep and lifestyle factors with personality dimensions (Table 6-3). Controls were not considered in these analyses as groups were selected according to their sleep and lifestyle differences so any correlations including controls would have been the result of dichotomy between groups. However, DSWPD and controls combined indicated the same trend of results as DSWPD group alone, with higher correlations and greater statistical power due to the larger group size and greater range of values. A group of 3 N24SWD patients is not reliable enough to report correlations so this group was omitted. Due to a small sample in DSWPD ($N = 16$) most correlations did not reach statistical significance. Therefore, correlation coefficients that indicate at least a medium effect size according to Cohen's benchmarks (Cohen, 1988) are bolded in back ink within the tables.

For the DSWPD patient group, results generally show that greater DSWPD severity is negatively associated with Extraversion, Agreeableness, and Conscientiousness but positively correlated with Neuroticism. DSWPD patients with later-timed circadian rhythms, sleeping patterns and evening-type diurnal preferences, typically indicated lower Extraversion, Agreeableness and

Conscientiousness but more Neuroticism. Those that scored lower on Extraversion, Openness, Agreeableness and Conscientiousness reported more disruption to aspects related to their social lives. Unsurprisingly, patients with higher Neuroticism reported more depressive symptoms on the $DASS_{21}$, while those low on Conscientiousness tended to reported more depression, anxiety and stress. Although DSWPD and controls differed significantly on the Neuroticism dimension, it seem that within the DSWPD group there is no systematic relation with neuroticism except later circadian phase and depression.

Particularly, the Conscientiousness personality dimension appeared to be strongest predictor of circadian, sleep, and lifestyle disturbances or generally greater DSWPD severity. Those low on conscientiousness reported later/longer circadian phase, later sleep timing/sleep preference and significant lifestyle and psychological disturbances according to the SDS and $DASS_{21}$, but a greater motivation to improve their sleeping patterns.

Table 6-3

Circadian Rhythm, Sleep–Wake Variables Lifestyle Factor and Personality Dimension Correlations for DSWPD Patients.

	Neuroticism	Extraversion	Openness	Agreeable-ness	Conscientious-ness
Circadian Parameters					
DLMO	.66**	-.21	-.02	-.21	-.51**
Mτ	.10	-.26	-.40	-.40	-.46*
Tmin	.20	-.35	-.32	-.40	-.50**
Tτ	.05	.31	-.28	-.03	-.21
Sleep/Wake Pattern					
Average Bedtime	.02	-.36	-.34	-.10	-.36
Average Get up time	.12	-.48*	-.45*	-.15	-.36
Work Day Bedtime	.26	-.03	-.05	.19	-.31
Work Day Get up time	.05	-.20	-.17	-.12	-.37
Free Day Bedtime	.08	-.52**	-.27	-.05	-.36
Free Day Get up time	.11	-.64**	-.57*	-.15	-.09
Lifestyle Factors					
MEQ score	-.29	.22	.24	.09	.61**
MSF^{SC}	.04	-.05	-.23	-.31	-.68**
Sleep Quality	.04	-.54**	-.42	-.26	.09
Work/school disruption	.40	.00	-.13	.08	-.17
Social life disruption	.11	-.58**	-.44*	-.38	-.17
Family life disruption	.06	-.10	-.13	-.33	-.53
Overall disruption	.15	-.31	-.31	-.30	-.41
Days of absence	.08	-.29	-.45*	-.57**	-.40
Reduced productivity	.00	-.17	-.05	-.55**	-.50*
Motivation to advance sleep	.24	.29	-.15	.24	.50*
Depression	.60*	.09	.05	.21	-.25
Anxiety	.02	-.01	.05	-.11	-.31
Stress	.08	.27	.33	.00	-.30

*one tailed <.05; **two tailed <.05; *** <.001

Abbreviations: DSWPD: Delayed Sleep-Wake Phase Disorder; Mτ: Melatonin tau; Tτ: Temperature tau; DLMO: Dim Light Melatonin Onset; Tmin: Temperature minimum; MEQ: Morningness-Eveningness Questionnaire; MSF^{SF}: Mid-sleep on free days corrected for accumulated sleep debt during the workweek.

Correlation coefficients r > .30 are bolded in back ink and indicate at least a medium effect size according to Cohen's benchmarks.

With respect to circadian timing, we found that the sample of DSWPD patients could be dichotomized into two separate groups. Approximately half of the DSWPD patients exhibited circadian phases closely comparable to the cluster of controls, while the other half of patients showed notably later Tmin and/or DLMO times (Figure 6-1). Hence, the DSWPD patients could be circadian-delayed or relatively circadian-entrained but still rigorously qualify as DSWPD based on clinical symptoms.

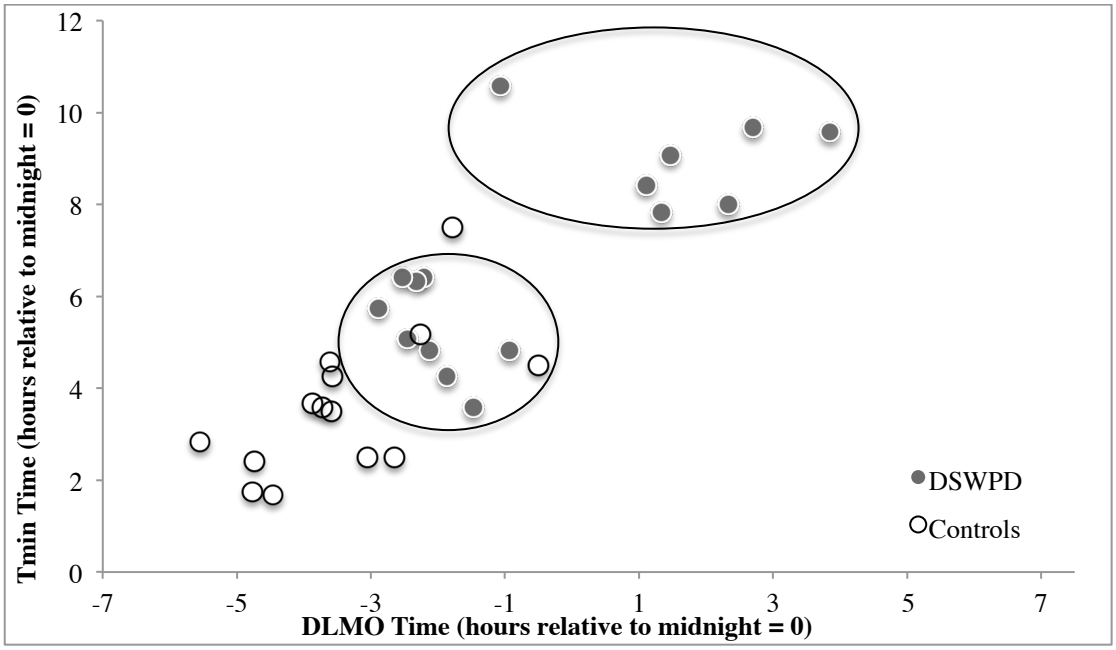

Figure 6-1. DSWPD patients and controls' dim light melatonin onset (DLMO) timing relative to core temperature minimum (Tmin) from the first evening of an 80h ultradian routine. Phase markers were positively correlated although two distinct groups were identified (large ovals). These groups include patients who were delayed in their circadian timing and those who exhibited relative circadian entrainment.

Upon further investigation of personality and psychosocial factors between the circadian-delayed and circadian-entrained, we found that patients were not a homogeneous group. Circadian-delayed patients exhibited generally greater DSWPD symptom severity and a more negative personality profile. Specifically, DSWPD patients who presented with an endogenous circadian delay scored higher on depression, anxiety, and stress and significantly lower on all Conscientiousness factors of

the five-factor model, except Order (see Table 6-4) compared to those who exhibited circadian entrainment. Circadian-entrained patients present with NEO scores that were intermediate to circadian-delayed DSWPD patients and controls. Thus, the DSWPD patients' associations between circadian delays and personality factors appear to be linear and sit on a continuum as presented in Table 6-4. However, not surprisingly many of the clinical symptoms that define DSWPD such as MEQ, MSF^{SC}, and psycho-social disruption show large differences between the circadian entrained DSWPD and controls and less difference between the DSWPD entrained and delayed patient.

Table 6-4

Between Group Differences in DSWPD Patients who are Circadian-Delayed compared to Circadian-Entrained.

	Mean ± SD			Cohen's *d*	
	(1) Circadian-delayed	**(2) Circadian-entrained**	**(3) Controls**	1 - 2	2- 3
Personality Dimensions					
Neuroticism	106.3 ± 30.6	93.0 ± 17.0	81.1 ± 23.8	0.56	0.56
Extraversion	95.6 ± 21.1	108.9 ± 14.7	116.1 ± 16.2	0.75	0.46
Openness	113.9 ± 18.5	120.3 ± 22.9	126.9 ± 12.3	0.30	0.39
Agreeableness	93.3 ± 22.5	106.8 ± 15.9	117.9 ± 14.4	0.71	0.74
Conscientiousness	89.0 ± 22.6	120.6 ± 22.9	123.4 ± 17.7	**1.39**	0.14
C1 - Competence	17.0 ± 2.0	21.7 ± 3.8	22.0 ± 3.1	**1.49**	0.09
C2 - Order	15.6 ± 6.4	18.1 ± 5.5	17.3 ± 6.9	0.42	0.13
C3 - Dutifulness	17.4 ± 4.7	23.6 ± 4.6	23.2 ± 4.4	**1.34**	0.09
C4 - Achievement Striving	13.9 ± 3.1	19.7 ± 5.5	20.6 ± 4.3	**1.23**	0.19
C5 - Self Discipline	10.9 ± 5.1	17.8 ± 5.9	20.9 ± 4.8	**1.24**	0.55
C6 - Deliberation	19.8 ± 2.2	14.3 ± 6.6	17.4 ± 4.9	**1.19**	0.55
Lifestyle Factors					
MEQ	30.1 ± 3.7	34.8 ± 6.8	58.5 ± 6.0	0.83	**3.75**
MSF^{SC}	6.5 ± 2.0	5.8 ± 1.8	3.4 ± 0.9	0.37	**1.69**
Sleep Quality	7.6 ± 3.7	4.9 ± 3.6	1.6 ± 1.7	0.74	**1.27**
Work/school disruption [b]	6.0 ± 2.5	6.3 ± 2.7	1.4 ± 1.7	0.11	**2.29**
Social life disruption [b]	5.6 ± 2.6	3.4 ± 2.6	2.1 ± 2.3	0.85	0.54
Family life disruption [b]	5.0 ± 1.6	4.8 ± 3.8	0.9 ± 1.8	0.07	**1.42**
Overall disruption [b]	16.6 ± 2.8	14.6 ± 7.9	4.4 ± 5.2	0.32	**1.60**
Days of absence	2.4 ± 2.6	0.5 ± 1.1	0.5 ± 0.8	1.00	0.00
Reduced productivity	3.9 ± 1.7	2.9 ± 2.6	0.6 ± 0.9	0.44	**1.31**
Motivation to advance sleep	2.3 ± 1.1	2.4 ± 1.2	1.3 ± 1.1	0.90	**0.97**
Depression	19.4 ±15.7	11.6 ± 9.0	4.5 ± 4.5	0.63	**1.08**
Anxiety	14.6 ± 8.9	5.6 ± 5.5	3.4 ± 2.9	**1.26**	**1.29**
Stress	19.4 ± 8.1	12.0 ± 9.1	6.9 ± 7.1	0.85	**0.64**
Mτ (h)	24.71 ± 0.15	24.51 ± 0.30	24.28 ± 0.22	0.79	.87
Tτ (h)	24.38 ± 0.55	24.57 ± 0.40	24.21 ± 0.40	0.41	**.90**

Note: Scores with Means, Standard Deviations, p-values and Cohen's d reported. Cohen's d Values Shown in Bold Indicate Statistically Significant Differences Between Groups.

6.5 Discussion

The present study examined the personality characteristics of DSWPD patients and N24SWD patients compared to healthy control sleepers. In line with the findings of Wilhelmsen-Langeland et al., (2013b), our results indicate that patients with DSWPD report significantly greater scores on the Neuroticism dimension and substantially lower scores on the Conscientiousness and Extraversion factors. DSWPD patients are also less Agreeable compared to controls, which was not a significant finding in Wilhelmsen-Langeland et al.'s study (2013b). Similar findings of DSWPD also extended to N24SWD patients compared to controls, apart from mean neuroticism score. Findings are subsequently discussed in greater detail.

6.5.1 Conscientiousness

The associations between Conscientiousness and sleep may be particularly relevant to DSWPD. In line with Wilhelmsen-Langeland et al., (2013b) DSWPD patients reported significantly lower scores within this dimension relative to controls. Patients scored particularly low in competence, achievement striving and self-discipline. These scores indicate that patients have a low internal locus of control, feel unprepared to deal with life, tend to procrastinate, quit easily and lack ability to persevere with tasks that are mundane (Costa, & McCrae, 1992a; Costa, & McCrae, 1995). Such personality traits can impact on bedtime routines and practices around sleep timing resulting in delays of bedtime and out-of-bed time. Further still, they can be applied to low adherence to treatment (Barion & Zee, 2007).

This facet was best associated with circadian, sleep/wake, psychosocial and lifestyle aspects of patients' lives, apart from sleep quality and disruptions to social lives and school/work commitments (Table 6-3). Patients were likely to report later bed/rise times, indicate more symptoms of depression, anxiety and stress, as well as greater disruption to lives as a result of their sleeping pattern. An important result is the positive association between motivation to improve

sleeping pattern and conscientiousness scores. It suggests that although those low in conscientiousness indicate greater disturbance across multiple life factors, they are less motivated to deal with their problems. This addresses clinicians' long-held suspicion surrounding patients' levels of motivation to improve their sleep and further highlights the need to address this aspect and implement therapeutic procedures such as motivational interviewing to produce better treatment outcomes (Alvarez et al. 1992; Dagan et al., 1996; Gradisar et al. 2011a; Gradisar, Smits, & Bjorvatn, 2014; Regestein & Pavlova, 1995; Wilhelmsen-Langeland et al., 2013b).

Although Conscientiousness showed a significant difference between patients and controls, there were also differences within the circadian "entrained" and "delayed" DSWPD sub-groups. Those who showed more severe circadian delay also exhibited significantly lower conscientiousness scores (Table 6-4) while the circadian "non delayed" did not differ from controls. Similarly, Wilhelmsen-Langeland et al., (2013b) reported that 62.5% of their sample of 40 DSWPD patients scored particularly low on the conscientiousness dimension and the remainder scored within the norms, comparable to controls. Wilhelmsen-Langeland et al. (2013b) propose that this finding could be due to the remaining 37.5% not internalizing the view of themselves as being lazy and lackadaisical. However, this would not explain why the more Conscientious DSWPD patients still experience sleep and lifestyle problems when they do not possess such a significant circadian delay.

Perhaps those in the DSWPD circadian entrained group who are not lower than normal on Conscientiousness may exhibit biological disturbances that explain their persistent delay. We hypothesized that longer circadian taus in this DSWPD group could explain why they suffer similar issues as their low-Conscientiousness counterparts. However, considering melatonin and temperature rhythm taus together, there was no systematic difference between the two subgroups. It seems other biological mechanisms such as abnormal responsiveness to light (Aoki, Ozeki & Yamada, 2001) or alterations in the homeostatic build-up of sleep ((Barion & Zee, 2007; Uchiyama

et al., 2000b) may explain the disturbance in these patients. Furthermore, the less circadian delayed and more conscientious patients may have developed sleep onset insomnia that contributes to their reported distress and clinical symptoms (Lack & Wright, 2007; Richardson, Gradisar & Barbero, 2015). These hypotheses need to be empirically tested.

6.5.2 Neuroticism

DSWPD patients scored higher on the Anxiety, Depression and Self Consciousness facets of the Neuroticism dimension. This factor is identified by increased instability and fluctuations in moods, self-defeat, worrying and self-pity (McCrae & Costa, 1992a; McCrae, Costa & Busch, 1986). Elevation in this dimension is typically associated with psychiatric conditions (Costa & McCrae, 1992b). Reports of such maladjustments are described in various papers on DSWPD and fit with the view that these emotional dispositions develop as a result of DSWPD (Alvarez et al., Dagan et al., 1996; Shirayama et al., 2003; Takahashi et al., 2000). DSWPD patients commonly present with an array of psychological disorders such as personality, learning, hypochondria, obsessive compulsive disorder (OCD), etc. (Alvarez et al., 1992; Dagan et al., 1996; Shirayama et al., 2003; Takahashi et al., 2000). Neuroticism, depression and anxiety scores according to the $DASS_{21}$ and NEO-PI-R (Tables 6-1 and 6-4, respectively) suggest that neurotic tendencies and psychiatric issues in our DSWPD sample exist and depression, is particularly correlated with patients' neurotic tendencies.

Neuroticism was not heightened in the present sample of N24SWD as was predicted. According to previous literature and the outcome of $DASS_{21}$ and NEO-PI-R scores in the present study, neuroticism and psychiatric issues in N24SWD could be as prominent as they are in DSWPD (Hayakawa et al., 2005; Takahashi et al., 2000; Uchiyama & Okawa, 2007). Other studies identify schizophrenia, bipolar disorder, depression, OCD or schizoid personality following the onset of N24SWD. Perhaps the one outlier within the N24SWD sample could be explained by a lack of external pressures for this patient to adjust and improve their sleeping pattern. This may have provided some protection against the development of psychiatric symptoms (Shiryama et al., 2003).

This N24SWD patient may have a healthier psychological profile because they only recently developed the condition or had less societal demands that enforce pressure to adjust their sleep times (i.e., was unemployed). In the largest review of sighted N24SWD patients (Hayakawa et al., 2005), it was found that 28% of the 57 patients, who were studied presented with psychiatric problems prior to the onset of the sleep disorder. On the other hand, 34% of the patients developed depression after the onset of the circadian misalignment (Hayakawa et al., 2005). In DSWPD, development of the sleep disorder seems to be bi-directionally associated to psychosocial problems (Takahashi et al., 2000). This may also extend to N24SWD patients such that those who do not have elevated Neuroticism scores prior to the onset of N24SWD may begin to experience psychiatric vulnerability and increase in neuroticism as a result of their apparently uncontrollable sleeping pattern. This stands to be empirically tested using a longitudinal study with a larger sample of N24SWD patients as well as treatment studies. It would be important to answer if those who are successfully treated and remain stable, gradually show improvements in their personality variables, Moreover, perhaps patients who can be successfully treated already possess healthier psychological profiles. This would be an important area of investigation.

There is a difference between DSWPD and N24SWD patients on the impulsiveness facet, suggesting that N24SWD patients better tolerate frustration and resist temptation (Costa & McCrae, 1992b). This could inform the psychological distinction between DSWPD and N24SWD, suggesting that N24SWD patients are more placid and will more readily accept issues surrounding their sleep disturbance, hence allowing themselves to 'free-run'. Instead, DSWPD patients may weave between acceptance and attempts to improve their lifestyles, which may or may not be advantageous to their circadian alignment (Wilhelmsen-Langeland et al., 2012).

6.5.3 Extraversion

Relative to controls, both patient groups reported lower Extraversion and particularly feel positive emotions. Similarly, Wilhelmsen-Langeland et al., (2013b) and Shirayama et al.[14] also found that

DSWPD lacked control of emotional expression and exhibited elevated social nervousness. These findings can now extend to N24SWD patients who appear the greatest introverts. It is atypical that DSWPD patients are less Extraverted since DSWPD patients are characteristically evening-types whose diurnal preference has been associated with greater Extraversion (Adan et al. 2012; Randler, 2008). It is suggested that DSWPD patients feel more lonesome and do not perceive themselves as sociably active (Wilhelmsen-Langeland et al., 2013), which also extends to N24SWD.

Coupled with patients' neurotic tendencies and disagreeableness, introversion could result in greater likelihood to maintain wakefulness during the conventional sleep time in an attempt to shy away from society. Evidence of social withdrawal could result in loss of social cues that typically facilitate circadian rhythm entrainment (Shirayama et al., 2003). This vicious cycle may in turn further exacerbate the disorder. In this instance, introversion is more causative rather than the result of DSWPD. A longitudinal or treatment study would reveal the order in which these develop.

6.5.4 Agreeableness

Lower agreeableness in the patient samples was not an expected finding, as Wilhelmsen-Langeland et al. (2013) did not report differences in their DSWPD sample. Still, the findings of the present study relate to previous literature on diurnal preferences, with significant and positive associations between morningness and agreeableness (Adan et al. 2012; Randler, 2008; Tsaousis, 2010). Hence, lower agreeableness scores are usually associated with evening-type scores that are typically reported by DSWPD patients.

In particular, DSWPD and N24SWD patients reported notably lower scores on the facets of Trust and Straightforwardness, indicating skepticism of others, proneness to deception, hiding true feelings and stretching the truth (Costa & McCrae, 1992a; Costa & McCrae, 1995). This aspect may be a precipitating factor in the maintenance of the disorders, as patients may feel cynical toward help from others (e.g., clinicians, family, friends). These factors could further exacerbate patients'

likelihood to shy away from society and isolate themselves (Ehlers, Frank, & Kupfer, 1988; Frank, Swartz, & Boland, 2007).

This personality profile would pose barriers for patients to request or accept help from others to resolve issues. Those who attempt to intervene with patients' issues (e.g., parents, clinicians) should be urged to develop inter-relational trust when dealing with disagreeable patients (Ehlers, Frank, & Kupfer, 1988; Frank, Swartz, & Boland, 2007). Building dependence could prompt patients to accept advice of people they trust and feel comfortable to speak openly when they are struggling with compliance or cannot implement specific interventions into their lives (Baron & Zee, 2007). In addition to addressing their low conscientiousness traits, developing trust may help account for the decreased success in treating DSWPD and mitigating their higher relapse rate.

6.5.5 Conclusions

This investigation leads to hypotheses that these personality dimensions could be viewed as causes and/or effects of DSWPD and N24SWD. The conscientiousness dimension seems a causal predisposing factor towards DSWPD and N24SWD, particularly lower dutifulness, achievement striving, and especially self-discipline. Conversely, dimensions of neuroticism (i.e., anxiety, depression, vulnerability), higher DASS scores and lower positive emotions in extraversion, competence in conscientiousness, trust in agreeableness, and feelings/aesthetic in openness could be the consequence of living with the conditions. Future work would beneficially inform the disorders by tracking personality changes during pubertal development and adolescence (i.e., when lifestyle and sleeping patterns undergo profound changes and incidence of DSWPD markedly increases) to understand the cause and effect of these variables.

Understanding basic emotional, interpersonal, experiential, attitudinal and motivational styles is important for successfully implementing clinical treatments for many types of psychological disorders (Costa & McCrae, 1992a). This study gives further support that the NEO-PI-R constitutes an important diagnostic tool for DSWPD and N24SWD. Motivational interviewing has been

recently proposed as effective in changing sleep behaviours (Cain, Gradisar, & Moseley, 2011; Gradisar, Smits, & Bjorvant, 2014). Gradisar, Smits, & Bjorvant, (2014) provide a model for motivational stages to improving adolescents' sleep habits. This method would be particularly useful for those seemingly more susceptible to extreme circadian delays, who are also low on conscientiousness and motivation to change. Findings of the present study suggest addressing low extraversion and agreeableness factors by improving patients' competency for social inclusion and building interpersonal trust. These factors could enhance social cues that in turn increase the ability to entrain circadian systems.

CHAPTER 7 GENERAL DISCUSSION

7.1 Overview of Discussion

The broad aim of this dissertation was to use an innovative laboratory method to determine circadian rhythm-based causes of DSWPD. Its purpose was to provide results that will allow the development of effective treatments and prevention programs for this sleep disorder. This final chapter of the thesis provides an overview of the aims and the main findings presented in Chapters 3 to 5. We discuss the implications of the thesis findings in the theoretical context and apply the updated findings of aetiology to the nosology of the disorder. Clinical significance of these results, methodological considerations and future research directions are discussed before concluding remarks are summarised.

7.2 Overview of Dissertation Aims

The potential for circadian regulation to influence human physiological, psychological and social variables (Dagan et al., 1996) is unsurprising considering the ubiquity of circadian systems in most living organisms. Circadian misalignment can be experienced in numerous contexts, such as incorrect timing of the sleep/wake cycle, the feeding rhythms, or misalignment of central and peripheral rhythms (Barion & Zee, 2007). In situations where the circadian misalignment has consequences for the individual (i.e. quality of life, impaired functioning), a diagnosis of a specific disorder is made. The most pertinent diagnoses are Circadian Rhythm Sleep-Wake Disorders (CRSWD; AASM, 2014, APA, 2013). These may consist of transient, 'extrinsic' disorders such as jetlag, shift-work typically caused by external circumstantial factors, or 'intrinsic' disorders that are considered of organic origin, such as Delayed Sleep-Wake Phase Disorder and Non-24-hour Sleep-Wake Rhythm Disorder (N24SWD; AASM, 2014; APA, 2013). It remains unclear how circadian rhythms particularly in intrinsic CRSWD, become misaligned and why they are persistently difficult to treat.

Empirical literature indicates several mechanisms are involved in manifestation of DSWPD and that it is likely of heterogenous nature (Regestein & Monk, Dagan & Eisenstein, 1999). Circadian misalignment in humans and particularly DSWPD, has been associated with: (1) polymorphisms of circadian clock genes suggesting a genetic basis (2) alterations between circadian phase-markers relative to sleep timing (3) aberration in homeostatic sleep drive (4) longer circadian period length (5) increased responsivity to phase-delaying evening-light and/or decreased responsivity to phase-advancing morning-light (6) psychological, environmental and behavioral aspects (Bartlett, Biggs, & Armstrong, 2013; Bass, 2012; Gradisar et al., 2011; Hiller et al., 2014; Jones et al., 2013; Lam & Levitan, 2000; Regestein & Monk, 1995; Wilhelmsen-Langeland et al., 2013b). The aim of the present dissertation was to further investigate the biological and behavioural basis of Delayed Sleep-Wake Phase Disorder (DSWPD). Most of the aforementioned theories and hypotheses regarding the cause of DSWPD have limited experimental support and are not reinforced by experimental or clinical evidence. Similarly, current treatments based on these theories and clinical treatment outcomes have limited empirical backing (Auger et al., 2015).

7.3 Summary of Findings

7.3.1 Chapter 3. Main Aims

The immediate problem of DSWPD patients is their delayed sleep/wake pattern. A delay in the circadian oscillator is presumed to drive their delayed sleep/wake pattern. Chapter 3 aimed to address two theoretical questions including 1) do the rhythms in DSWPD remain delayed because of significantly longer *taus* compared to normal sleepers? 2) Are the longer *taus* in DSWPD significant contributing factors that prevent patients from phase-advancing their sleep/wake cycle and circadian rhythms, which would presumably resolve their DSWPD problem? These questions can be addressed firstly by confirming that DSWPD and N24SWD patients do, indeed, have longer taus than entrained normal sleepers.

To our knowledge, this was the first study to use gold standard measures of melatonin for circadian rhythm *tau* assessment in DSWPD, and the third to explore if DSWPD may be caused by a longer than normal circadian temperature rhythm *tau*. Previous works by Campbell and Murphy (2007) and Micic et al., (2013) supported the proposed explanation in very limited samples. The present study aimed to expand previous findings in a larger group that may be more widely representative of this patient population. Our results confirmed the long-standing suspicion that DSWPD patients have longer *taus* of both circadian temperature and melatonin rhythms. This suggested that longer circadian *taus* contribute to stronger tendencies for DSWPD patients to phase delay and thus greater difficulty in phase advancing.

Furthermore, Non 24-hour Sleep-Wake Rhythm Disorder (N24SWD) patients are quite rare in the general population thus it was serendipitous that we discovered four in the process of recruiting DSWPD patients. Studying their rhythms could elucidate important information about the aetiology of N24SWD. Hence, when we encountered 4 patients with N24SWD during recruitment, we included them for this protocol. By comparing N24SWD and DSWPD, results could also inform distinctions and similarities between these CRSWD. We found that *taus* are longer still in N24SWD patients compared to DSWPD and controls. The persistent pressure to delay sleep-wake rhythms would be great in DSWPD and even greater still in N24SWD. Yet the delayed sleep timings and severity of DSWPD symptoms was explained by only some of the variance in length of *taus* exhibited in a time free, highly controlled environment. Indeed, not all DSWPD and N24SWD patients presented with abnormally long *taus*. A secondary finding was that in approximately half of the DSWPD sample, circadian rhythms were not abnormally delayed or significantly longer than normal. These results give further validation that DSWPD is a heterogeneous condition and in accordance with previous empirical findings, suggest that a combination of biological, genetic, social and psychological factors would contribute to the disorder (Alvarez et al. 1992; Dagan, 2002;

Gradisar et al. 2014; Lack & Wright, 20112; Micic et al., 2016; Regestein & Pavlova, 1995; Takahashi et al., 2000).

7.3.2 Chapter 4. Behavioural Rhythms

In this chapter we further analysed the *taus* of the vigilance, subjective and objective sleepiness rhythms as measures of behavioural circadian tendency in our highly controlled laboratory environment. Our aim was to investigate if phase angles of entrainment (PAE) differed between DSWPD, N24SWD patients and controls. Specifically, based on previous empirical evidence in morning- and evening-types (Lack et al., 2009), we explored if *taus* of behavioural measures were significantly longer in patients relative to controls, thus leading to a larger PAE between their selected sleep time and circadian phase. This was based on the notion that sleepiness is an important determiner of sleep timing in people's habitual environment (Gradisar & Lack, 2004). Hence, if DSPWD patients tend to have an evening-chronotype (AASM, 2014), they may have longer sleepiness rhythms, thus a stronger tendency to postpone sleep times thus later light exposure contributing to circadian phase delays.

We did not find evidence that vigilance, subjective and objective sleepiness rhythm *taus* differed between the three groups. However, we did find that DSWPD patients had a significant delay in the interval between their sleep propensity rhythm and core temperature rhythm relative to controls and N24SWD patients. Furthermore, the PAE was significantly correlated with participants' melatonin and core temperature *taus*, such that those with larger PAE between Tmin and the timing of sleep propensity mesor had longer *taus*. This provides further evidence that alterations in PAE can contribute to the stronger tendency to phase delay in the DSWPD (Ozaki et al., 1996; Uchiyama et al., 2000a; Watanabe et al., 2003).

In the laboratory environment, the DSWPD patients had a 2.5-hour lag in the timing of their sleep propensity rhythms relative to the core body temperature rhythms. This was a significantly longer time interval compared to control sleepers whose sleep propensity rhythms followed core

body temperature rhythms by less than an hour. We did not find supporting evidence of longer sleepiness or vigilance rhythms nor PAE differences in N24SWD relative to controls. For this group lower cross correlations between rhythms indicated that greater internal de-synchronisation in measured rhythms, as well as significantly longer core body temperature and melatonin *taus*, were the contributors to the disorder.

7.3.3 Chapter 5. Melatonin Profiles

This chapter explored differences in melatonin secretion profiles between DSWPD patients and controls. The present dissertation and previous empirical literature indicate that melatonin rhythms are delayed in DSWPD. Furthermore, there are significant positive correlations between sleep and melatonin phase markers. Interestingly, while analysing melatonin data for the present investigation, we noticed a decrement in the early rise of the melatonin profiles of DSWPD patients relative to controls. After thoroughly searching the literature, we did not specifically find previous reports of this phenomenon. Rahman and colleagues (2009) also noted a similar effect during their partial DLMO protocol of DSWPD patients. They reported that DSWPD patients might have reduced rates of melatonin secretion onset that relates to a slower offset of melatonin secretion. Indeed, we found a diminished initial surge of melatonin secretion following DLMO but it was not related to a reduced rate of melatonin secretion offset. The diminished rise of melatonin secretion could contribute to delays and the aetiology of DSWPD. Alternatively, one could suggest that the more robust early surge of melatonin secretion in the normal sleepers could help to protect them against phase delays despite them having >24h *taus*.

7.3.4 Chapter 6. Personality Profiles

In 2013 a novel line of research into DSWPD was published assessing the personality differences between this clinical sample and healthy, normal sleeping individuals. This prompted the secondary aim of the present study to further investigate the personality profiles of DSWPD patients as well as

N24SWD patients relative to controls. The results confirmed that personality dimensions assessed by the Big Five Factors significantly differed between the three groups.

DSWPD patients indicated higher scores of neuroticism and significantly lower conscientiousness, extraversion and agreeableness compared to normal sleepers. Furthermore, N24SWD patients were also significantly lower on the conscientiousness, extraversion and agreeableness factors when compared to controls. This finding has the potential to inform the causality and bi-directionality of personality traits and development of sleep issues related to CRSWD. For the DSWPD group conscientiousness was related to lifestyle, circadian and sleep parameters. These results have implications for CRSWD suggesting an important relationship between personality profiles of patients' and their circadian misalignment. Although causal mechanisms between these factors cannot be concluded, this information can inform assessment and treatment of these disorders. The study highlighted the need to assess personality factors to implement appropriate therapies and develop effective long-term N24SWD and DSWPD treatments.

7.4 Implications of Thesis Findings

7.4.1 Longer Circadian *taus*

Present findings suggest that an abnormally long *tau* contributes to the chronic timing delay of the circadian system and the clinical symptoms of DSWPD (AASM, 2014). Comparing circadian rhythms of the two clinical subgroups, DSWPD and N24SWD, was advantageous to further understand how circadian misalignments and sleep delays occur in CRSWD. This information is crucial to elucidate how circadian rhythms become delayed and are persistently difficult to entrain in both DSWPD and particularly for N24SWD. The large effect sizes of *tau* differences between N24SWD patients and controls indicate that the free-running nature of sleeping patterns of N24SWD patients are likely to be predominantly biologically driven. The near-25-hour pace at

which patients' SCN tends to oscillate would make it nearly impossible for rhythms to synchronise to the 24-hour day. A daily pressure to phase-delay by one hour would require the N24SWD patient to effectively phase advance by that same amount each day to remain entrained or at least stabilised on a 24-hour period but a later circadian phase as are the DSWPD patients. Such findings of de-synchrony are particularly prominent in totally blind patients (Nakagawa, Sack, & Lewy, 1992; Sack, Brandes, Kendall & Lewy, 2000; Miles Raynal & Wilson, 1977) who cannot receive photic stimulation necessary to entrain to the natural environment (Reppert & Weaver, 2002; Saper, Scammel & Lu, 2005). In essence, blind patients' sleep patterns passively 'free-run' temporally as they cannot be entrained by the exogenous light/dark cycle. However, since our patients are fully-sighted individuals who should be able to utilise external time cues (i.e., *zeitgebers*), they are apparently insufficient to overcome an endogenous SCN pressure to delay by approximately an hour each day.

7.4.2 Circadian *tau* Variability

Approximately half of the DSWPD patients who show *taus* comparable to controls cannot be ignored and suggest a different causal mechanism for sleep delays in this sub-group. Differences in *taus* have been found in other non-clinical subsamples including shorter *taus* of morning- versus evening-types (Duffy, Rimmer, & Czeisler, 2001), African-Americans versus European-Americans (Eastman, Suh, Tomaka, & Crowley, 2015), females versus males (Duffy et al., 2011) and elder versus older adults (Kripke et al., 2005). In all of the aforementioned studies, the measured *taus* ranged from 23.79-24.77h. According to measures of melatonin in Chapter 3, 37% of our DSWPD patients fall into the range of having *taus* within the 4 N24SWD patients. Hence, these DSWPD patients' difficulty to maintain earlier, desired sleep times could be attributed to mechanisms similar to N24SWD patients.

7.4.3 Other Aetiological Factors

While some of our DSWPD patients have *taus* that are still longer compared to these sub populations, others fall within the range of normal sleepers in the present study, as well as those aforementioned. As such, there are likely to be other explanations why this subpopulation appear unable to entrain to their expressed desired earlier sleep times. While *taus* make a significant contribution to the aetiology of both DSWPD and N24SWD, we have found differences in personality characteristics, phase angle of entrainment and less robust melatonin secretion, that also appear involved in the aetiology of the disorders. Hence, for example, one DSWPD patient may present with significantly longer *taus* and persistent delays in circadian timing that they cannot overcome. Another patient however, can possess personality factors (e.g., lower conscientiousness and high neuroticism) as well as alterations in PAE. This patient may have a normally entrained circadian timing, however due to lower conscientiousness and high sleep propensity at the time they are required to wake-up, they may 'snooze' or sleep-in. In turn, this would mask light exposure necessary for most humans to entrain (Czeisler et al., 1999) and remain stabilised to the solar light/dark cycle (Khalsa et al., 2003).

Perhaps a limitation of the present study is that such strict confinement conditions cannot quantify the effect of behaviours, psychological variables and self-selected exposure to light at different time/intensities on circadian timing in DSWPD. Even in our 'clean' sample of diagnosed patients, thoroughly screened for caffeine, alcohol, BMI, depression, anxiety, stress, etc., there is greater variability of lifestyle measures than in controls. These factors also have associations with, and the potential to influence sleep and circadian timing. Like light, inappropriate timing of food (Reid et al., 2014; Stokkan, Yamazaki, Tei, Sakaki & Menaker, 2001), caffeine (Sherman et al., 2011) and alcohol (Ruby, Brager, Depaul, Prosser & Glass, 2009) intake can result, either directly or indirectly, in circadian disturbances. Depression seems to be linked to circadian misalignment although this relationship is not yet elucidated (Kripke et al., 2008; Lovato & Gradisar, 2014; Quera

Salva et al., 2011). While depression is typically associated with early morning awakening, patients with depression tend to exhibit diurnal fluctuations in mood such that positive feelings occur later in the evening (Peeters et al., 2006). In non-clinical samples positive emotional affect also exhibits a robust circadian fluctuation with enhanced mood associated with temperature maximum (Murray et al., 2009). Therefore, evening-type individuals may defer bedtime to extend the 'feeling good' period that for them occurs later in the evening. Furthermore, negative feelings of sleepiness, grogginess, etc., could contribute to 'avoiding' the morning (Lack & Wright, 2012). As per previous evidence and findings of the present dissertation, this would delay the light/dark cycle and thus circadian rhythms (Ozaki et al., 1996; Uchiyama et al., 2000a; Watanabe et al., 2003).

7.4.4 Non-Circadian DSWPD

The subgroup of DSWPD patients without an abnormal circadian-based delay, also support previous and current claims that DSWPD is a heterogeneous disorder stemming from a physiological tendency to sleep late (Dagan & Eisenstein, 1999; Regestein & Monk, 1995). Our results reinforce the notion that this tendency is due to a multitude of factors and not one sole contributor. Exposure to morning light seems the most protective factor of entrainment for individuals' circadian system (Khalsa et al., 2003). If individuals omit adequate and appropriately timed light exposure in the morning, the circadian system and associated sleep time are likely to shift later due to *taus* being spontaneously longer than 24-hours – observed not only in DSWPD and N24SWD patients but also most healthy controls. As such, for most people sleeping in late in the morning and avoiding morning light exposure, or gaining too much light exposure in the evening will likely result in a phase delay irrespective of *tau* length. Even normal sleepers with presumably normal *taus* show approximately 23-minute delays of circadian rhythm on days in which they sleep in 2-3 hours later than normal (Taylor et al. 2008).

This factor is becoming particularly problematic in 24h societies where light is easily manipulated, and can be overly abundant late at night contributing to phase delay. Furthermore, it

can be dimmed at inappropriate times in the morning that could oppose the endogenous tendency to phase delay. In essence, while light is considered the most significant zeitgeber, individual behaviours (Badia, Myers, Boecker, Culpepper & Harsh, 1991; Stokkan, Yamazaki, Tei, Sakaki & Menaker, 2001), emotions (Shirayama et al., 2003; Wilhelmsen-Langeland et al., 2012; Wilhelmsen-Langeland et al., 2013b) and lifestyle choices (Dijk, 2013) can have equally pivotal effects on the timing of light exposure, and resultant phase-shifts of SCN and sleep/wake timing. These factors can potentially explain phase delays in those DSWPD who do not exhibit longer *taus*.

7.4.5 Phase Angle of Entrainment

Chapter 4 presents further evidence that larger phase angles between sleep propensity and core temperature rhythms in DSWPD relative to controls are associated with longer *taus*, even in highly controlled laboratory conditions. These results support notions that PAE differences contribute to DSWPD aetiology (Ozaki et al., 1996; Uchiyama et al., 2000a; Watanabe et al., 2003).

The behavioural tendency as a sleep-delaying factor alone could be more malleable and overwritten by social and other external factors (Badia, Myers, Boecker, Culpepper & Harsh, 1991; Stokkan, Yamazaki, Tei, Sakaki & Menaker, 2001). Chapter 4 indicates that behavioural tendencies explain why DSWPD patients. On Table 3-1, addressing ICSD-3, Diagnostic Criterion C, sleep diary/actigraphy data indicate that DSWPD patients slept longer on days of no commitments compared to commitment days, but these differences were not statistically different relative to controls. This phase angle information suggests that DSWPD patients ignore the biological inclination to go to bed and defer their sleep onsets/offsets, particularly on free days. They may be less inclined to do this on nights before commitment days, due to next-day obligations and a required earlier awakening. On nights accompanied by social pressure to awaken early, DSWPD patients seem to be able to fall asleep slightly earlier, at their biologically delayed time. However, on days free of commitments DSWPD patients seem to put off or procrastinate getting to bed, possibly due to behavioural or personality tendencies (Dagan & Eisenstein, 1999; Gradisar et al.,

2011; Wilhelmsen-Langeland et al., 2012; Wilhelmsen-Langeland et al., 2013b). Hence, their tendency to sleep-in appears to contribute to sleep delays by masking the exposure to light at a period that is necessary for circadian entrainment to the 24hour light/dark cycle.

However, our results do not elucidate why other studies have not found PAE differences (Chang et al., 2009; Wyatt et al., 2006; Saxvig et al., 2013). One explanation may be that inter-individual differences between samples of different studies could contribute to inconsistency in previous findings. Perhaps there are abnormalities in homeostatic process in patient cases where PAE differences are observed and not when patients have abnormally long *taus* (Uchiyama et al., 2000a). Future studies are important to unravel these associations.

7.4.6 Personality Factors in Circadian Misalignment

Consistent with previous findings of personality dimension in DSWPD (Wilhelmsen-Langeland et al., 2013b), we found our patients also reported lower conscientiousness, agreeableness and extraversion, but higher neuroticism scores on the NEO-PI-R. Initially, we expected that the less circadian delayed group of DSWPD would exhibit greater psychological difficulties including increased neuroticism, less extraversion, and less conscientiousness. This was based on the assumption that all patients had a DSWPD diagnosis, therefore those with DLMOs within the normal range (e.g. < 23:00 h) could present with sleep delays due to greater differences in personality characteristics. For example, those with normal DLMO time could perhaps exhibit high neurotic tendencies, thus enforcing a delayed or erratic sleep/wake schedule.

This seemed a good rationale, however our findings formed a simpler, unexpected explanation. Instead of finding a negative correlation between more extreme personality dimensions (i.e., higher neuroticism, less conscientiousness) in the less delayed DSWPD, just the opposite was the case. Circadian and sleep phase delays were highly correlated with the psychological measures of neuroticism, introversion, less agreeableness, and less conscientiousness as well as higher depression scores on the $DASS_{21}$. We screened out higher moderate to severe depression

(Depressions > 18 on the $DASS_{21}$) and perhaps lost as many as 10-12 potential DSWPD with higher depression scores, so we had a somewhat truncated range of depression and severity of sleep phase delay. Even so, we had significant correlations with DLMO, sleep phase timing, and depression in our remaining DSWPD participants.

Misalignments in sleep timing or awakenings throughout the night are commonly associated with psychological problems (Bartlett, Biggs, & Armstrong, 2013; Lovato & Gradisar, 2014; Okawa & Uchiyama, 2007; Regestein & Monk, 1995; Richardson, Gradisar, & Barbero, 2015). Thus, it is unsurprising that DSWPD patients report other psychosomatic issues related to their sleep (Takahashi et al., 2000). Our conclusions form the view that instead of circadian delay and psychological impairments being complementary and reciprocal in our DSWPD sample, they lay on a continuum of DSWPD severity reflected in sleep delay, circadian delay, psychological impairments, and distress.

7.4.6.1 Chicken or Egg… What comes first?

However, these findings raise important questions regarding the development of the sleep disorder and specific traits. That is, do personality characteristics lead to poor sleep behaviours and eventually DSWPD, or does the development of DSWPD eventuate to changes in personality traits? Given the findings of this dissertation are correlational, there is no basis to infer cause and effect. It seems intuitive that personalities cannot be affected by sleeping patterns and instead, personalities are more likely to influence individuals' sleeping pattern. Hence, personality dimensions will affect choices in bedtime and, in turn, bedtime and rise time will produce phase delays in circadian timing.

However, qualitative studies indicate that individuals' sleeping difficulties could have implications on their self-image, as society tends to attribute individuals with delayed sleep characteristics as being lazy and unmotivated (Wilhelmsen-Langeland et al., 2012). Perhaps through uncertainty, self-defeat and learned helplessness, patients take on this identity to attempt to come terms to with their problems (Wilhelmsen-Langeland et al., 2012). Few studies have investigated

primary or secondary DSWPD, where 'primary' indicates the onset of DSWPD before other morbidities, contrary to 'secondary' DSPWD where sleep delays are the result of other morbidities. Preliminary psychological studies suggest that puberty and age of onset are the greatest predictors of developing 'primary' DSWPD, while psychological triggers including mental disorders, as well as professional and personal issues lead to development of 'secondary' DSWPD (Takahashi et al., 2000; Yamadera et al., 1998). Future prospective and treatment studies of the development of both DSWPD and N24SWD are important to understand bi-directionality of issues related to the disorders. These are subsequently discussed in 'Directions for Future Research'.

7.5 Application of Aetiology to Future Nosology

This project gives further evidence that DSWPD and N24SWD cannot be taken simply as endogenous biological rhythm disorders (AASM, 2014; APA, 2013). Despite thorough and careful screening of what seemed a homogenous group of DSWPD patients for the present study (i.e., surface symptoms of sleep delays and inability to sleep earlier), outcome measures indicate heterogeneity in the presentation of the disorder. We provide supporting evidence for general phase delays, a longer *tau*, a blunted melatonin profile, phase-shifts of behavioural sleep rhythms, and contributions of personality factors. Perhaps we selected DSWPD patients whose disorder stems from various aetiologies.

7.5.1 Heterogeneity of DSWPD

For long-term, clinical success and management, it is important to begin to dissect the disorder into practicable components that facilitate treatment of the actual perpetuating issues rather than surface symptoms. This thesis presents some categorisation of DSWPD at least from the biological, behavioural and personality bases. We propose that underlying biological processes are most likely bearers of predisposition to DSWPD in those with significantly longer *taus*. However, in other instances where *taus* are within the normal human range, personality and psychosocial factors may

lead to specific lifestyles, habits and attitudes that drive delays in sleep. These may also have some familial basis, for example, the habits learned from parents, other family members or peers.

If future research can 'phenotype' classes of DSWPD, there will be much greater potential to tailor treatments specific to different DSWPD causes and achieve better long-term outcomes for patients. Phenotyping would entail running large-scale studies to investigate the different causal mechanisms for the delay in sleep timing (i.e., proposed in the present study and previous empirical literature). This would enable differential diagnoses and catering treatment according to specific defects rather than surface problems (i.e., using 'one size fits all' approach), as is the current conventional intervention (i.e., melatonin and bright light therapy).

7.5.2 Differentiating DSWPD

There is a possibility of DSWPD subtypes and different drivers of the disorder (Campbell et al., 1999; Lack & Wright, 2012; Regestein & Pavlova, 1995). As such, the most efficacious interventions are those devised upon individual symptoms and physiological anomalies. Given evidence indicates alternate mechanisms for DSWPD such as longer *taus*, personality differences, altered zeitgeber responsivity (Aoki, Ozeki, & Yamada, 2001; Cain et al., 2014) and homeostatic sleep dysfunction (Uchiyama et al., 2000b), possible prospective diagnostic options may therefore include in-lab assessments of patients' ECP *tau* lengths, PRC curves or measures of multiple sleep latency tests (MSLT) to investigate sleep drive.

However, these would likely require extended periods of time for continuous overnight stays in temporal isolation. This precision in diagnosis is unlikely to justify the expense of running such analyses and using resources for lengthy protocols. Following our assessment of circadian *tau* using the present protocol, it is estimated that similar clinical assessments would cost patients approximately US$3000 for core temperature *tau* evaluations and ±US$3700 for DLMO assessments. A 24h MSLT constant routine to measure homeostatic sleep drive alone, would be a less costly alternative, nevertheless would still cost patients a minimum of US$1100. In addition,

both of these estimates disregard indirect costs such as the time and effort required from the patient, or hours of missed paid work due to temporal isolation. Therefore, it would be advantageous for future studies to identify clinical predictors of underlying aetiological factors of DSWPD that are most cost- and time-effective.

Indeed, the ability for 'pen-and-paper' questionnaires to predict underlying aetiological factors (i.e., *tau*, DLMO, sleep homeostatic pressure) would advance the clinical field. Taillard et al. (2003) reveal associations between morningness-eveningness and reduced sleep homeostasis, while Emens et al. (2009) suggest morningness-eveningness and phase-angle differences are highly related. Further work can elucidate these relationships to develop a quick, inexpensive tool to clinically predict sleep homeostatic reduction/phase-angle differences based on eveningness scores. A self-reported DSWPD severity index derived from habitual and required sleep time discrepancies may further facilitate more accurate diagnoses.

Molecular experiments indicate significant, positive correlations between longer *taus* and later sleep parameters (Hida et al., 2013). Perhaps assessments of circadian in vitro *taus*, that culture patient cell types to determine period length, could also offer a more affordable detection tool.

While the ICSD-3 recommendation for chronotype assessment is important, the use of excessive sleepiness and insomnia scales may also be warranted for clinical diagnosis and determination of severity. Symptoms of insomnia and excessive sleepiness are highlighted as major indicators of CRSWD in diagnostic manuals (AASM, 2014; APA, 2013) and appear to be a prominent process in DSWPD (Richardson, Gradisar, & Barbero, 2015). Even in our present sample DSWPD patients indicated longer sleep latencies relative to controls (please see Table 5-1). Therefore, an insomnia index, as well as a daytime sleepiness measure could facilitate more quantifiable and precise diagnosis. These techniques could inform and assist clinicians to better tailor successful interventions in the future.

7.6 Clinical Implications and Significance of Findings

7.6.1 Chronobiologic Treatments

The findings from the main aims of the present investigation that explored circadian *tau* differences give important clinical information regarding treatment of DSWPD and N24SWD. Outcomes suggest that overall DSWPD patients have significantly longer *taus* of salivary melatonin and core temperature than controls, but not as long as the *taus* of N24SWD patients. Results give further support for rigorous and persistent morning bright light therapy to reset the timing of the sleep-wake cycle and circadian rhythms. Furthermore, evening melatonin administration would assist to ensure a sufficient rise in melatonin at the appropriate time in the evening. In particular, very small doses (0.1-0.3mg) may be effective closer to bedtime (2-3 hours) and still assist with higher than physiological levels following DLMO. These methods of treatment have been successful for treating DSWPD and N24SWD. In particular, combination treatments of both bright light therapy and melatonin administration show greatest efficacy in advancing patients' sleep timing (Crowley & Eastman; 2015Saxvig et al., 2014; van Maanen 2013; Wirz-Justice et al., 2004). In this section we apply results of the study to this mainstream treatment of circadian misalignment. Importantly we extend treatment recommendations to novel findings from the present dissertation.

7.6.1.1 Bright Light Therapy (BLT).

As has been highlighted, post-awakening BLT can reset the time of sleep-wake cycles by advancing circadian phase, thus consolidating *taus* (Czeisler et al., 1989; Duffy & Wright, 2005; Khalsa et al., 2003; Rosenthal et al., 1990). Still, poor compliance to BLT tends to truncate patient outcomes (Barion & Zee, 2007) and with DSWPD patients' strong predisposition to avoid morning light, blue light may be a more successful option than white light, since it can still be effectively implemented later in the morning (Revell, Molina & Eastman, 2012). In effect, it is unsurprising that BLT compliance is low in DSWPD patients considering their low conscientiousness and typically unmotivated to improve their sleep. Coupled with aversion to morning bright light (Lack & Wright,

2012) and sleep restriction to ensure morning-light exposure, would deter most patients from complying with BLT. Therefore, to maintain the effectiveness of BLT on physiological outcomes, alternative strategies need to be considered. For example, behavioural tendency to delay, sensitivity to light and patients' general motivation/willingness comply should be taken into account when applying therapies to facilitate better BLT outcomes.

Other approaches need to accompany BLT to supplement its efficacy on the SCN and retiming circadian rhythms. Indeed, empirical literature shows that 2,000-10,000 lux light intensity is necessary to advance the circadian system (Chesson et al., 1999; Duffy & Wright, 2005). However, when BLT is used in conjunction with another form of therapy, such as Cognitive Behavioural Therapy (CBT), circadian rhythms can be reset at less intense light levels (Gradisar et al., 2011). This indicates that other cognitive and behavioural factors can explain patients' treatment success. One recent study indicates a novel technique of passive BLT administration that could potentially improve outcomes for DSWPD patients (Zeitzer, et al., 2014). Participants who were exposed to 2-msec of light flashes (~2995 lux at corneal level) administered every 30-sec 2-3 hours after habitual bedtime, exhibited a significant phase delay of 0.48 h in the timing of their DLMO and their sleep architecture was not affected. Hence, this study stands to be replicated and particularly in a clinical sample with evidence of significant phase-advancement since this change is typically harder to achieve due to the natural SCN tendency to delay. Nevertheless, similar novel approaches and modified strategies of BLT may be necessary to devise and empirically test to improve compliance and efficacy of BLT in DSWPD patients.

7.6.1.2 Melatonin Treatment.

According to clinical practice guidelines for treatment of intrinsic CRSWD (Auger et al., 2015), strategically timed melatonin administration appears an effective and practical form of DSWPD treatment, although empirical evidence remains rather weak and scarce. Timed melatonin seems most efficacious in combination with post-awakening BLT and behavioural treatments, albeit

further research is necessary to confirm these conclusions. The results of decreased melatonin secretion during the first half of the nocturnal secretion period provide further evidence that exogenous melatonin could supplement the apparent secretion deficiency post DLMO in DSWPD patients and assist to advance the SCN timing. On the other hand, sustained or slow release melatonin preparations should be avoided for this purpose since they may maintain plasma melatonin long enough to stimulate the phase delay portion of the melatonin PRC resulting in contra-indicated outcome.

7.6.2 Clinical Management

It is crucial to recognize that significantly longer *taus* can explain the frequently reported long-term relapse following current form of therapy (Regestein and Monk, 1995, Wilhelmsen-Langeland 2013a, Saxvig et al., 2014). While commonly used treatments for DSWPD and N24SWD may allow patients to wake up at a regular and conventional time, the difficulties of maintaining this schedule on a long-term basis could make them susceptible to relapse. In some patients, even one day of untimely exposure or lack of exposure to zeitgebers has the potential to delay circadian timing by up to an hour. Therefore, if compliance to treatment decreases following therapy, circadian rhythms will likely extend back to the endogenous SCN timing, resulting in desynchrony with the 24-hour day. Sleep education and knowledge about circadian rhythms may be of supplementary help to combined conventional treatment. Informing DSWPD patients of their potentially longer *taus* and high susceptibility to relapse may facilitate treatment compliance. This information may prompt patients to become more stringent to gaining appropriately timed exposure to zeitgebers, thus augmenting treatment, especially in the long-term. This information could augment compliance, at least for those who are motivated to change their sleep behaviours. Even more broadly, informing ‘high-risk’ prevalence populations (e.g., adolescents) can reduce susceptibility of developing DSWPD (Cain, Gradisar, & Moseley, 2010).

Due to the large amount of overlap in *tau* distributions between the groups, interventions addressing other causes also need to be considered. At present treatment for DSWPD is quite uniform and typically utilise interventions aimed at re-aligning the timing of circadian rhythms. However, our results further support the heterogeneity of the disorder, and many patients encompass a combination of biological and lifestyle factors that perpetuate the delay in DSWPD. Findings of the present dissertation may further support this claim, with a substantial proportion of DSWPD patients who did not exhibit significantly longer *taus* or even delayed circadian rhythms compared to normal sleepers. These patients indicated other potential mechanisms contributing to their delayed sleep including suppressed melatonin secretion, altered phase angles and/or personality differences. This group of patients may suffer adjustment difficulties perpetuated by other factors, rather than an abnormal circadian rhythm characteristic (Regestein & Monk, 1995; Dagan & Eisenstein, 1999). Hence, for this clinical subsample, in addition to conventional bright light and melatonin therapy, we recommend Cognitive Behavioural Therapy (CBT; Gradisar et al., 2011; Trauer et al., 2015) to correct behaviours and teach good sleep hygiene.

7.6.2.1 Chronobiologic Treatment for Non-Circadian DSWPD.

Whether both aforementioned chronobiologic therapies are therapeutic for the non-circadian delayed DSWPD sub-group or phenotype is an interesting question. It may be the case that these patients have a consistently abnormal phase relationship between sleep/wake timing and circadian phase with an abnormally large phase angle (e.g. relatively late sleep onset time compared to DLMO). In this phenotype circadian timing may still have a strong effect on sleep/wake timing but that places or determines sleep at an abnormally delayed circadian phase. In this case phase advancing circadian phase with the use of chronobiologic therapy should still be efficacious.

7.6.2.2 Cognitive Behavioural Therapy (CBT).

In addition to biological mechanisms, it is important to address any behavioural and cognitive elements that may contribute to DSWPD in order to optimize treatment outcomes (Barion & Zee,

2007; Bartlett, Biggs, & Armstrong, 2013; 2013; Gradisar et al., 2011; Hiller et al., 2014; Jones et al., 2013; Lack & Wright, 2007; Wilhelmsen-Langeland et al., 2013b). Previous literature readily suggests that DSWPD patients typically show heightened arousal in the evening, during a time when conventional sleepers are falling asleep (Dagan & Eisenstein, 1999; Gradisar et al., 2011). Certainly in some cases, this may be the result of an endogenous delay that drives the wake maintenance zone to an unfavourably late time when patients would prefer to be falling asleep. However, in cases where a circadian delay is not apparent, psychological and diurnal preferences may be driving the inclination to stay up later. For example through association with positive reinforcement, positive feelings, and when patients know they will be most productive they remain awake to make the most of their good feeling period.

7.6.2.3 Conditioned Sleep Onset Insomnia.

Perhaps the opposite is true for some patients, such that they feel anxious to attempt sleep due to repeated inability and past failures of initiating sleep quickly. These individuals may have developed psychophysiological or conditioned sleep onset insomnia (Lack & Wright, 2012; Richardson, Gradisar, & Barbero, 2015). Table 5-1 shows the DSWPD group sleep latencies around 54 minutes even at their later bedtimes on free days that are and significantly longer than the controls. The DSWPD group on the whole indicate some level of sleep onset insomnia. This is not surprising given numerous occasions of trying to fall asleep at earlier bedtimes on work nights but being relatively unsuccessful. Their failure to fall asleep, or trying to initiate sleep during the WMZ and becoming anxious or frustrated knowing of their fixed wake-up time in the morning and their impending impairment from inadequate sleep (Lack & Wright, 2007).

As such, it may well be the case that the delayed sleep pattern in these non-circadian delayed DSWPD is not due to circadian phase and, therefore, chronobiologic treatments would be ineffective. This group of patients may suffer adjustment difficulties perpetuated by other factors, rather than an abnormal circadian rhythm characteristic (Dagan & Eisenstein, 1999; Regestein &

Monk, 1995). Hence, for this clinical subsample, CBT aimed at increasing behavioural control over sleep/wake schedules, teaching good sleep hygiene and circadian education, and mitigating maladaptive beliefs that contribute to inappropriate sleep/wake schedules would be most indicated (Gradisar et al., 2011). For sleep onset insomnia stimulus control therapy (Morin et al., 2006) would be indicated in addition to morning BLT. Evening melatonin can be soporific when administered before DLMO on those early night occasions (Lack & Wright, 2007). These therapy suggestions clearly require empirical investigation.

However, without a measure of circadian phase in the clinical diagnosis of DSWPD, all tools in the armamentarium including chronobiologic and CBT would be indicated. Irrespective of the aetiology, additional cognitive behavioural therapy (CBT) is effective for correcting behaviours and cognitions, as well as teaching good sleep hygiene particularly in adolescents (Gradisar et al., 2011). All treatments should also be tailored based on degree of phase delay, severity of associated symptoms, lifestyle circumstance, feasibility of adherence, as well as motivation to comply (Barion & Zee, 2007; Gradisar, Smits, & Bjorvatn, 2014).

7.6.2.4 Sleep and Circadian Education.

Although light is ubiquitous in all lifestyles, knowledge of circadian systems is rare in the general population. With constant emergence of technological devices that transmit light and cause alerting affects, it is becoming increasingly more important to educate individuals about circadian systems and give insight about factors that exacerbate delayed sleep (Chang et al., 2015; Cajochen et al., 2011; Cajochen, 2015). Teaching good behaviours around sleep hygiene and circadian timing is beneficial at least in adolescents (Cain, Gradisar, & Moseley, 2011). Thus it would be beneficial to inform patients about how exercising, consuming caffeine/energy dense food at inappropriate times, as well as keeping irregular sleep schedules such as sleeping in late on weekends may aggravate their condition. This may promote better sleep and help to avoid circadian delay in most instances.

7.6.2.5 Motivational Interviewing.

In Chapter 6 we discuss how and why Motivational Interviewing might benefit patients. The proposed differences in personality profiles, can be used to augment treatment outcomes by giving clinicians insight into the dilemmas patients face. For example, Wilhelmsen-Langeland et al., (2013b) suggest using the (NEO-PI-R) to assess personalities and derive treatments to suit the characteristics of patient personalities. If the standard 240 item full version is seen as impractical the short version (NEO FFI) is only 60 items with only a marginal loss of internal consistency. Those low on achievement-striving for example, may benefit from inclusion of motivational interviewing as a component of treatment to evoke patients' intrinsic motivations for changing sleeping behaviour (Costa & McCrae, 1992a). While those less organized may need assistance in keeping appointments and reminders about treatment implementation.

Expenses of using DLMO to confirm endogenous circadian delay have been elucidated throughout the literature (Gradisar, Smits, & Bjorvatn, 2014; Keijzer, Smits, Duffy & Curfs, 2014; Molina & Burgess, 2011). Perhaps an alternative to measuring DLMO to gauge circadian misalignment could be supplemented by personality investigations. Our results suggest that low conscientious personality characteristics are more endogenously delayed and more difficult to treat. According to our findings in Chapter 6 the association between Tmin/DLMO and the conscientiousness dimension was relatively strong in a small sample (r=0.52). Therefore this dimension can potentially be a predictor of the circadian phase delay. The treatment for these individuals would focus on restructuring lifestyles, rebuilding and establishing patient relationships with their social support structures as well as clinicians. The aim of this would be to redevelop their connection with the society and 24-hour rhythm of societal demands (Frank, Swartz, & Boland, 2007). It would aim to create a client-psychologist connection such that the patient complies to treatments and reports accurate feedback to the practitioner. Such feedback could be translated to seek alternate intervention methods that would better suit the lifestyles of patients who score low on

conscientiousness, are lackadaisical and would otherwise likely cease treatment and attempts to fix their sleeping pattern.

7.6.2.6 Behavioural Treatments.

In DSWPD, it is important to address external contributors of the disorder (e.g., poor sleep hygiene and other maladaptive behaviours). DSWPD can cause significant and permanent reductions to patients' quality of life (Alvarez et al., 1992; Crowley et al., 2007), which in turn can further exacerbate the condition. Depression, as well as misuse of substances, tobacco, alcohol and caffeine is greater in patients with DSWPD than healthy individuals (Baron & Reid, 2014; Crowley et al., 2007; Kripke et al., 2008; Lovato et al., 2013). Previous studies and our results indicate significant associations between DSWPD and health/social disruptions, such as increased depression and anxiety symptoms, as well as co-morbidity with other disorders (e.g., personality, attention-deficit, learning, obsessive-compulsive, affective disorder; Alvarez et al., 1992; Crowley et al., 2007), Moreover, empirical literature indicates that patients report higher medication and substance use such as antacids, hypnotics, tobacco, alcohol and caffeine while indicating poor academic achievement, failure, absenteeism, loss of employment. These behaviours may be, in part, a response to the DSWPD condition but also exacerbate it. Therefore addressing these maladaptive behaviours and lifestyle choices should be considered adjunct to effective therapy outcomes.

7.6.2.7 Chronotherapy Treatment.

Intuitively, DSWPD and N24SWD patients with longer *taus* are likely to benefit most from chronotherapy since their tendency to delay is greatest. If predictors of *tau* can be determined, this is something clinicians could consider in the future when developing patient-specific treatment regimes. Chronotherapy may also be preferred for those who display delayed behavioural tendencies, enjoy staying up late and avoid morning light.

However, this method of treatment of CRSWD, particularly for those patients who are less conscientious, may not be advisable for the following reasons. Some case studies show favourable

outcomes of chronotherapy for DSWPD patients (Czeisler et al., 1981). However, controlled clinical trials do not exist to support its efficacy or safety and studies indicate high relapse rates from this treatment method (Auger, 2009). Furthermore, another patient reported development of N24SWD from their original DSWPD diagnosis following chronotherapy thus resulting in an outcome worse than the original disorder (Oren & Wehr, 1992).

As mentioned in the introduction, the treatment period during which sleep time is progressively delayed, may lead to extreme disturbances to sleep/wake schedules (Lack & Wright, 2007). Due to aggravated sleep disturbance during the entrainment period and considering patients' other precipitating factors associated with their condition, this therapy could worsen the impact for patients. The transition to stabilized sleep period from the forced delay regime may be too abrupt or very difficult for those who are low on conscientiousness or high on neuroticism. The apparently easier method of retiming the body clock in this manner may suit the personality and behavioural tendencies of these patients. As such abruptly enforcing rigorous sleep/wake schedules to maintain the achieved preferred time could pose difficulty for these patients, thus causing relapse. For this reason, omitting the use of chronotherapy and focussing strictly on early evening melatonin administration, morning BLT and CBT may be produce better long-term outcomes. These methods stabilize and maintain sleep times, are less intrusive on lifestyles and will ensure patients are instilled with beneficial information regarding sleep behaviour, rather than counter-intuitive strategies.

7.6.2.8 Lifestyle Adjustments.

Lifestyle adjustments may be a favourable outcome for some DSWPD and N24SWD patients. It seems that DSWPD is predominant in evening-types who have a tendency and preference to remain awake past conventional bedtimes. One strategy may be to create 'pro-zeitgeber' bedroom environments for those vulnerable to or suffering from DSWPD or N24SWD. Clinicians could instruct patients not to turn on overhead lights and only use dimmers. In extreme cases, they may

design strategies that deploy 'circadian-friendly' evening and morning environments considering artificial light has a profound effect for alertness. In the evening, clinicians may suggest installation of ceiling lights with low levels of lux and long wavelength (yellow to red) and suggest use of software programs for devices that filter aversive and potentially phase-delaying short wavelength light. This could be extended to mornings by mounting high-intensity bedside lamps with blue-green wavelength to immediately expose the patient to SCN advancing stimuli. Even unmotivated, non-compliant patients could benefit from this non-invasive, passive environmental setup.

Patients with particularly long *taus* and repeated failure to respond to treatment may discover favourable outcomes by simply coming to terms with their disorder. Although this by no means treats either DSWPD or N24SWD, it could be an alternative strategy for reducing the impact and burden of the disorders for some patients and their families. If the patients and surrounding social networks can accommodate the condition, this may be an appropriate alternative to safeguard associated psychological and mental burden of living with the disorder. This could include seeking employment that facilitates work during patient's biological 'feel best times'. Working from home, working nightshifts or late in the evenings may be some strategies for example. In this manner, patients may not be defeated about their issues and perhaps feel they have more control over their health and lifestyle, thus improving their psychological health and quality of life. For example, one of the N24SWD participants with the longest *tau* had a relatively healthy personality profile in contrast to the other two with personality measures. Anecdotally, this participant had accurately understood the circadian physiology underlying his condition and had accommodated to a continuously delaying sleep pattern without apparent negative impact on his personality.

Importantly, these substantial adjustments to lifestyles and patient management should be undertaken under strict supervision of a sleep professional who can monitor the outcomes of therapies and make amendments where necessary.

7.7 Directions for Future Research

Directions for future research have been given extensive consideration in all chapters of this thesis and this section will only extend a few ideas that have not been mentioned previously.

In terms of methodology, Chapter 3 highlights the need to validate the present protocol. It would be important to investigate if short ultradian forced de-synchrony routines yield similar results to longer forced de-synchrony routines (e.g., 28 'hour' days). This would be important not only for research, but for the implementation the ultra-short constant routine in clinical settings. This method could be applied to various diseases and disorders that exhibit underlying circadian rhythm disturbances (e.g., ADHD, Parkisonson's disease).

The next objective would be to attempt phenotyping the DSWPD patients according to the underlying mechanisms contributing predominantly to their disorder and inability to advance their delayed sleep pattern. Although this may prove difficult in a limited sample of 26 DSWPD, it is a logical and essential step forward to subcategorizing the disorder. This research is necessary to better understand its multifaceted aetiology and inform clinicians on efficacious treatments based on patients' specific underlying cause. Future studies employing larger cohorts of the clinical sample would be pivotal to appropriately and sufficiently address this aim.

Scrutiny of personality factors would contribute to the phenotyping of CRSWD disorders and determine if personalities contribute to exacerbating the circadian delays. Given DSWPD onset is typically around pubescence, a longitudinal study of pre-pubescent children into adolescence (i.e., 4-6 year study) may reveal the order of development of the abnormal personality dimensions. Which of these dimensional abnormalities exist prior to DSWPD and which develop following the development of DSWPD? This would be an important question to answer.

Moreover, it would be important to investigate the bi-directionality of personality dimensions and circadian misalignments. Treatment studies that remedy sleep-wake misalignment and measure personality factors as a dependent variable would provide important information regarding the

impact of sleep disturbance on personality dimensions. Contrarily, studies that correct 'self-image' (i.e., adverse personal cognitions and behaviours that lead to sleep disturbance), improve measures on personality dimensions and treat sleep timing as the dependent variable would elucidate if sleep behaviours can resultantly be improved.

7.8 Methodological Considerations and Strengths

7.8.1 Methodological Considerations

Methodological considerations and limitations have been discussed throughout this dissertation where appropriate. However, there are several other weaknesses in the methodology that should be noted when interpreting the findings of the current study.

We cannot explain with certainty why salivary melatonin and core temperature are not more strongly correlated ($r = 0.32$) when previous studies using a different methodology find higher coherence between the variables (Carskadon et al., 1999). Also, melatonin markers exhibit greater associations with some sleep parameters while in some instances, core body temperature, and particularly Tmin, are more affiliated with sleep delays. If the markers are modulated by separate mechanisms, they may take on different *taus* particularly in a highly controlled, constant routine where the SCN does not receive entrainment stimuli to consolidate the peripheral clocks. Laposky and colleagues' (2008) study on sleep loss, may help to elucidate these findings, indicating that during episodes of sleep deprivation, internal rhythms can become desynchronised from one another. Given DSWPD suffer sleep loss more often than controls, this could lead to some desynchrony of rhythms upon commencement of the modified-constant routine. Also, it may be the case that like N24SWD, the DSWPD patients have a much more variable sleep/wake schedule than the controls and thus might have greater internal de-synchronisation (Yamazaki et al., 2000).

Therefore, a transition effect could occur when individuals are placed in a time free environment that might cause the *tau* to shorten or lengthen temporarily. Available results from the

present study did not capture this phenomenon. However, having hourly salivary melatonin samples throughout the entire protocol would have elucidated answers to enable direct comparisons between core temperature and melatonin rhythms. Unfortunately this was not financially feasible within the present study. Therefore, it is recommended that future studies extend the methodology for 5 or 6 days and sample melatonin hourly throughout the protocol. This will enable determination of possible transition effects.

Another consideration is that there are at least 4 known methods for determining DLMO thresholds. Other methods may take into account the area under curve (AUC), upslope of secretion and peak value to derive DLMO thresholds (Molina and Burgess, 2011; Voultsios et al., 1997). The different threshold criteria may result in somewhat different DLMO times on the first evening of the ultradian routine relative to the last night. There is no evidence to suggest which out of the possible methods to determine threshold are most valid, thus our resolve to use an absolute threshold that remained the most constant across all participants and sampling evenings seems most probable in maintaining greatest accuracy.

Hypersensitivity to light has been recently highlighted as a potential contributing factor to DSWPD. When this finding became apparent, one concern was whether the bedroom environment of <10 lux was sufficiently dim to ensure light did not induce melatonin suppression. Recent findings published by Crowley and colleagues (2015) provide supporting evidence that such dim levels could not have affected melatonin secretion in the present sample. Although 15 lux could suppress melatonin in pre- and mid-pubertal adolescents, older adolescents and adults similar to the sample of the present study did not show any effect of suppression at 15 lux. Therefore, in our study the dimmer lights of <10 lux are not likely to mask the endogenous melatonin secretion.

Lastly, Wilhelmsen-Langeland et al.'s publication in 2013b triggered our interest to investigate the personality profiles of our participants. Because we had already started our data collection, we were unable to include the total sample (69%) in the measurement of the NEO 5

personality measures. There is a possibility (although slight) that the earlier recruited participants for whom we do not have personality data may have differed in this data. However, the fact that our results are very consistent with those of Wilhelmsen-Langeland et al. (2013b) suggests that our sample with personality data is representative of the DSWPD population. Nevertheless, circadian differences and available demographics in the sample of respondents and non-respondents to the NEO-PI-R were compared to investigate if any differences were evident between groups and none were noted.

7.8.2 Strengths

To the investigators' knowledge, this was the largest clinical sample to be studied using a forced desynchrony protocol. The current protocol was theoretically justified and achieved promising results that were comparable to past studies. The highly controlled lighting conditions of the present study ensure light as a zeigeber did not affect the measurement of circadian phase or *tau* lengths. All measures of circadian rhythms show clear, robust curves suggesting that careful control of the laboratory environment had effectively eliminated unwanted effects. A predominant strength of the present study was that biological *taus* were measured by salivary melatonin and core temperature, two gold-standard markers of circadian rhythm. Additionally, melatonin could be verified against the measure of core body temperature. It is also the first study to investigate associations between carefully measured, sophisticated biological sleep variables and personality characteristics.

7.9 Conclusions

Based on the findings of the present thesis, it can be concluded that DSWPD patients present with significantly longer endogenous *taus*. Nevertheless, it is not merely a circadian-based disorder. While longer circadian *taus* explain some variance in circadian and sleep delays of DSWPD patients, other pathophysiological and psychological factors contribute to the variability in findings. Among other possible aetiologies, the present dissertation also identified biological, behavioural

and personality factors that contribute to sleep delays in this disorder. Results indicate that altered phase angles of entrainment, lowered initial rates of melatonin secretion, conditioned sleep onset insomnia, as well as personality differences are all potential contributors to DSWPD. Future studies recruiting large cohorts of this clinical population are necessary to identify and differentiate the various aspects contributing to this heterogeneous DSWPD population. Our findings suggest that clinical diagnosis of DSWPD should use all available tools in the armamentarium to diagnose and treat patients. The results of the thesis maintain the importance of using traditional treatment methods of combined morning bright light therapy and strategically timed evening low dose immediate release exogenous melatonin administration to palliate DSWPD. Furthermore, results also support the use of therapies that address cognitive and behavioural factors in DSWPD.

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APPENDICES

APPENDIX A

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PARTICIPANT INFORMATION SHEET

Title of the project

An investigation of the biological body clock in individuals with different sleeping patterns.

Researchers

This is a PhD project conducted by Gorica Micic, under the supervision of Professor Leon Lack and Dr Nicole Lovato.

Invitation to participate

You are invited to participate in this research project but you do not have to be involved, whether you wish to or not is entirely up to you. Whether you take part or not, your medical care/relationship with the university/the services which you receive will not be affected in any way.

Selection

Upon contact, you will be asked to complete five online questionnaires that assess your general health, sleep timing, and sleep quality. If found suitable, you will be invited to participate in our study. If you accept, we will ask you to visit the sleep laboratory, where you will:

- *receive a full explanation of the study, and have any questions answered*
- *be familiarised with the rooms and procedures (e.g., polysomnography – a device used to monitor physiological sleepiness/alertness, saliva collection apparatus, computer tasks, questionnaires)*

At this time, we will ask you to take home and complete a 14-day sleep/wake diary and wear a wrist activity monitor across this same 14-day period. Arrangements will also be made for you to return these to the sleep laboratory at the end of this period. Based on your responses to the initial questionnaire, if you are classified as a suitable, evening-type sleeper, you will be asked to keep a sleep/wake diary and wear an activity watch for an additional 3 days and nights. During this period, we will ask you to go to bed and attempt to initiate sleep at an earlier time compared to your habitual sleep time.

Aims of the project

The project aims to investigate the differences in individuals' biological body clocks, using a 3 day laboratory protocol.

Commitments

Initially, you will be required to respond to a set of five online questionnaires, which will collectively take a maximum of 30 minutes to complete. If selected for the study, you will be asked to attend the sleep laboratory two times over approximately two weeks. The first visit will be approximately half an hour long. We will use this time to acquaint you with the sleep laboratory and the procedures of the study. We will also provide you with an Actigraph and a sleep/wake diary to take home. Two weeks later, you will be asked to attend the sleep laboratory to return the equipment and begin an 80-hour laboratory session, starting on Thursday night (i.e., 6pm) and concluding on early Monday morning (i.e., 2 am).

Summary of procedures

The study procedure will formally commence at 6pm on Thursday night and conclude early on Monday morning (i.e., at 2am).

Before coming to the laboratory

From 6pm Wednesday evening, we ask that you do not consume any alcohol or caffeinated drinks such as coffee, tea, and cola. If you experience any distress during the study, as a result of your withdrawal from caffeine, or for any other reason, you will be free to discontinue your participation in the experiment. On Thursday evening, please have your last meal at home before 4pm. Please avoid eating cheese, chocolate, bananas, asparagus and tomatoes as these foods may interfere with our measures. We advise that you wash your hair and shower at home prior to arrival and refrain from applying any make-up or cosmetics on your face. If possible, also please refrain from using conditioner and avoid the use of other hair products after washing your hair. We also ask that you wear loose fitting clothes (e.g., tracksuits, t-shirts) as these will be worn for the full duration of your laboratory stay.

At the sleep laboratory

On arrival, you will be required to swallow a core temperature ingestible capsule which will monitor your temperature during the laboratory stay. A PSG will then be attached to you (with sticky electrodes), to monitor your sleep and alertness the entire duration of the experiment. Prior to the formal commencement of the experiment we will ask you to insert a disposable rectal thermistor intended for one-time use only. At this time, you will be invited to settle into the bedroom you will be occupying for the remainder of the experiment and asked to complete a 30-minute personality questionnaire. During the full period of the study, you will be confined to a bed in a dimly lit room with no time cues (e.g. no windows, clocks, computers, etc.) and will be asked to continuously stay in bed, sitting in a dimly lit room. Over the 3-day protocol, you will have the opportunity to sleep for a third of your time in the sleep laboratory (i.e., the recommended quota of required daily sleep) at short, regular and frequent intervals. During periods of wakefulness you will be required to provide saliva samples, complete questionnaires, and perform a short computer task. In your spare time you will be able to do homework, read, watch DVDs, listen to music (no television or other time cues such as mobile phones, computers MP3/iPods, watches will be permitted). You will not be served standard meals since consumption of large food portions may interfere with our measures, however small snacks will also be provided at regular, frequent intervals to equate with your daily nutritional requirements.

Monday morning

At the completion of the laboratory session you will be debriefed about the study and will be allowed to shower and have a recovery sleep in the laboratory or alternatively, return home as a passenger in a taxi or with a friend/relative. In the unlikely situation that you experience sleep difficulties following your involvement in the study, you will be offered free treatment from co-investigator Professor Leon Lack, who has extensive experience in the treatment of sleep disorders.

Benefits

You may not receive any direct benefits from participating in this project.

Risks and adverse effects

Some sleep restriction may occur during the sleep study, however you will be provided with enough sleep opportunities throughout the experiment to obtain the recommended sleep quota. Please be aware that you will be expected to avoid caffeine from the Wednesday preceding your protocol to the Sunday (inclusive). We advise that you may or may not react to withdrawal from caffeine (e.g. poor alertness, impaired functioning, headaches, etc). If you feel that these side effects will occur, it is suggested that you do not take part in this protocol. Alternatively, you may decide to reduce or withdraw from caffeine at least a week before entering the laboratory session. Additionally, if you experience any distress during the laboratory session, as a result of your withdrawal from caffeine, or for any other reason, you will be free to discontinue your participation in the experiment.

Compensation

If you suffer injury as a result of participation in this research or study, compensation might be paid without litigation. However, such compensation is not automatic and you may have to take legal action to determine whether you should be paid. Participants in this study are insured under the Flinders University insurance scheme underwritten by Uni Mutual.

Confidentiality

All records containing personal information will remain confidential & anonymous, and no information which could lead to your identification will be released, except as required by law. You will not be identifiable in any reports relating to this project. Information obtained throughout the selection process will only be accessible to the researchers directly involved in this study and will not be given to third parties. Furthermore, data will be de-individualised and averaged for analysis purposes. Information from participants who did not meet all of the stages of the selection process will be shredded.

Publication

The final reports of the project will be available in the Flinders University Library as well as the Thesis Library in the School of Psychology at Flinders University. Furthermore, these outcomes may be published in peer reviewed journals and presented at conferences.

Withdrawal

Your participation in this study is entirely voluntary and you have the right to withdraw from the study at any time without giving a reason. If you decide not to participate in this study, or if you withdraw from the study, you may do so freely, without consequences.

Outcomes

You will not be directly informed of the results of the study.

Expenses and payments

All participants will be financially compensated proportional to their time invested in the project. You will receive a reimbursement of $500 for your time if you complete the experiment in its entirety (i.e. screening and experimental procedures). In the event that you withdraw from the study at any stage you will receive compensation proportional to your duration of involvement.

Contact

For further information about participation and the project please feel free to contact the following persons:

Primary Researcher:

Gorica Micic: e-mail: mici0004@flinders.edu.au telephone: 8201 2377

Project Supervisor and Co-supervisor

Leon Lack: e-mail: leon.lack@flinders.edu.au telephone: 8201 2391

Nicole Lovato: e-mail: nicole.lovato@flinders.edu.au telephone: 8201 2377

Complaints

This study has been reviewed by the Southern Adelaide Clinical Human Research Ethics Committee. If you wish to discuss the study with someone not directly involved, in particular in relation to policies, your rights as a participant, or should you wish to make a confidential complaint, you may contact the Executive Officer on 8204 6453 or email research.ethics@health.sa.gov.au

APPENDIX B

FLINDERS UNIVERSITY
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CONSENT TO PARTICIPATION IN RESEARCH

PRE-SCREENING

I, ...

(first or given names) (last name)

request and give consent to my involvement in the research project: The analysis of the body clock in individuals with different sleep patterns.

I acknowledge the nature, purpose and contemplated effects of the research project, especially as far as they affect me, have been fully explained to my satisfaction by Gorica Micic and my consent is given voluntarily.

I acknowledge that the detail(s) of the following has/have been explained to me, including indications of risks; any discomfort involved; anticipation of length of time; and the frequency with which they will be performed:

I have been fully informed of the purpose and details of all procedures in which I may participate, including:

- Answering questionnaires that seek sensitive information about health issues and sleep habits.
- Undertaking preliminary procedures such as wearing a monitor that detects movements during sleep and completing a sleep/wake diary.

I accept that the screening procedure will take approximately 3 weeks. After the completion of the screening routine I will be notified if I am eligible to participate in the study.

I understand that all personal information obtained during the study will remain confidential and only selected PhD and Honours students, as well as supervisors will have access to this information. It will not be given to unrelated third parties.

I acknowledge that my involvement in this research project may not be of any direct benefit to me. I understand that I am free to withdraw my consent and participation at any stage without reason or explanation without affecting my rights or the responsibilities of the researchers in any respect.

If I choose to discontinue my participation, I am aware that I may not receive the full reimbursement of $500 for my time.

I am aware that should I be selected for the laboratory protocol, I will be expected to avoid caffeine from the Wednesday preceding this protocol to the Sunday (inclusive). I have been informed that this withdrawal may have effects on my wellbeing. I am aware that I can choose to discontinue my participation in the experiment at any time.
I have been given a copy of this consent form, and the researcher has shown me proof of identity in the form of a student card.

I have understood and am satisfied with the explanations that I have been given.

I have been provided with a written information sheet.

I declare that I am over the age of 18 years.

I acknowledge that I have been informed that should I receive an injury as a result of taking part in this study, I may need to start legal action to determine whether I should be paid compensation for the injury.

Signature of Research Participant: .....

Date: .....

I, Gorica Micic..... have described to .....

the research project and nature and effects of procedure(s) involved. In my opinion he/she understands the explanation and has freely given his/her consent.

Signature: .....

Date: .....

Status in Project: Project Leader.....

APPENDIX C

GH&MQ

Thank you for your interest in our study on "Biological Body Clocks & Sleeping Patterns".
We would like to find out some information about your lifestyle and how it may affect your sleeping pattern.

The questionnaire will take approximately 30 minutes to complete and there are 104 questions in total.

Your responses are confidential and there are no right or wrong answers, so please provide responses which most accurately apply to you and your lifestyle.

1. Please enter the unique ID code that was provided to you:

ID code: _____

2. Do you speak and read fluent English?

	Yes	No
.	◯	◯

3. What is your occupation?

_____

4. Do you have any health disorders or disabilities?

	Yes	No
.	◯	◯

If yes, please specify:

_____

5. Do you have any sleep disorders?

	Yes	No
.	◯	◯

If yes, please specify:

_____

6. Have you had a history of substance abuse in the past 12 months?

	Yes	No
.	◯	◯

7. Have you had any psychiatric disorders in the past 12 months?

	Yes	No
.	◯	◯

8. Have you traveled outside of Australia in the past 2 months?

	Yes	No
.	◯	◯

9. Have you worked a night shift in the past 2 months?

(a nightshift is defined as a work period between the hours of 10pm and 8am for 6 or more hours per shift)

	Yes	No
.	◯	◯

10. Have you taken recreational drugs in the past 6 months?

	Yes	No
.	◯	◯

11. Are you currently taking any medication?

(this includes over the counter (OTC) medicines or herbal substances)

	Yes	No
.	◯	◯

If yes, please specify:

12. If yes, are you able to abstain from taking herbal substances (not including OTC medicines) for 2 weeks prior to and 3 days during the experimental protocol?

	Yes	No
.	◯	◯

GH&MQ

13. Approximately how many standard alcoholic drinks do you consume PER WEEK?

Examples of 'one standard drink':
30mL shot glass of spirit
150mL glass of wine
400mL can or a small bottle of beer

14. Please describe which caffeinated beverage type you consume most frequently.

- ☐ Instant coffee
- ☐ Roasted and ground coffee
- ☐ Tea
- ☐ Soft drink
- ☐ Energy drink
- ☐ N/A

Other, please specify:

15. Approximately how many caffeinated drinks do you consume PER DAY?

16. If you consume caffeine, are you able to abstain from caffeine consumption for 3 days during the experimental protocol?

	Yes	No
.	◯	◯

17. Do you smoke?

	Yes	No
.	◯	◯

If yes, approximately how many cigarettes do you smoke per day?

18. If yes, are you able to abstain from smoking for 3 days during the experimental protocol?

	Yes	No
.	◯	◯

19. If female, please enter the date of your last menstruation (i.e., beginning of menses).

	DD	MM	YYYY
Date	/	/	

20. If female, typically, how regular are your menstrual cycles?

- ◯ 1-3 days of difference between months
- ◯ 3-5 days of difference between months
- ◯ 5-7 days of difference between months
- ◯ more than 1 week of difference between months

21. If female, do you use birth control?

	Yes	No	N/A
.	◯	◯	◯

If yes, please specify what form of birth control you use:

22. Are you currently pregnant or lactating?

	Pregnant	Lactating	Neither	N/A
.	◯	◯	◯	◯

23. Please indicate your height (cm) and weight (kg).

Height (cm)	
Weight (kg)	

STQ

24. Approximately, how many hours of combined commitments do you have per week? e.g., school, work, sports trainings, etc.

Number of hours:

25. On average, how many days per week do these commitments occur?

Number of days per week:

26. What time of the day do you usually have to wake up in order to meet your commitments?

- ◯ Early morning: 6am-9am
- ◯ Late morning: 9am-midday
- ◯ Early afternoon: midday- 3pm
- ◯ Late afternoon: 3pm-6pm
- ◯ I have no commitments

27. How would you describe the commencement schedule of your current commitments?

- ◯ I have no commitments
- ◯ I am free to choose my commencement times
- ◯ A regular schedule that commences at the same time each day.
- ◯ A variable commencement schedule that changes from day to day.

28. If you have a variable commencement schedule that changes from day to day, approximately how many hours/minutes of difference is there between the commencement times of commitments between days?

Hours:

Minutes:

29. If you had to rise at 6am everyday for a commitment, what do you think it would be like?

- ◯ It would always be very difficult and unpleasant
- ◯ It would be rather difficult and unpleasant but would gradually improve with time
- ◯ It would be a little unpleasant but no great problem
- ◯ It would be easy and not unpleasant

STQ: Current Sleeping Pattern

30. Please indicate the time when your "body clock" currently allows you to fall asleep most easily?

	HH	MM	AM/PM
Time:		:	

31. How long do you think it would take you to fall asleep at this time?

Minutes:

32. How difficult would you find it to fall asleep at this time?

	Not at all difficult	A little difficult	Somewhat difficult	Very difficult	Extremely difficult
Difficulty:	◯	◯	◯	◯	◯

33. Please indicate the time when your "body clock" currently allows you to wake up most easily?

	HH	MM	AM/PM
Time:		:	

34. How difficult do you find it to wake up at this time?

	Not at all difficult	A little difficult	Somewhat difficult	Very difficult	Extremely difficult
Difficulty:	◯	◯	◯	◯	◯

STQ: Preferred Sleeping Pattern

35. Disregard you "body clock" and only consider your daytime obligations and social commitments for the question below.

Please indicate the time when you would PREFER to fall asleep most easily?

	HH	MM	AM/PM
Time:		:	

36. With your CURRENT sleeping pattern in mind, how long do you think it would take you to fall asleep at this PREFERRED time?

Minutes:

37. With your CURRENT sleeping pattern in mind, how difficult do you think it would be to fall asleep at this PREFERRED time?

	Not at all difficult	A little difficult	Somewhat difficult	Very difficult	Extremely difficult
Difficulty:	◯	◯	◯	◯	◯

38. Disregard you "body clock" and only consider your daytime obligations and social commitments for the question below.

Please indicate the time when you would PREFER to wake up most easily?

	HH	MM	AM/PM
Time:		:	

39. With your CURRENT sleeping pattern in mind, how difficult do you think it would be to wake up at this PREFERRED time?

	Not at all difficult	A little difficult	Somewhat difficult	Very difficult	Extremely difficult
Difficulty:	◯	◯	◯	◯	◯

40. On the following scale, please indicate how motivated you are to change the timing of your CURRENT sleeping pattern to your PREFFERED?

	Not at all motivated	A little motivated	Somewhat motivated	Very motivated	Extremely motivated	N/A
Motivation:	◯	◯	◯	◯	◯	◯

PSQI

41. During the past month, what time have you usually gone to bed at night?

	HH	MM	AM/PM
Usual bed time:		:	

42. During the past month, how long (in minutes) has it usually taken you to fall asleep each night?

Number of minutes:

43. During the past month, what time have you usually gotten up in the morning?

	HH	MM	AM/PM
Getting up time:		:	

44. During the past month, how many hours of ACTUAL SLEEP did you get at night? (This may be different than the number of hours you spent in bed.)

Hours:

Minutes:

For each of the remaining questions, check the one best response. Please answer ALL questions.

45. During the past month, how often have you had trouble sleeping because you:

	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
Cannot get to sleep within 30 minutes	◯	◯	◯	◯
Wake up in the middle of the night or early morning	◯	◯	◯	◯
Have to get up to use the bathroom	◯	◯	◯	◯
Cannot breathe comfortably	◯	◯	◯	◯
Cough or snore loudly	◯	◯	◯	◯
Feel too hot	◯	◯	◯	◯
Feel too cold	◯	◯	◯	◯
Had bad dreams	◯	◯	◯	◯
Have pain	◯	◯	◯	◯

46. During the past month, how often have you had trouble sleeping because of other reasons?

	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
Other:	◯	◯	◯	◯

Please describe the reason(s):

47. During the past month, how would you rate your sleep quality overall?

	Very good	Fairly good	Fairly bad	Very bad
Sleep quality:	◯	◯	◯	◯

48. During the past month, how often have you:

	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
Taken medicine (prescribed or "over the counter") to help you sleep?	◯	◯	◯	◯
Had trouble staying awake while driving, eating meals, or engaging in social activity?	◯	◯	◯	◯

49. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?

	No problem at all	Only a very slight problem	Somewhat of a problem	A very big problem
.	◯	◯	◯	◯

SDS

50. Please indicate to what extent your sleeping pattern has disrupted the following aspects of your life, over the past month.

0 = Not at all
1-3 = Mildly
4-6 = Moderately
7-9 = Markedly
10 = Extremely

	0	1	2	3	4	5	6	7	8	9	10	N/A
Work/School	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Social life	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Family life/Home responsibilities	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯

For the next two questions, "symptoms" may include (but are not restricted to) daytime sleepiness, reduced productivity, poor concentration, lower performance, reduced reaction time, etc.

51. On how many days in the last week did the symptoms related to your sleeping pattern cause you to miss school or work, or leave you unable to carry out your normal daily responsibilities?

Number of days:

52. On how many days in the last week did you feel so impaired by the symptoms related to your sleeping pattern, that even though you went to school or work, your productivity was reduced?

Number of days:

MEQ

53. Approximately what time would you get up if you were entirely free to plan your day?

	5:00 AM – 6:30 AM	6:30 AM – 7:45 AM	7:45 AM – 9:45 AM	9:45 AM – 11:00 AM	11:00 AM – 12 noon
.	◯	◯	◯	◯	◯

54. Approximately what time would you go to bed if you were entirely free to plan your evening?

	8:00 PM – 9:00 PM	9:00 PM – 10:15 PM	10:15 PM – 12:30 AM	12:30 AM – 1:45 AM	1:45 AM – 3:00 AM
.	◯	◯	◯	◯	◯

55. If you usually have to get up at a specific time in the morning, how much do you depend on an alarm clock?

	Not at all	Slightly	Somewhat	Very much
.	◯	◯	◯	◯

56. How easy do you find it to get up in the morning (when you are not awakened unexpectedly)?

	Very difficult	Somewhat difficult	Fairly easy	Very easy
.	◯	◯	◯	◯

57. How alert do you feel during the first half hour after you wake up in the morning?

	Not at all alert	Slightly alert	Fairly alert	Very alert
.	◯	◯	◯	◯

58. How hungry do you feel during the first half hour after you wake up?

	Not at all hungry	Slightly hungry	Fairly hungry	Very hungry
.	◯	◯	◯	◯

59. During the first half hour after you wake up in the morning, how do you feel?

	Very tired	Fairly tired	Fairly refreshed	Very refreshed
.	◯	◯	◯	◯

60. If you had no commitments the next day, what time would you go to bed compared to your usual bedtime?

	Seldom or never later	Less than 1 hour later	1-2 hours later	More than 2 hours
.	◯	◯	◯	◯

61. You have decided to do physical exercise. A friend suggests that you do this for one hour twice a week, and the best time for him is between 7-8 AM.

Bearing in mind nothing but your own internal "clock", how do you think you would perform?

	Would be in good form	Would be in reasonable form	Would find it difficult	Would find it very difficult
.	◯	◯	◯	◯

62. At approximately what time in the evening do you feel tired, and, as a result, in need of sleep?

	8:00 PM – 9:00 PM	9:00 PM – 10:15 PM	10:15 PM – 12:45 AM	12:45 AM – 2:00 AM	2:00 AM – 3:00 AM
.	◯	◯	◯	◯	◯

MEQ

63. You want to be at your peak performance for a test that you know is going to be mentally exhausting and will last two hours. You are entirely free to plan your day.

Considering only your "internal clock", which one of the four testing times would you choose?

	8:00 AM – 10:00 AM	11:00 AM – 1:00 PM	3:00 PM – 5:00 PM	7:00 PM – 9:00 PM
.	◯	◯	◯	◯

64. If you got into bed at 11 PM, how tired would you be?

	Not at all tired	A little tired	Fairly tired	Very tired
.	◯	◯	◯	◯

65. For some reason you have gone to bed several hours later than usual, but there is no need to get up at any particular time the next morning.

Which one of the following are you most likely to do?

	Will wake up at usual time, but will not fall back to sleep	Will wake up at usual time, and will doze thereafter	Will wake up at usual time, but will fall asleep again	Will not wake up until later than usual
.	◯	◯	◯	◯

66. One night you have to remain awake between 4-6AM in order to carry out a night watch. You have no time commitments the next day.

Which one of the alternatives would suit you best?

	Would not go to bed until the watch is over	Would take a nap before and sleep after	Would take a good sleep before and nap after	Would sleep only before the watch
.	◯	◯	◯	◯

67. You have two hours of hard physical work. You are entirely free to plan your day.

Considering only your internal "clock", which of the following times would you choose?

	8:00 AM – 10:00 AM	11:00 AM – 1:00 PM	3:00 PM – 5:00 PM	7:00 PM – 9:00 PM
.	◯	◯	◯	◯

68. You have decided to do physical exercise. A friend suggests that you do this for one hour twice a week. The best time for her is between 10-11 PM.

Bearing in mind only your internal "clock", how well do you think you would perform?

	Would be in good form	Would be in reasonable form	Would find it difficult	Would find it very difficult
.	◯	◯	◯	◯

69. Suppose you can choose your own work hours. Assume that you work a five-hour day (including breaks), your job is interesting, and you are paid based on your performance.

At approximately what time would you choose to begin?

	5 hours starting between 4-8 AM	5 hours starting between 8-9 AM	5 hours starting between 9 AM – 2 PM	5 hours starting between 2-5 PM	5 hours starting between 5 PM – 4 AM
.	◯	◯	◯	◯	◯

70. At approximately what time of day do you usually feel your best?

	5-8 AM	8-10 AM	10 AM - 5 PM	5-10 PM	10 PM - 5 AM
.	◯	◯	◯	◯	◯

71. One hears about "morning types" and "evening types".

Which one of these types do you consider yourself to be?

	Definitely a morning type	Rather more a morning type than an evening type	Rather more an evening type than a morning type	Definitely an evening type
.	◯	◯	◯	◯

DASS

72. Please familiarise yourself with the 4 descriptions across the top of the matrix shown below, then read each statement carefully.

Select the description which best indicates how much the statement applied to you over the past week.

Do not spend too much time on any statement.

	Did not apply to me at all	Applied to me to some degree, or some of the time	Applied to me to a considerable degree, or a good part of time	Applied to me very much, or most of the time
I found it hard to wind down	○	○	○	○
I was aware of dryness of my mouth	○	○	○	○
I couldn't seem to experience any positive feeling at all	○	○	○	○
I experienced breathing difficulty (e.g., excessively rapid breathing, breathlessness in the absence of physical exertion)	○	○	○	○
I found it difficult to work up the initiative to do things	○	○	○	○
I tended to over-react to situations	○	○	○	○
I experienced trembling (eg, in the hands)	○	○	○	○
I felt that I was using a lot of nervous energy	○	○	○	○
I was worried about situations in which I might panic and make a fool of myself	○	○	○	○
I felt that I had nothing to look forward to	○	○	○	○
I found myself getting agitated	○	○	○	○
I found it difficult to relax	○	○	○	○
I felt down-hearted and blue	○	○	○	○
I was intolerant of anything that kept me from getting on with what I was doing	○	○	○	○
I felt I was close to panic	○	○	○	○
I was unable to become	○	○	○	○

enthusiastic about anything				
I felt I wasn't worth much as a person	○	○	○	○
I felt that I was rather touchy	○	○	○	○
I was aware of the action of my heart in the absence of physical exertion (e.g., sense of heart rate increase, heart missing a beat)	○	○	○	○
I felt scared without any good reason	○	○	○	○
I felt that life was meaningless	○	○	○	○

MCTQ: Work Days

For the questions presented on this page please only consider the days where you have to wake up at specified times in the morning to meet social commitments (e.g. school, work, training, etc.).

73. I get up at ... o'clock (24-hour time).

74. I need ... minutes to wake up.

minutes

75. I regularly wake up before / with the alarmclock.

- ◯ Before
- ◯ With

76. I am fully awake from ... o'clock (24-hour time).

77. At around ... o'clock I have an energy dip (24-hour time).

78. On nights before workdays I go to bed at .... o'clock (24-hour time)...

79. ... and it takes me ... minutes to fall asleep at that time.

Minutes

80. True or False?

If I get the chance, I like to take a nap.

- ◯ True
- ◯ False

81. If I have a nap, then I sleep for ... minutes.

Minutes

82. If I take a nap at this time, I would feel terrible afterwards.

- ◯ Yes
- ◯ No

83. Average time per day, I spend outdoors, exposed to direct sunlight.

Hours

Minutes

MCTQ: Free Days

For the questions presented on this page please only consider those days free of social obligations such as late-night parties, school, work, etc.

84. My dream would be to sleep until ... o'clock (24-hour time).

85. I normally wake up at ... o'clock (24-hour time).

86. True or False?

If I wake up at around the normal (workday) alarm time, I try to get back to sleep.

- ◯ True
- ◯ False

87. If I get back to sleep, I sleep for another ... minutes.

Minutes

88. I need ... minutes to wake up.

Minutes

89. From ... o'clock I am fully awake (24-hour time).

90. At around ... o'clock, I have an energy dip (24-hour time).

91. On nights before free days, I go to bed at ... o'clock (24-hour time)...

92. ... and it then takes me... minutes to fall asleep.

Minutes

93. True or False?

If I get a chance I like to take a nap.

- ◯ True
- ◯ False

94. If I have a nap, then I sleep for... minutes.

Minutes

95. True or False?

If I take a nap at this time, I would feel terrible afterwards.

- ◯ True
- ◯ False

96. Average time per day, I spend outdoors, exposed to direct sunlight.

Hours

Minutes

MCTQ: General

97. Once I am in bed, I like to read for... minutes...

Minutes

98. ... but generally fall asleep after no more than ... minutes.

Minutes

99. True or False?

I prefer to sleep in a completely dark room.

- ◯ True
- ◯ False

100. True or False?

I wake up more easily when morning light shines into my room.

- ◯ True
- ◯ False

Self assessment

After you have answered the preceding questions, you should have a feeling to which chronotype (time-of-day-type) you belong to. If for example, you like (and manage) to sleep quite a bit longer on free days than on workdays, or if you cannot get out of bed on Monday mornings, even without a Sunday-night-party, then you are more a late type. If, however, you regularly wake up and feel perky once you jump out of bed, and if you would rather go to bed early than to an evening concert then you are an early type. In the following questions, you should categorise yourself and your family members.

101. Please tick only one possibility for each of the following:

	extreme early type	moderate early type	slight early type	normal type	slight late type	moderate late type	extreme late type
I am...	◯	◯	◯	◯	◯	◯	◯
as a child I was...	◯	◯	◯	◯	◯	◯	◯
as a teenager, I was...	◯	◯	◯	◯	◯	◯	◯

102. Please tick only one possibility:

My parents are/were...

	extreme early type	moderate early type	slight early type	normal type	slight late type	moderate late type	extreme late type
Mother...	◯	◯	◯	◯	◯	◯	◯
Father...	◯	◯	◯	◯	◯	◯	◯

103. Please tick only one possibility:

My siblings are/were:

	extreme early type	moderate early type	slight early type	normal type	slight late type	moderate late type	extreme late type
Brother	◯	◯	◯	◯	◯	◯	◯
Sister	◯	◯	◯	◯	◯	◯	◯
Brother	◯	◯	◯	◯	◯	◯	◯
Sister	◯	◯	◯	◯	◯	◯	◯
Brother	◯	◯	◯	◯	◯	◯	◯
Sister	◯	◯	◯	◯	◯	◯	◯
Brother	◯	◯	◯	◯	◯	◯	◯
Sister	◯	◯	◯	◯	◯	◯	◯

104. Please tick only one possibility:

My partner (girl/boy friend, spouse, significant other) is/was:

	extreme early type	moderate early type	slight early type	normal type	slight late type	moderate late type	extreme late type
.	◯	◯	◯	◯	◯	◯	◯

Thank you!

105. Please use the following textbox to provide further information about your general sleeping patterns and/or lifestyle, if you wish.

Thank you for taking the time to answer our questions.
Your responses will be reviewed and you will be notified of your eligibility for the study shortly.

Kind regards,

Flinders University Sleep Research Team

APPENDIX D

7-DAY SLEEP/WAKE DIARY

Symbols

↓ in bed ● lights out ▬▬ asleep ↑ out of bed **P** – sleeping pill **N** – nap *min*

C – caffeinated drink (eg. Coffee, tea, cola) **A** – standard drink of alcoholic beverage **F** – food

Abbreviations

SOL – Time to fall asleep (in minutes)
WASO – Time spent awake during night – not including SOL (in minutes)
WD – Work Day (e.g., daytime commitments)
TIB – Time In Bed (in hours)
TST – Total Sleep Time (in hours)
FD – Free Day

Example

Day	9 AM	10	11	12 Noon	1	2	3	4	5 PM	6	7	8	9	10	11	12 Midnight	1	2	3	4	5 AM	6	7	8	9	Daytime fatigue	F/WD	SOL	WASO	TST	TIB
Mon			C	F	N 10					F	A		↓	●	▬	▬	▬	▬		▬	▬	↑	F	C		Hi (Med) Lo	WD	35	105	6	9

Name______________________________ **Start Day and Date** ____________________________

Set Sleep Time ____________________________

Day	9 a.m.	10	11	12 Noon	1	2	3	4	5 p.m.	6	7	8	9	10	11	12 Midnight	1	2	3	4	5 a.m.	6	7	8	9	**Fill out in the evening** Daytime fatigue	**Fill out in the morning** F/WD	SOL	WASO	TST	TIB
																										Hi Med Lo					
																										Hi Med Lo					
																										Hi Med Lo					
																										Hi Med Lo					
																										Hi Med Lo					
																										Hi Med Lo					
																										Hi Med Lo					

TST Week Total	TIB Week Total

INSTRUCTIONS FOR THE 7-DAY SLEEP/WAKE DIARY

The diary starts at **9 a.m.** on the first day.

Just before going to bed at night:

- Using the letters below, record the following activities at the appropriate times
  C – caffeine (one C for each cup of coffee, tea, chocolate, glass of cola etc.)
  A – alcohol (one A for each standard alcoholic beverage)
  F – food
  P – sleeping pill
  N – nap (write underneath the estimated minutes of sleep in the nap, see Example overpage)

- Rate your level of daytime fatigue in the Daytime Fatigue column by circling the appropriate level (Hi = High, Med = Medium, Lo = Low) for your feeling of fatigue generally across the day.

- Place a 'down arrow (🡻) at the time you go to bed

- Place a ● just before you turn out your light

When you get up in the morning:

- Mark the time you actually got out of bed with an 'up' arrow (🡹).

- Estimate how long (**minutes**) it took you to fall asleep after turning out the light and enter that estimate in the **SOL** column.

- Estimate how long (**minutes**) you felt you were awake during the night after initially falling asleep and before getting out of bed and enter that estimated time in the **WASO** column.

- Estimate how long you slept in total hours and enter that estimate in the **TST** column.

- Determine the amount of time in hours you spent in bed from (🡻) to (🡹) and enter that figure in the **TIB** column.

- In the **F/WD** colomn, indicate whether on this particular day you awoke to meet commitments or were able to sleep in to your desired wake up time.

APPENDIX E

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FLINDERS UNIVERSITY
ADELAIDE • AUSTRALIA

CONSENT TO PARTICIPATION IN RESEARCH

EXPERIMENTAL PROTOCOL

I, ...

.....

(first or given names) (last name)

request and give consent to my involvement in the research project: The analysis of the body clock in individuals with different sleep patterns.

I acknowledge the nature, purpose and contemplated effects of the research project, especially as far as they affect me, have been fully explained to my satisfaction by Gorica Micic and my consent is given voluntarily.

I acknowledge that the details of the following have been explained to me, including indications of risks; any discomfort involved; anticipation of length of time; and the frequency with which they will be performed:

- Being confined to bed in a time free environment within the Flinders University Sleep Laboratory for three continuous days.
- Completing questionnaires that relate to my health and sleep.
- Completing a 30-minute questionnaire that examines dimensions of my personality.
- Undertaking preliminary procedures such as ingesting a core temperature capsule, applying a rectal thermistor and providing saliva samples.
- Acknowledging that continuous sleep and alertness monitoring will be used during sleep opportunities.
- Accepting that I will follow the researcher's instructions that will detail when snacks are to be consumed and when sleep is allowed. This will involve being woken regularly over the experimental period.
- At the completion of the laboratory session I will be allowed to have a recovery sleep in the laboratory or return home as a passenger in a taxi or with a friend/relative.
- I understand that I will be contacted within a week from the time of my involvement in this experiment for a follow-up assessment and offered sleep counselling if needed.

I understand that all personal information obtained during the study will remain confidential and only selected PhD and Honours students, as well as supervisors will have access to this information. It will not be given to unrelated third parties.

I acknowledge that my involvement in this research project may not be of any direct benefit to me. I understand that I am free to withdraw my consent and participation at any stage without reason or explanation without affecting my rights or the responsibilities of the researchers in any respect.

If I choose to discontinue my participation, I am aware that I may not receive full reimbursement ($500) for my time.

I have been given a copy of this consent form, and the researcher has shown me proof of identity in the form of a student card.

I have understood and am satisfied with the explanations that I have been given.

I have been provided with a written information sheet.

I understand that my involvement in this research project may not be of any direct benefit to me and that I may withdraw my consent at any stage without affecting my rights or the responsibilities of the researchers in any respect.

I declare that I am over the age of 18 years.

I acknowledge that I have been informed that should I receive an injury as a result of taking part in this study, I may need to start legal action to determine whether I should be paid compensation for the injury.

Signature of Research Participant: .....

Date: .....

I,..... have described to .....

the research project and nature and effects of procedure(s) involved. In my opinion he/she understands the explanation and has freely given his/her consent.

Signature: .....

Date: .....

Status in Project:.....

APPENDIX F

FLINDERS UNIVERSITY
ADELAIDE • AUSTRALIA

School of Psychology
Flinders University
GPO Box 2100
Adelaide 5001 Australia

Telephone: (+61 8) 8201 2377

Email: gorica.micic@flinders.edu.au

PROTOCOL INFORMATION SHEET

After careful consideration of your application, you have been selected to take part in the 3 day sleep laboratory experiment at Flinders University.

*Your laboratory session will **begin** at **4:00pm** on*

Thursday.....

Please meet at the Flinders University Sleep Laboratory (room 137SSS) at this time.

*Your laboratory session will **end** at **2:00am** on Monday,.....*

Please read the following information carefully as it is important you adhere to these instructions prior to commencing the experiment. Deviation for these instructions may affect your eligibility. You will also find some handy tips that may be of use to you during the protocol.

Sleep pattern

- A week before you enter the laboratory, we ask that you maintain your typical sleep pattern. During this week, please do not consume any sleep medication (i.e. sleeping pills or herbal supplements).
- 24 hours before you enter the laboratory, please refrain from napping or engaging in intensive-moderate exercise (i.e activities that raise a sweat, such as running)

Meals

- 24 hours before entering the lab, you will be required to avoid caffeine and alcoholic beverages.
- It is highly recommended that you eat dinner before the protocol starts (i.e. Thursday night before 3pm). For this meal, please ensure you do not eat cheese, bananas, chocolate, and tomatoes or drink caffeine or alcohol.
- Remember you will be fed frequent snacks and drinks through the experiment. Please remind us if you have any allergies or dietary requirements.

Clothing & Hygiene

- During the 3 day protocol you will remain in bed. Thus, it is recommended that you wear layered, loose fitting comfortable clothing (i.e. a tracksuit).
- Showering facilities will not be available during the protocol. 24 hours before coming in the laboratory please do **NOT use any hair products** (i.e hair gel, hair spray, leave-in conditioner) **or conditioner**. All male participants, please ensure you are clean shaven for the protocol. These are very important instructions, since these factors will interfere with the electrodes that will be placed on your head/face, used to measure your brain waves. Also, it is highly advisable that female participants arrive at the laboratory wearing NO makeup.
- You will be permitted to brush your teeth but without toothpaste during the study so remember to bring a toothbrush. Also, it may be useful to bring some baby wipes which you may use to refresh yourself.
- Clean towels, body wash, shampoo & conditioner, as well as toothpaste will be made available upon completion of the experiment.

Entertainment

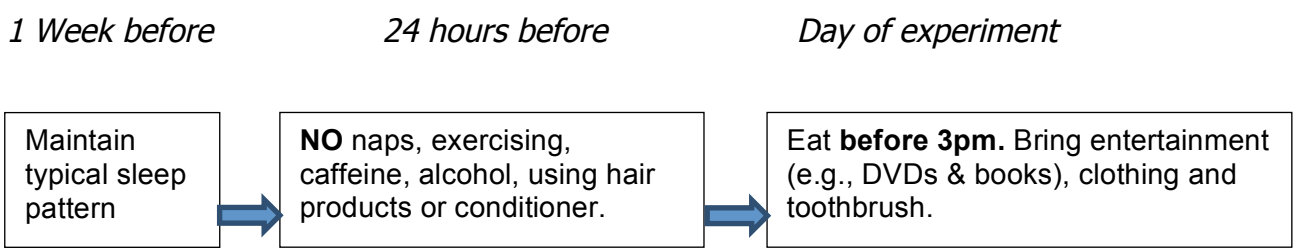
- During the times when you are awake, you are permitted to engage in quiet activities (i.e. watching DVDs, reading, studying etc.) The laboratory has a limited library of DVDs but it is recommended that you bring a few of your own as well as any books, study materials or music. Laptops are not permitted.
- Time keeping devices are strictly prohibited (i.e. watches, mobile phones, mp3 with display screens)
- Please note, any items that you bring into the sleep lab will be inspected. If it is of the researchers' opinion that an item is not suitable for the laboratory environment, it will be locked in a cabinet for the duration of the experiment.

End of protocol

- The protocol will end on a Monday at 2:00am. For safety reasons, please be aware you will not be permitted to drive home at this time. If you are unable to organise for a family member or friend to pick you up, you may sleep in the laboratory until 10am.

Please keep this information sheet as a future reference.

Summary of instructions



If you have any questions about the experiment, please feel free to contact me on 8201 2349 (gorica.micic@flinders.edu.au).

Kind Regards,

Gorica Micic

Psychology PhD Candidate

APPENDIX G

FLINDERS UNIVERSITY
ADELAIDE • AUSTRALIA

School of Psychology
Flinders University
GPO Box 2100
Adelaide 5001 Australia

Telephone: (+61 8) 8201 2377

Email:
gorica.micic@flinders.edu.au

EMERGENCY CONTACT FORM

Participant's name:_____

Emergency contact name:_____

Relationship to participant:_____

Phone:_____

Mobile:_____

Address:_____

I……(participant's name) give you permission to contact the aforementioned contact in the case of an emergency.

END OF EXPERIMENT ARRANGMENTS

Please complete the following questions in regards to your arrangements for the conclusion of the experiment. (Please tick).

- ☐ I will be sleeping at the sleep laboratory until 10am Monday morning
- ☐ I will be picked up by a family member or friend

  Family member/friend name……

  Contact phone number……..

  This person would like a reminder call or text at 1:30pm Sunday night

  - ☐ Yes
  - ☐ No

APPENDIX H

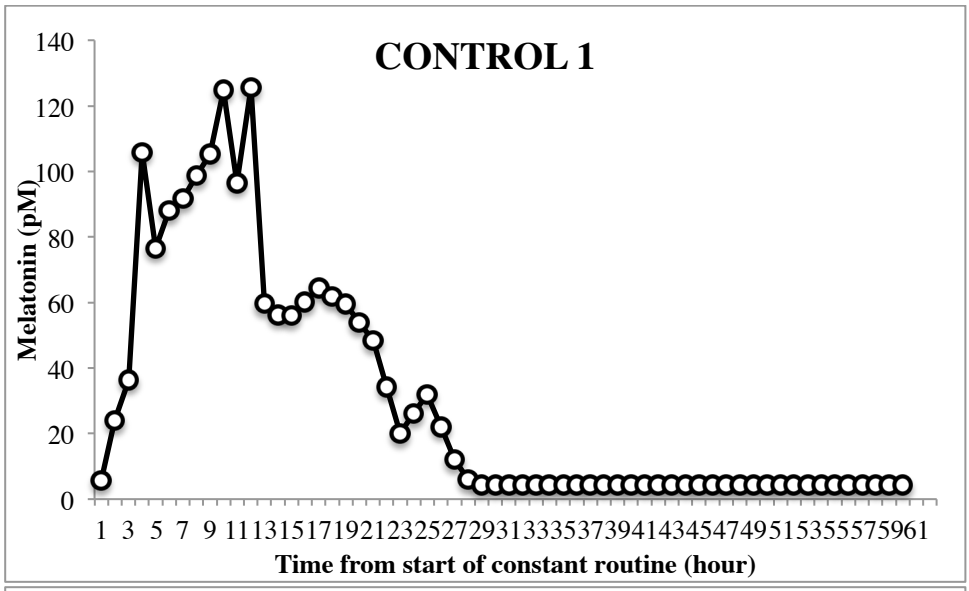
Supplementary Table 4-6

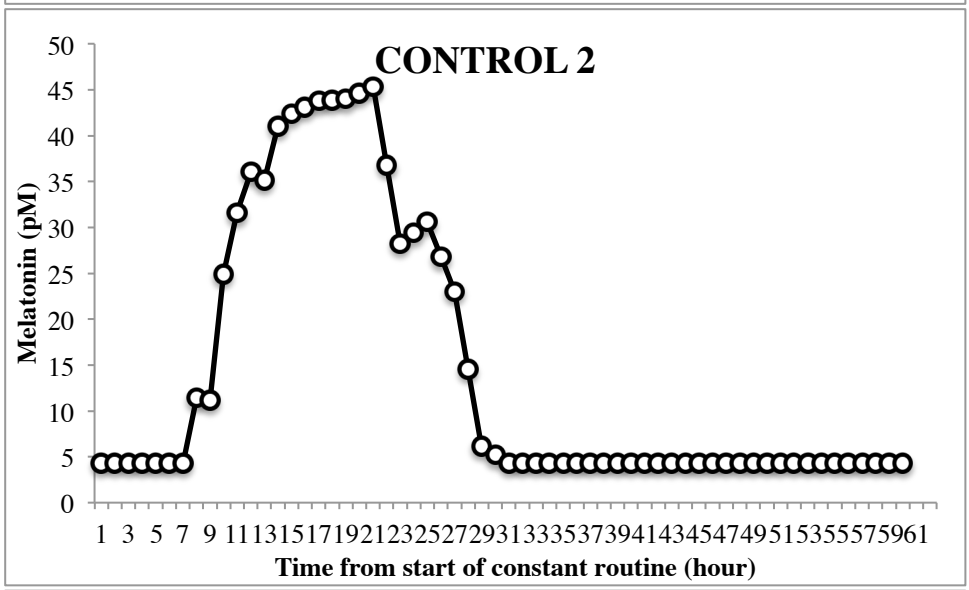
Rhythm associations between core body temperature, melatonin, subjective sleepiness, sleep propensity and vigilance from the initial 40h of the ultradian routine. Rhythm data was phase-lagged to obtain greatest correlation coefficients. Derived phase-lag differences between groups and highest associations between rhythms between for DSWPD, and N24SWD patients relative to controls.

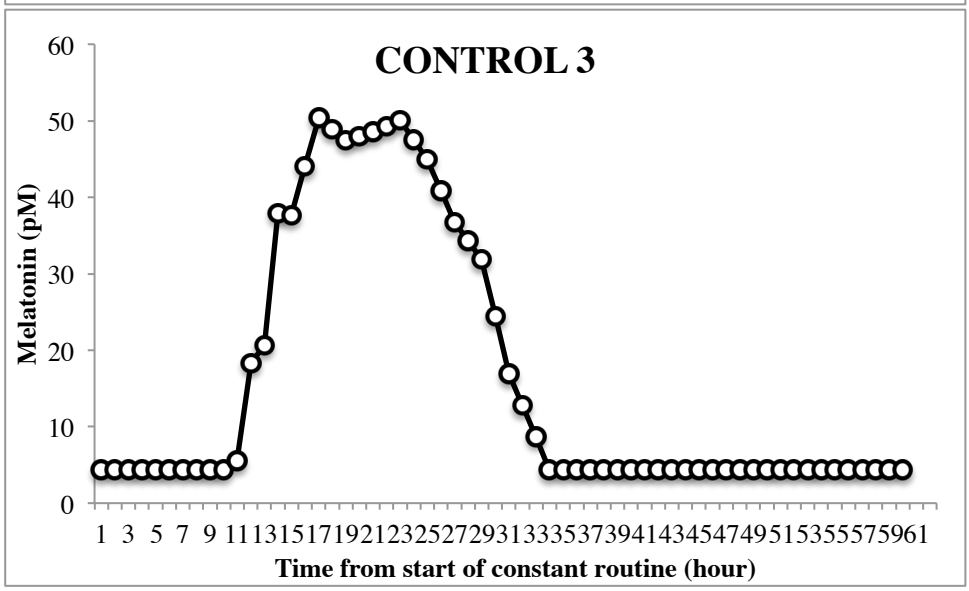
	Means ± Standard Deviation			Mean Difference ± Standard Error Cohen's *d*		
Rhythm Associations	Controls	DSPWD	N24SWD	DSPWD-Controls	N24SWD - Controls	N24SWD - DSPWD
Timing of SS relative to Temp	0.89 ± 1.84h *r*=-0.61 ± 0.16	0.23 ± 1.92h *r*= -0.62 ± 0.20	-1.75 ± 0.50h *r*= -0.44 ± 0.14	0.66 ± 0.58 h *d*= 0.35	**2.64 ± 0.95h** ***d*= 1.55**	**1.98 ± 0.98h** ***d*=0.93**
Timing of SP relative to Temp	0.56 ± 1.15h *r*= -0.63 ± 0.12	1.62 ± 2.26h *r*= -0.61 ± 0.16	1.25 ± 2.75h *r*= -0.52 ± 0.18	**1.06 ± 0.58h** ***d*= 0.54**	0.69 ± 1.40h *d*= 0.46	0.37 ± 1.25h *d*=0.16
Timing of V relative to Temp	0.06 ± 1.73h *r*= -0.51 ± 0.17	0.35 ± 2.10h *r*= -0.54 ± 0.13	-0.67 ± 2.08h *r*= -0.48 ± 0.23	0.29 ± 0.60h *d*= 0.15	0.72 ± 1.10h *d*= 0.41	1.01 ± 1.28h *d*= 0.15
Timing of Temp relative to Mel	1.00 ± 2.72 *r*= -0.78 ± 0.15	1.80 ± 2.68 *r*= -0.72 ± 0.35	0.75 ± 2.50 *r*= -0.83 ± 0.06	0.80 ± 0.85 *d*= 0.30	0.25 ± 1.49 *d*= 0.09	1.05 ± 1.43 *d*= 0.39
Timing of SS relative to Mel	2.71 ± 2.69 *r*= 0.69 ± 0.17	2.64 ± 2.36 *r*= 0.68 ± 0.12	8.75 ± 9.5 *r*= 0.77 ± 0.07	0.07 ± 0.78 *d*= 0.03	6.04 ± 2.51 ***d*= 1.34**	-6.11 ± 4.77 *d*= 1.58
Timing of SP relative to Mel	3.00 ± 1.50 *r*= -0.64 ± 0.15	2.80 ± 2.79 *r*= -0.64 ± 0.13	3.00 ± 3.37 *r*= -0.65 ± 0.10	0.20 ± 0.55 *d*= 0.08	0.00 ± 1.07 *d*= 0.00	-0.20 ± 1.13 *d*= 0.07
Timing of V relative to Mel	3.59 ± 2.79 *r*= -0.56 ± 0.19	2.88 ± 1.99 *r*= -0.63 ± 0.10	1.67 ± 2.52 *r*= -0.56 ± 0.26	0.71 ± .74 *d*= 0.30	1.92 ± 1.73 *d*= 0.70	1.21 ± 1.24 *d*= 0.59
Timing of SP relative to SS	0.39 ± 0.98h *r*= 0.64 ± 0.13	0.27 ± 1.04h *r*= 0.64 ± 0.14	1.00 ± 2.16h *r*= 0.46 ± 0.19	0.12 ± 0.31h *d*= 0.12	0.61 ± 1.10h *d*= 0.50	0.73 ± 1.10h *d*= 0.60
Timing of V relative to SS	-0.67 ± 1.08h *r*= -0.58 ± 0.25	-0.64 ± 0.95h *r*= -0.63 ± 0.13	0.00 ± 1.73h *r*= -0.47 ± 0.15	0.03 ± 0.31h *d*= 0.03	0.67 ± 0.73h *d*= 0.56	0.64 ± 0.63h *d*= 0.60
Timing of V relative to SP	-0.72 ± 0.27h *r*= -0.58 ± 0.11	-0.27 ± 0.72h *r*= -0.58 ± 0.09	-0.67 ± 0.58h *r*= -0.47 ± 0.09	**0.45 ± 0.23h** ***d*= 0.77**	0.06 ± 0.46h *d*= 0.15	0.40 ± 0.44h *d*= 0.57

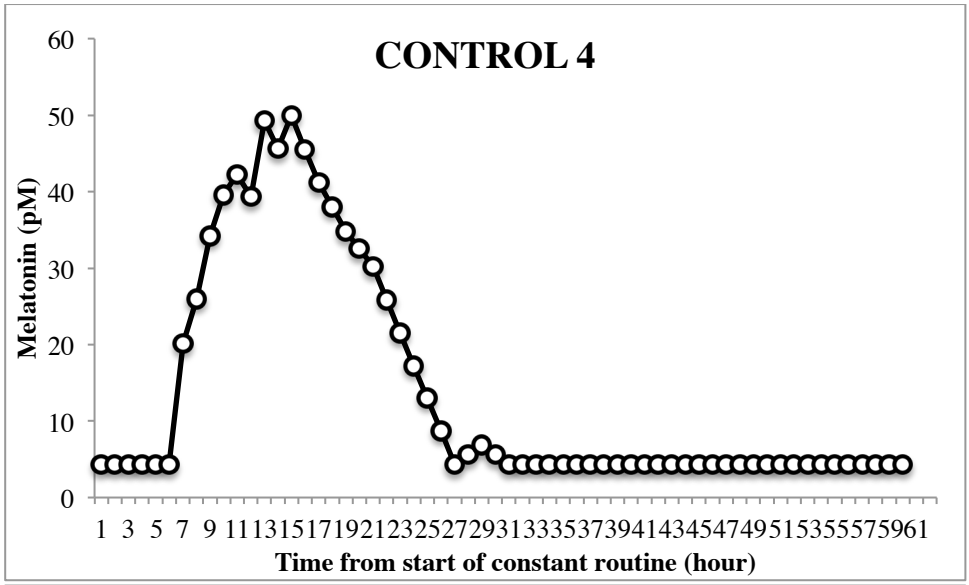
Note. Significant differences in *r*-values between N24SWD patients and controls for Temperature – Subjective Sleepiness rhythm (p=.07) and Subjective Sleepiness – Sleep Propensity rhythm associations (p=0.02) n=1 DSWPD and n=1 control lacked sufficient saliva to determine full melatonin profiles and were excluded from this analyses.
Increasing values of the SS and SP variables indicate greater physiological sleepiness. Lower values of the V and Temp measure indicate greater physiological sleepiness.

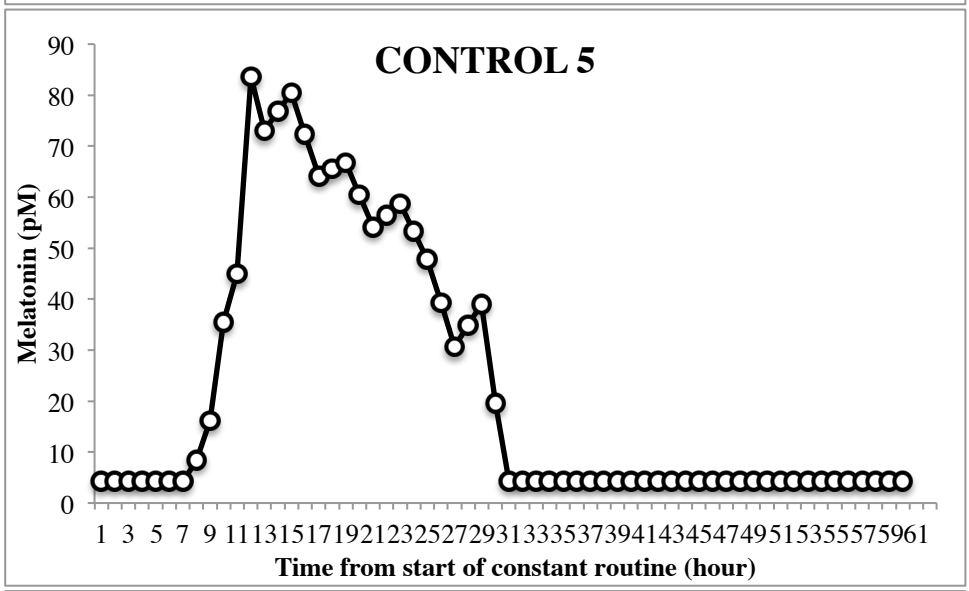
APPENDIX I

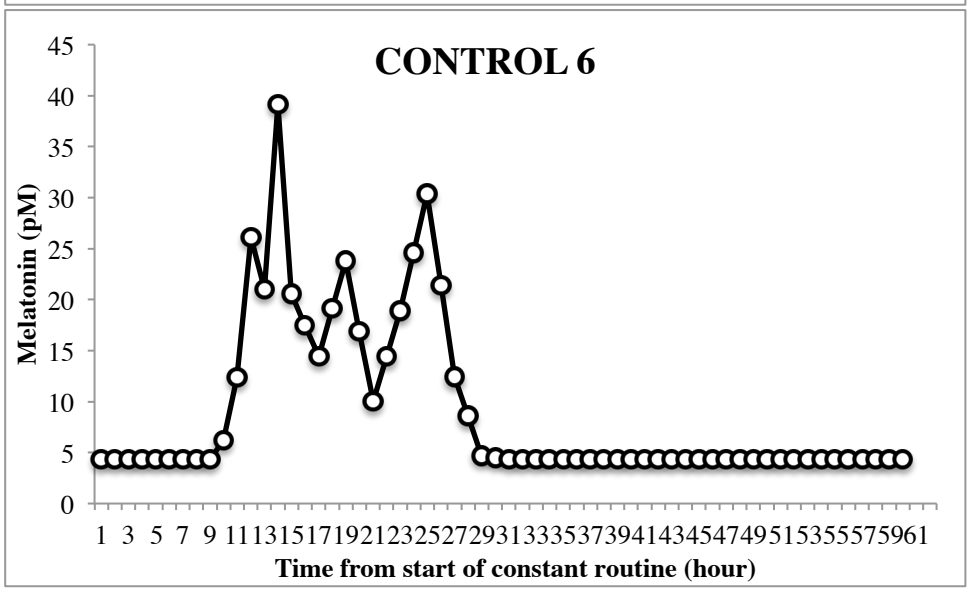

CONTROL 1
Melatonin (pM)
0
20
40
60
80
100
120
140
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61
Time from start of constant routine (hour)

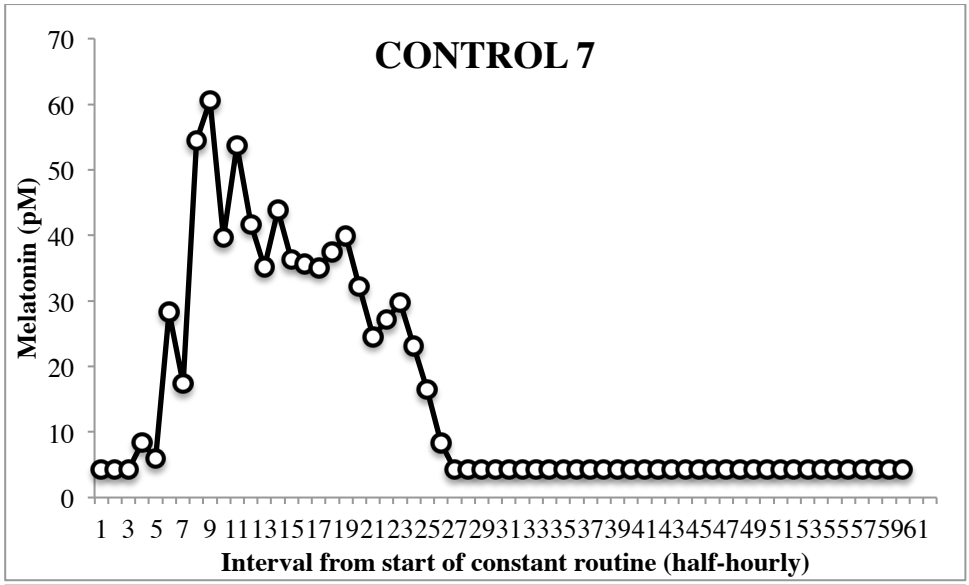

CONTROL 2
Melatonin (pM)
0
5
10
15
20
25
30
35
40
45
50
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61
Time from start of constant routine (hour)

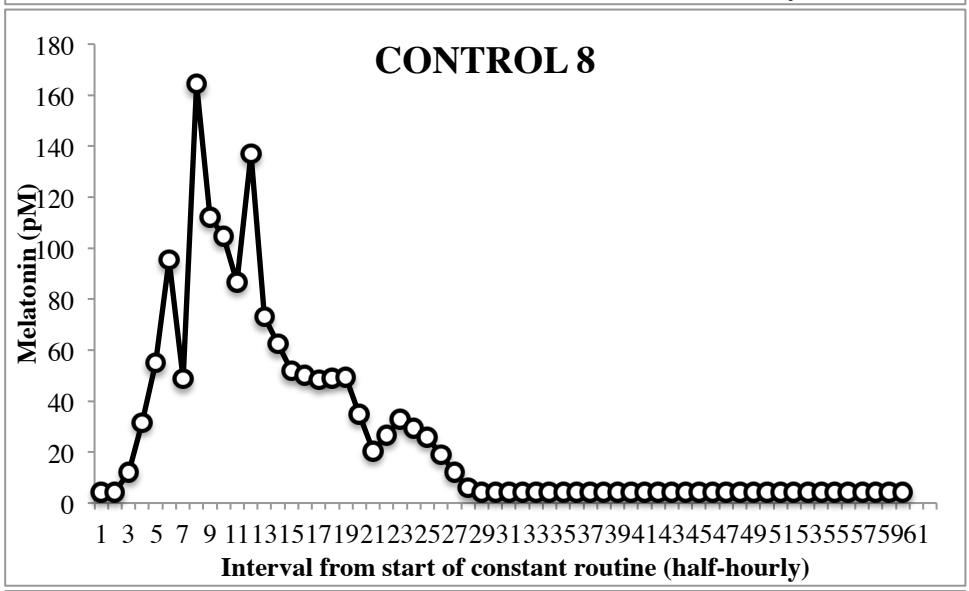

CONTROL 3
Melatonin (pM)
0
10
20
30
40
50
60
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61
Time from start of constant routine (hour)

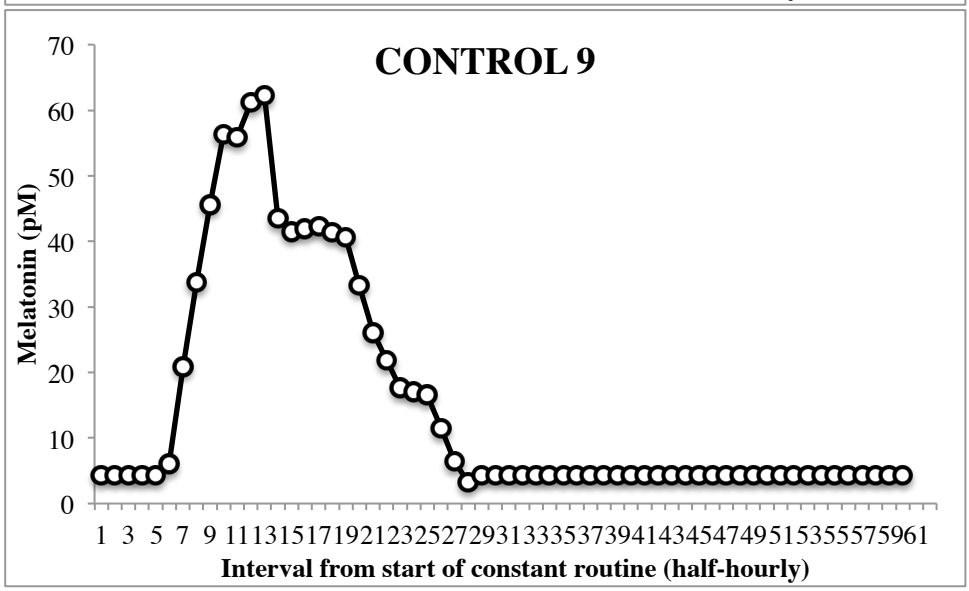

CONTROL 4
Melatonin (pM)
0
10
20
30
40
50
60
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61
Time from start of constant routine (hour)

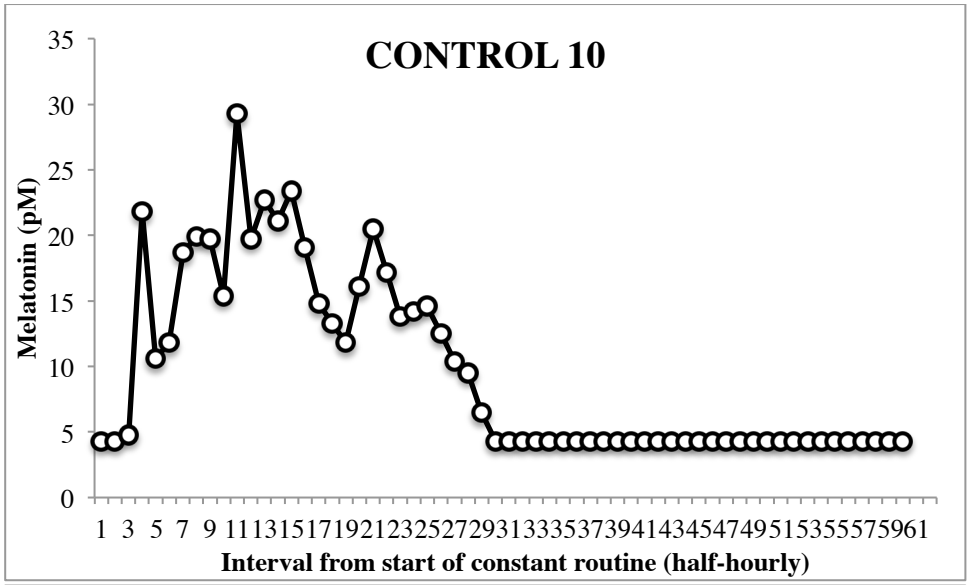

CONTROL 5
Melatonin (pM)
0
10
20
30
40
50
60
70
80
90
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61
Time from start of constant routine (hour)

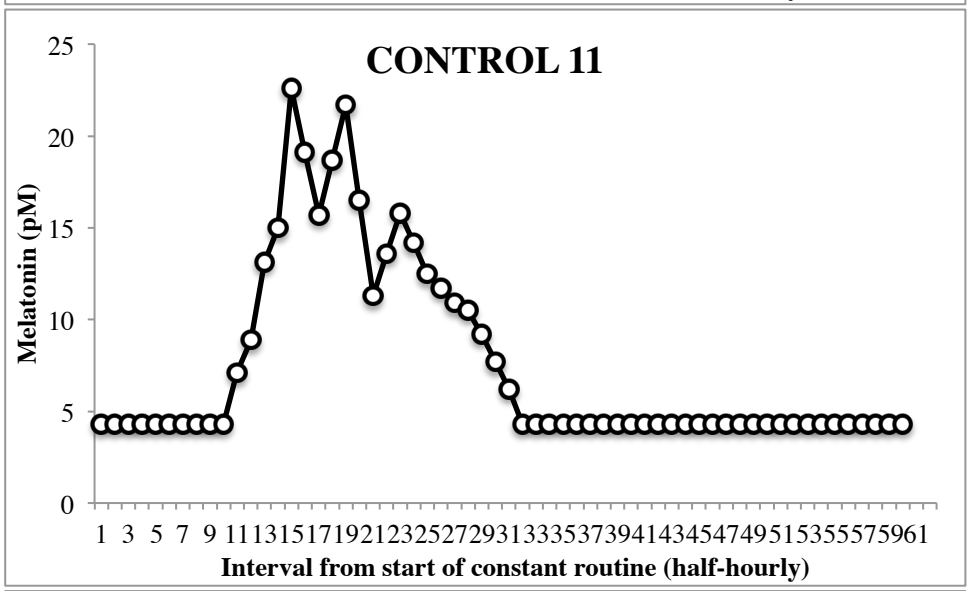

CONTROL 6
Melatonin (pM)
0
5
10
15
20
25
30
35
40
45
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61
Time from start of constant routine (hour)

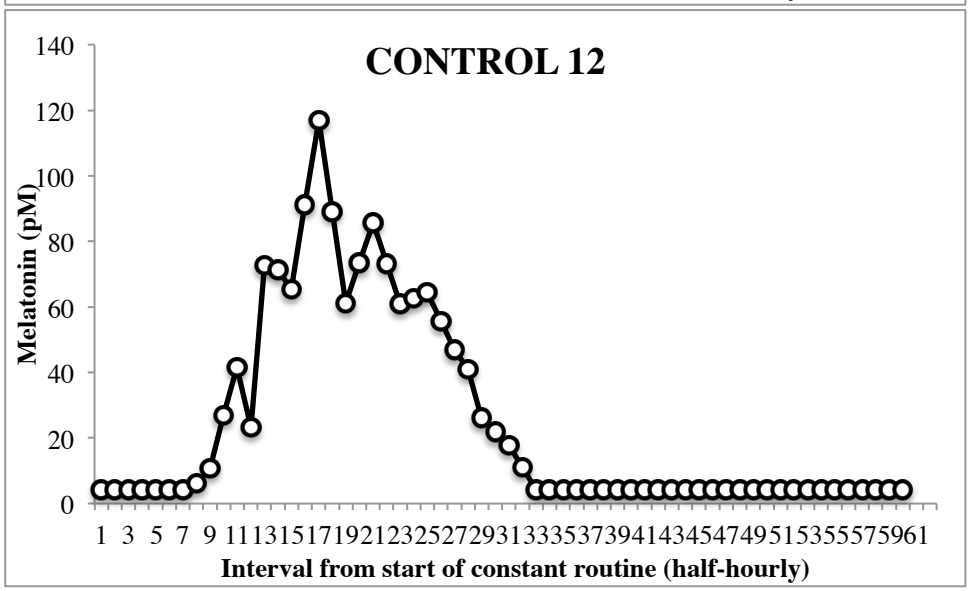

CONTROL 7
Melatonin (pM)
0
10
20
30
40
50
60
70
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61
Interval from start of constant routine (half-hourly)

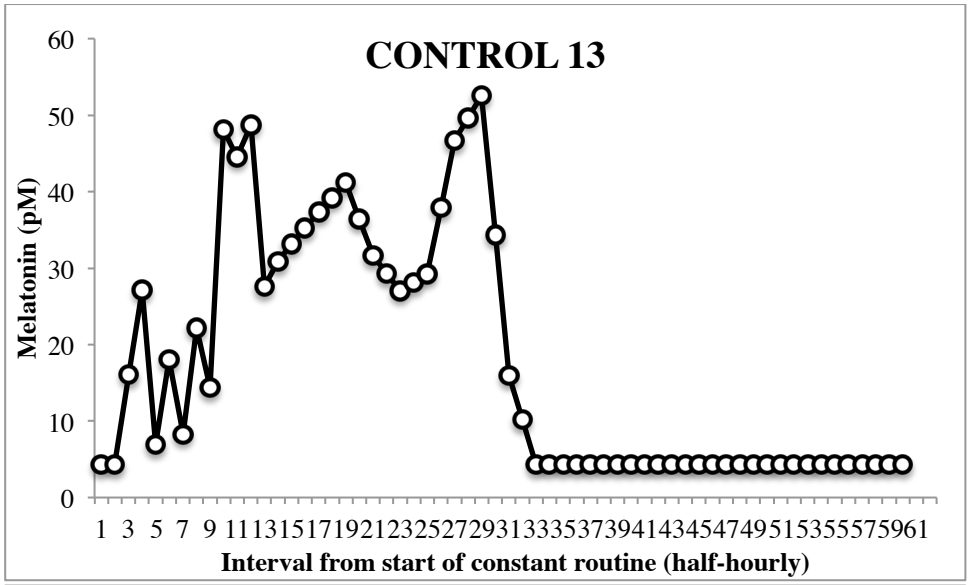

CONTROL 8
Melatonin (pM)
0
20
40
60
80
100
120
140
160
180
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61
Interval from start of constant routine (half-hourly)

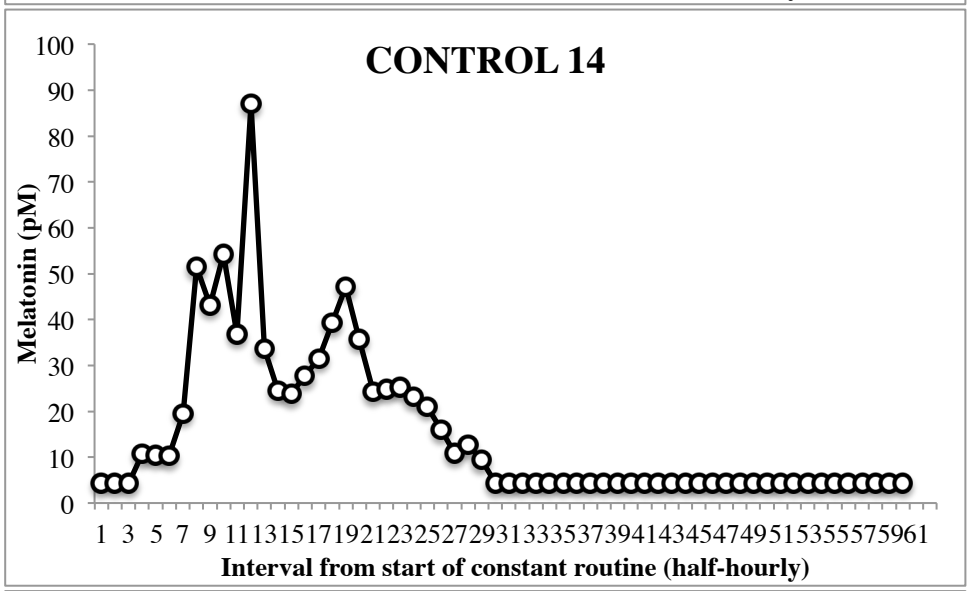

CONTROL 9
Melatonin (pM)
0
10
20
30
40
50
60
70
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61
Interval from start of constant routine (half-hourly)

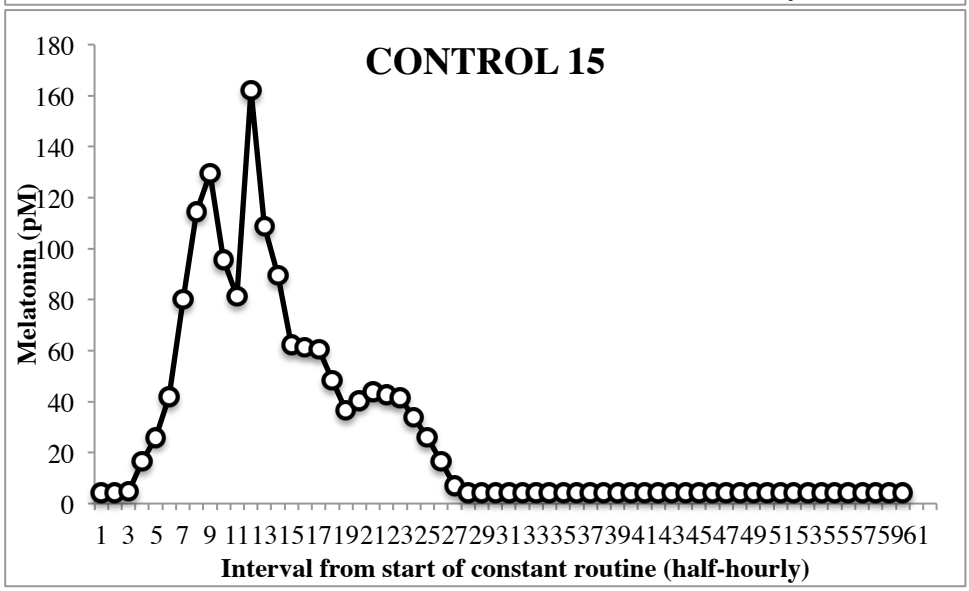

CONTROL 10
Melatonin (pM)
0
5
10
15
20
25
30
35
1 3 5 7 9 11131517192123252729313335373941434547495153555759 61
Interval from start of constant routine (half-hourly)

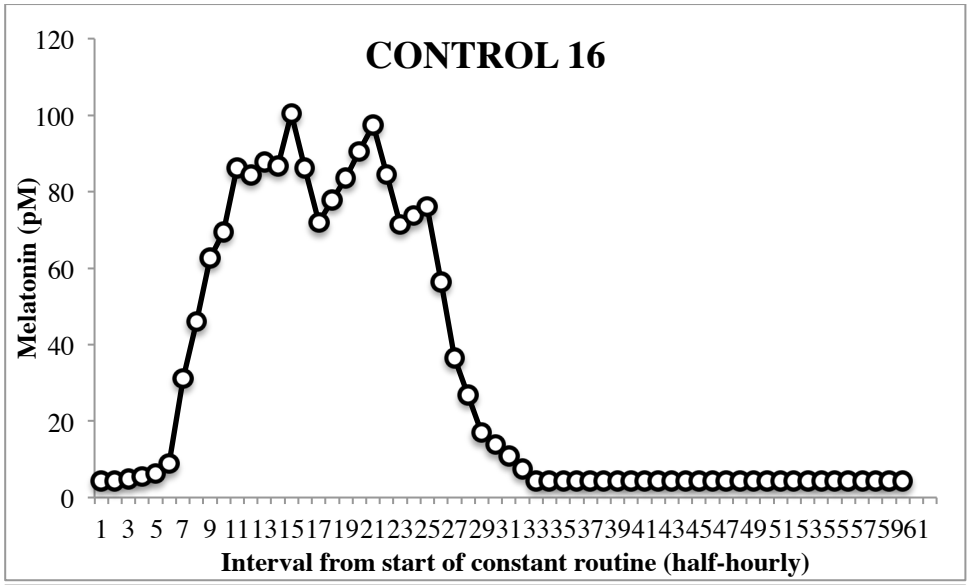

CONTROL 11
Melatonin (pM)
0
5
10
15
20
25
1 3 5 7 9 11131517192123252729313335373941434547495153555759 61
Interval from start of constant routine (half-hourly)


CONTROL 12
Melatonin (pM)
0
20
40
60
80
100
120
140
1 3 5 7 9 11131517192123252729313335373941434547495153555759 61
Interval from start of constant routine (half-hourly)


CONTROL 13
Melatonin (pM)
0
10
20
30
40
50
60
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61
Interval from start of constant routine (half-hourly)


CONTROL 14
Melatonin (pM)
0
10
20
30
40
50
60
70
80
90
100
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61
Interval from start of constant routine (half-hourly)


CONTROL 15
Melatonin (pM)
0
20
40
60
80
100
120
140
160
180
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61
Interval from start of constant routine (half-hourly)


CONTROL 16
Melatonin (pM)
120
100
80
60
40
20
0
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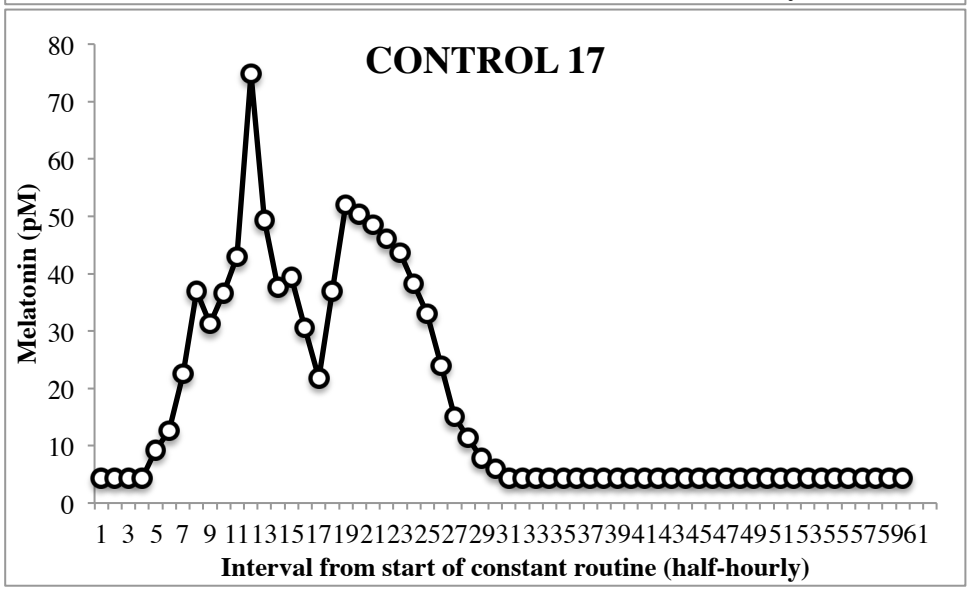
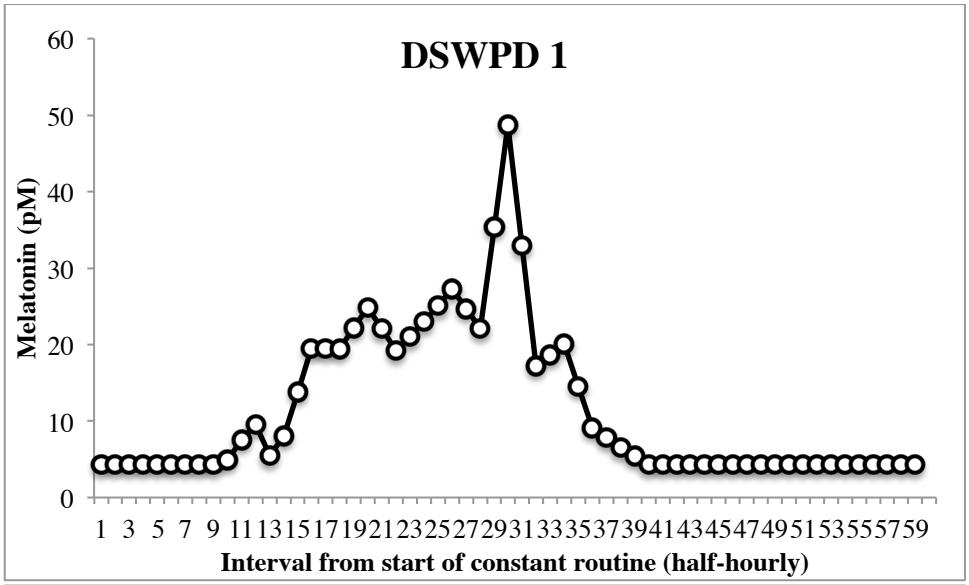
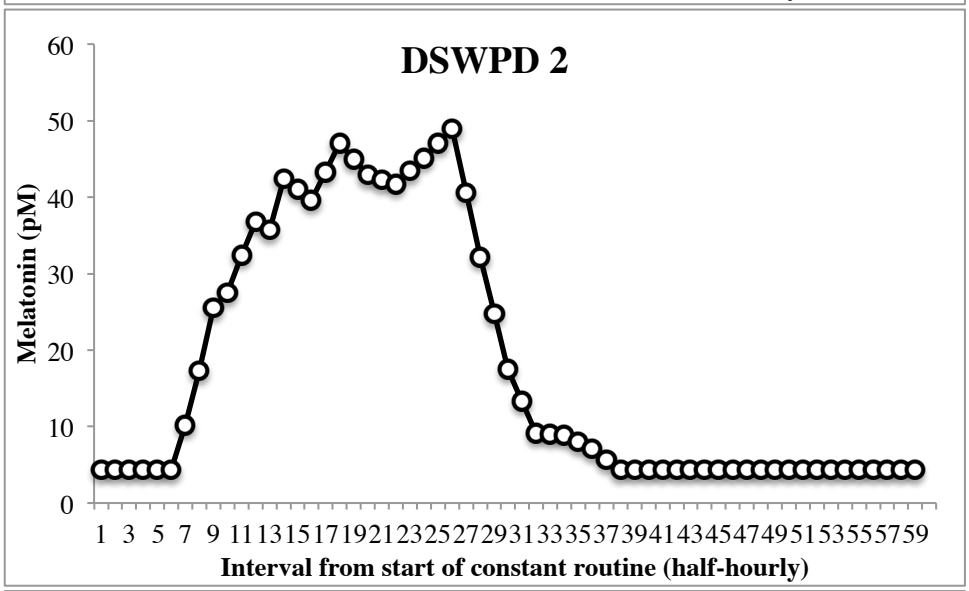
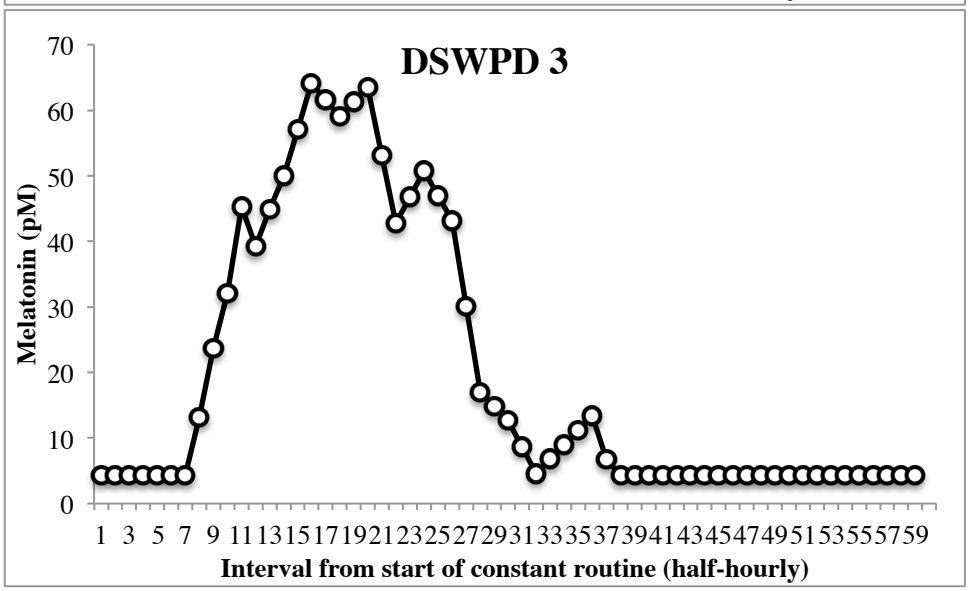
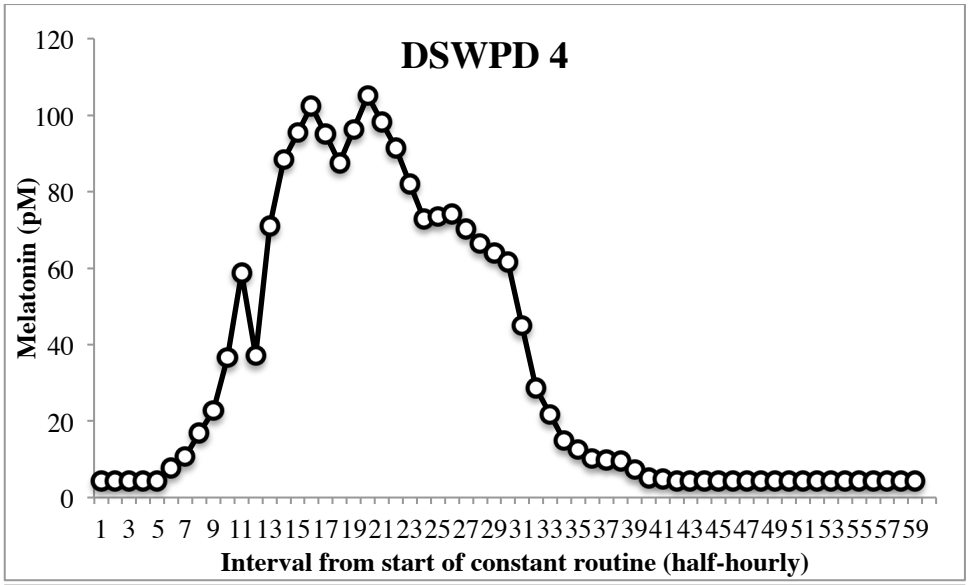
Interval from start of constant routine (half-hourly)

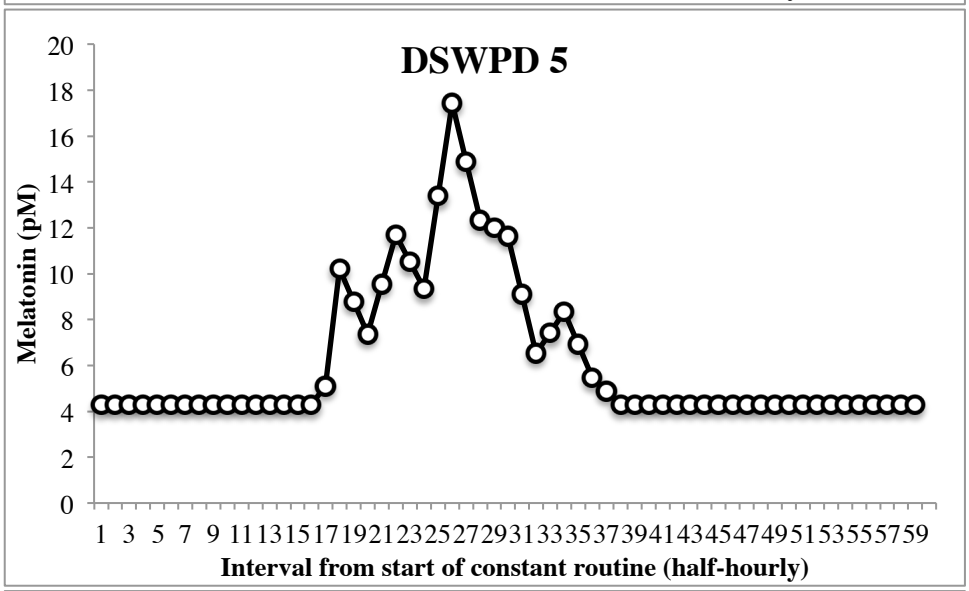

CONTROL 17
Melatonin (pM)
80
70
60
50
40
30
20
10
0
1 3 5 7 9 11131517192123252729313335373941434547495153555759 61
Interval from start of constant routine (half-hourly)

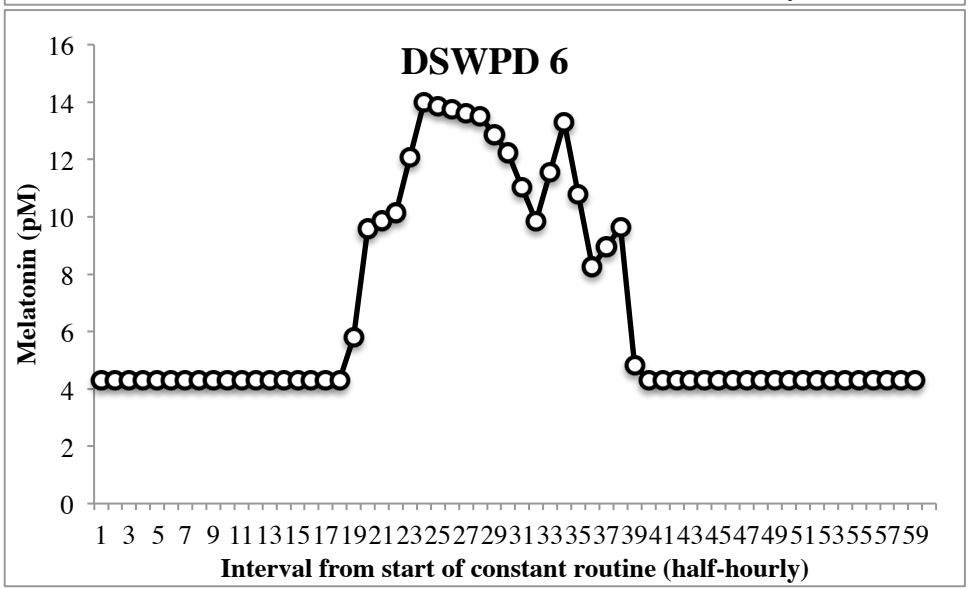

DSWPD 1
Melatonin (pM)
0
10
20
30
40
50
60
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)

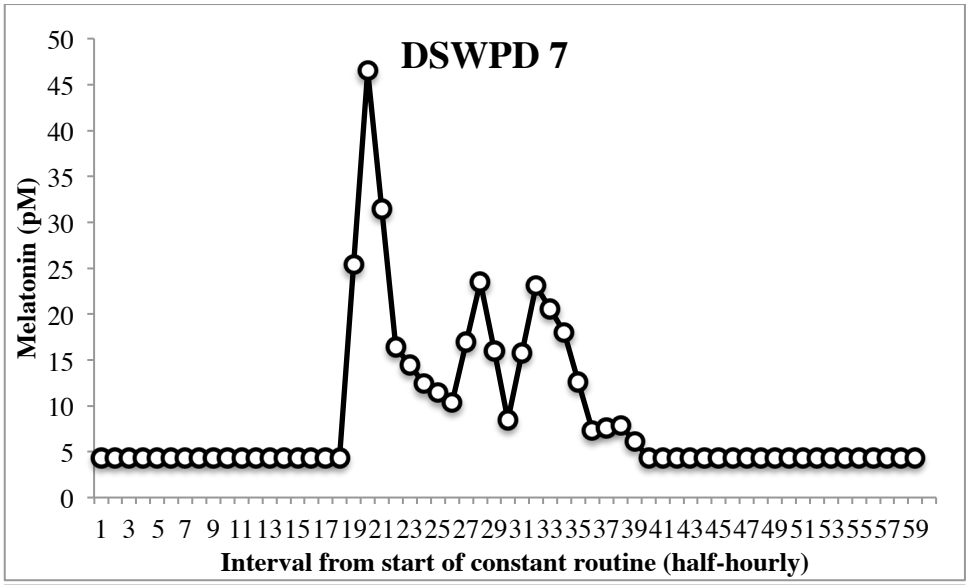

DSWPD 2
Melatonin (pM)
0
10
20
30
40
50
60
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)

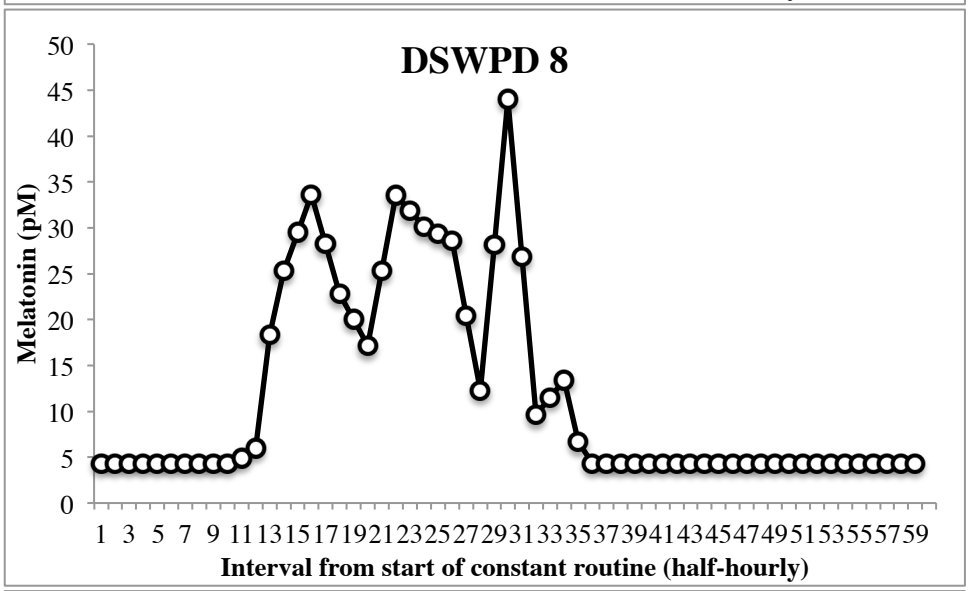

DSWPD 3
Melatonin (pM)
0
10
20
30
40
50
60
70
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)

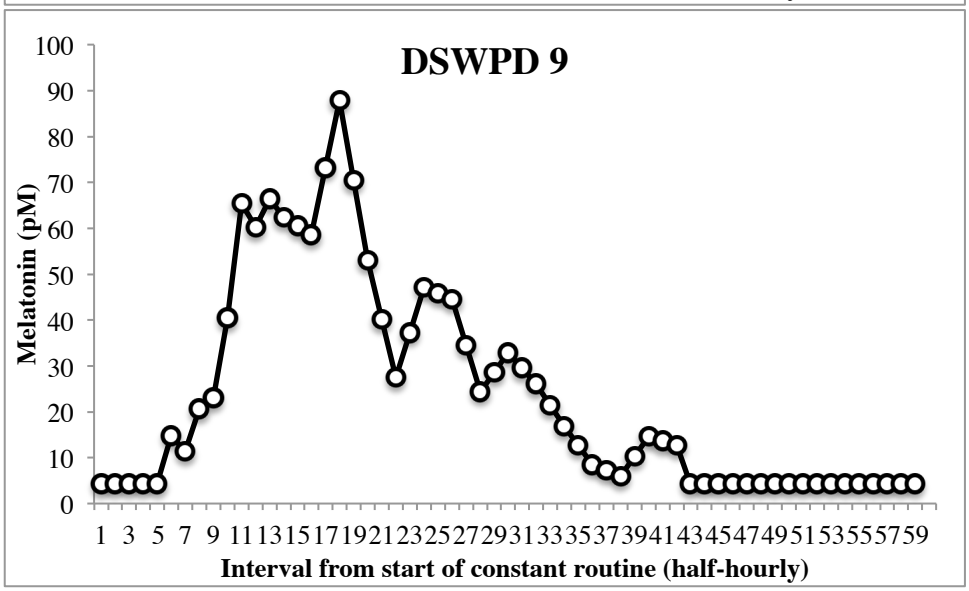

DSWPD 4
Melatonin (pM)
0
20
40
60
80
100
120
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)

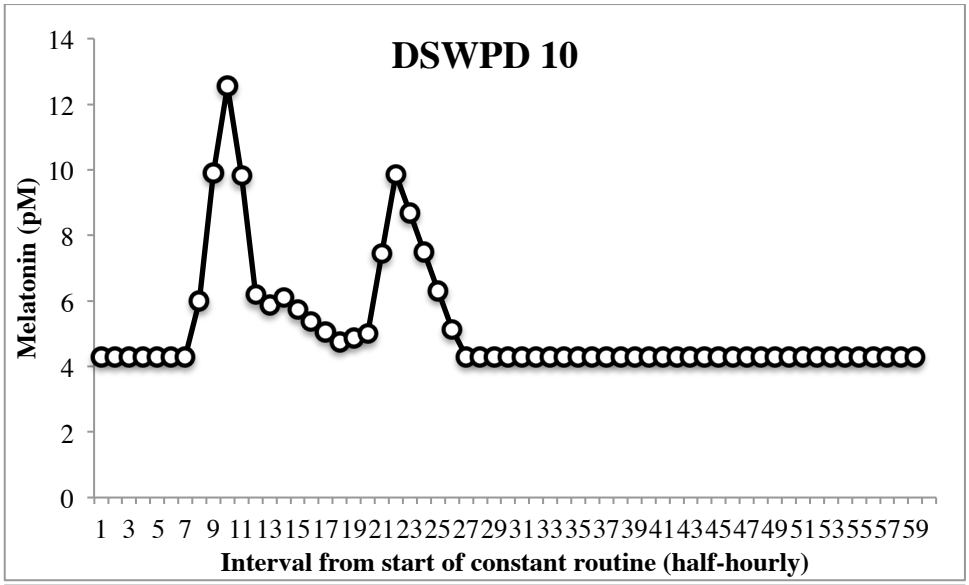

DSWPD 5
Melatonin (pM)
0
2
4
6
8
10
12
14
16
18
20
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)

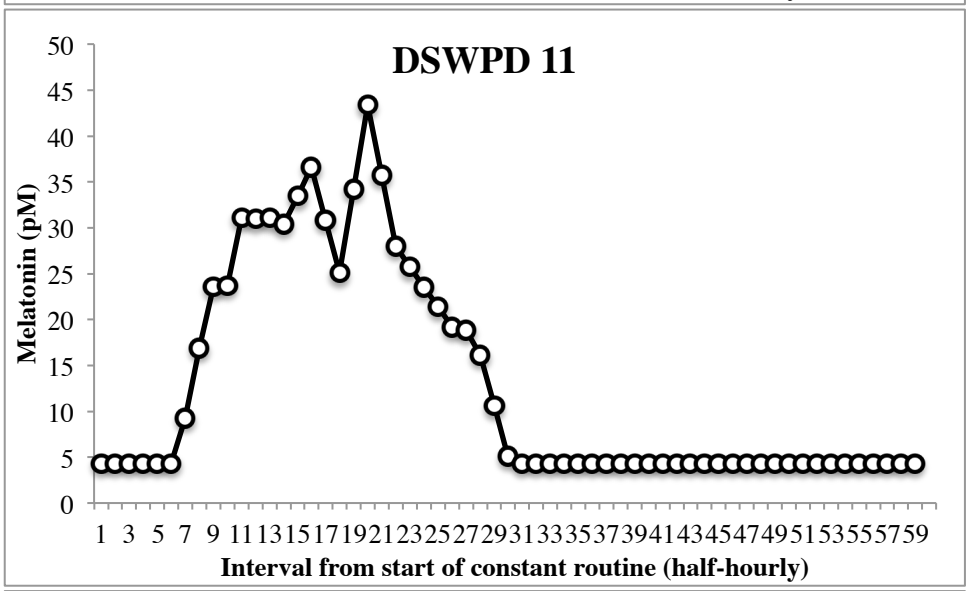

DSWPD 6
Melatonin (pM)
0
2
4
6
8
10
12
14
16
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)

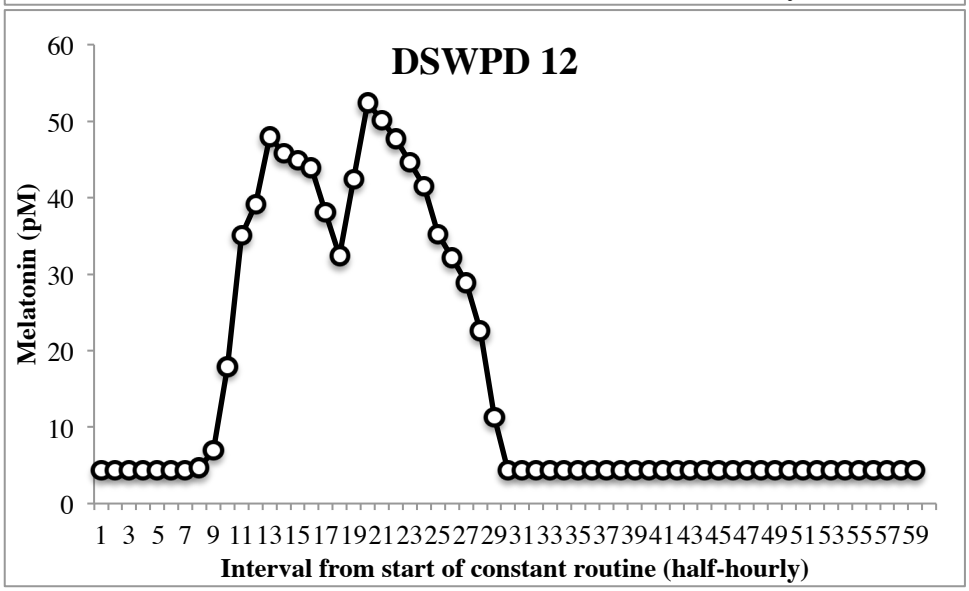

DSWPD 7
Melatonin (pM)
0 5 10 15 20 25 30 35 40 45 50
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)

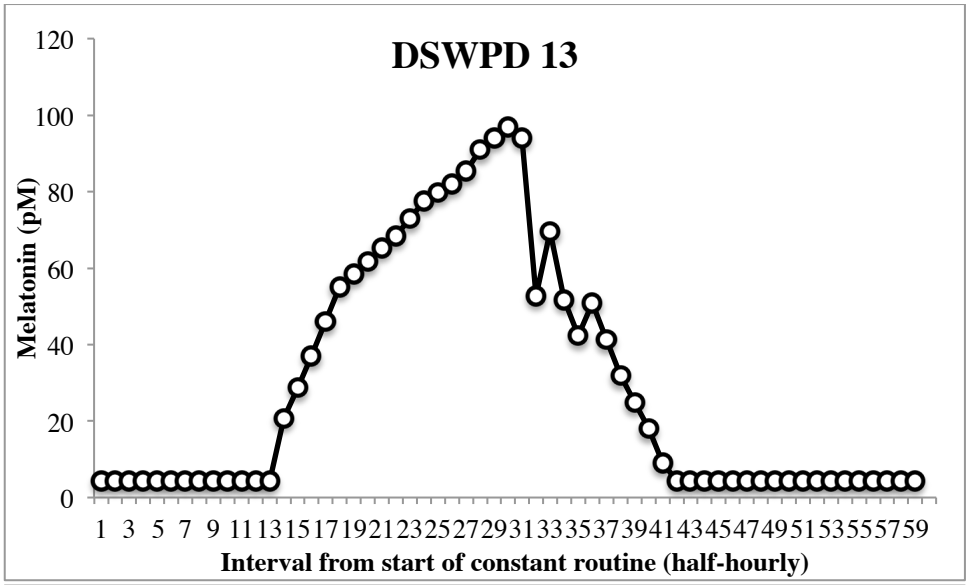

DSWPD 8
Melatonin (pM)
0 5 10 15 20 25 30 35 40 45 50
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)

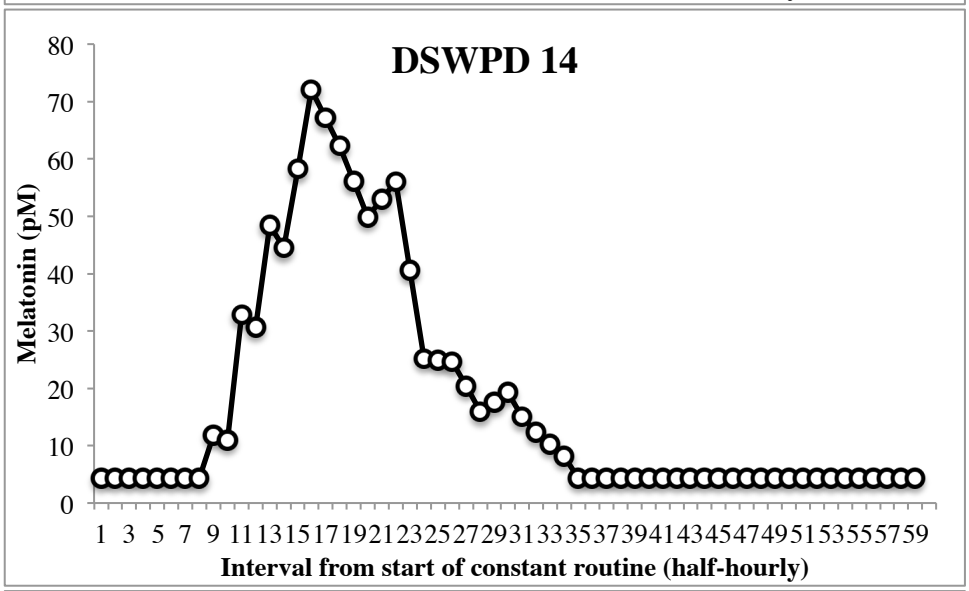

DSWPD 9
Melatonin (pM)
0 10 20 30 40 50 60 70 80 90 100
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)

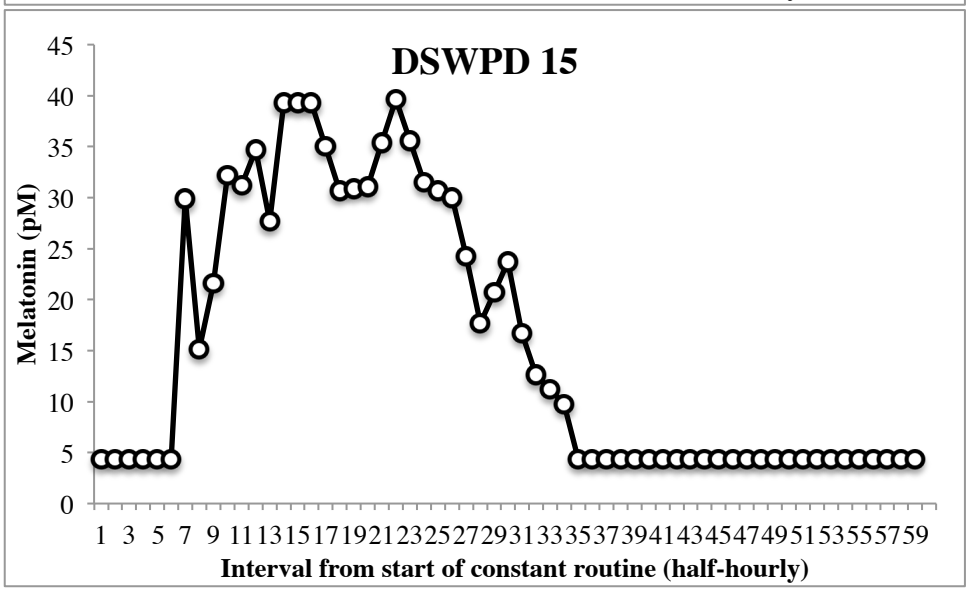

DSWPD 10
Melatonin (pM)
0
2
4
6
8
10
12
14
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)

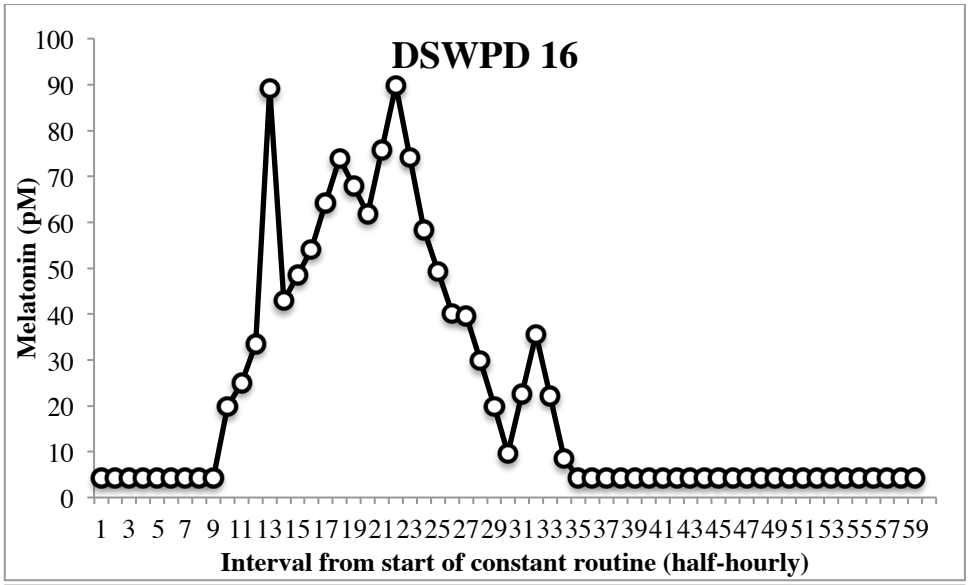

DSWPD 11
Melatonin (pM)
0
5
10
15
20
25
30
35
40
45
50
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)

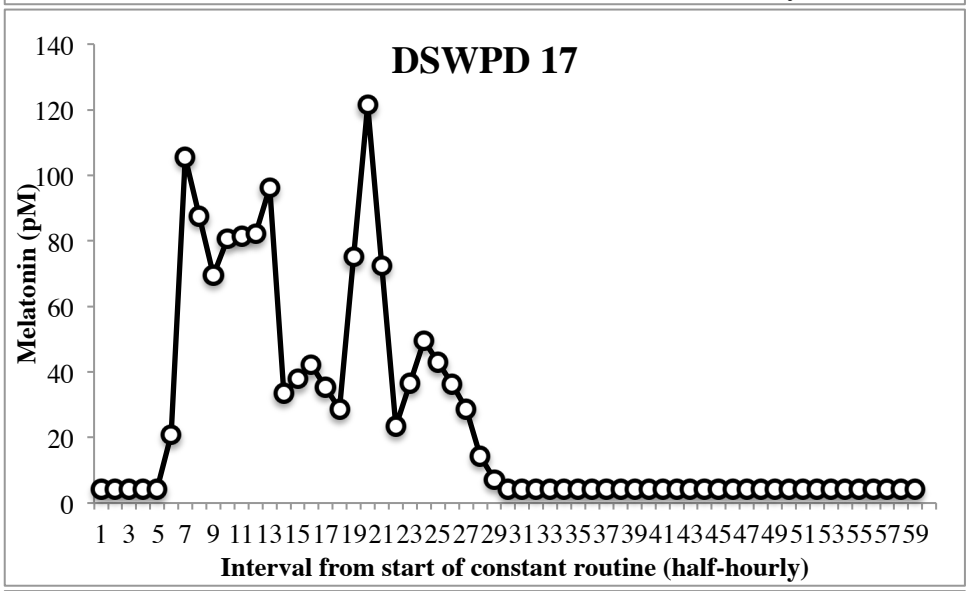

DSWPD 12
Melatonin (pM)
0
10
20
30
40
50
60
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)

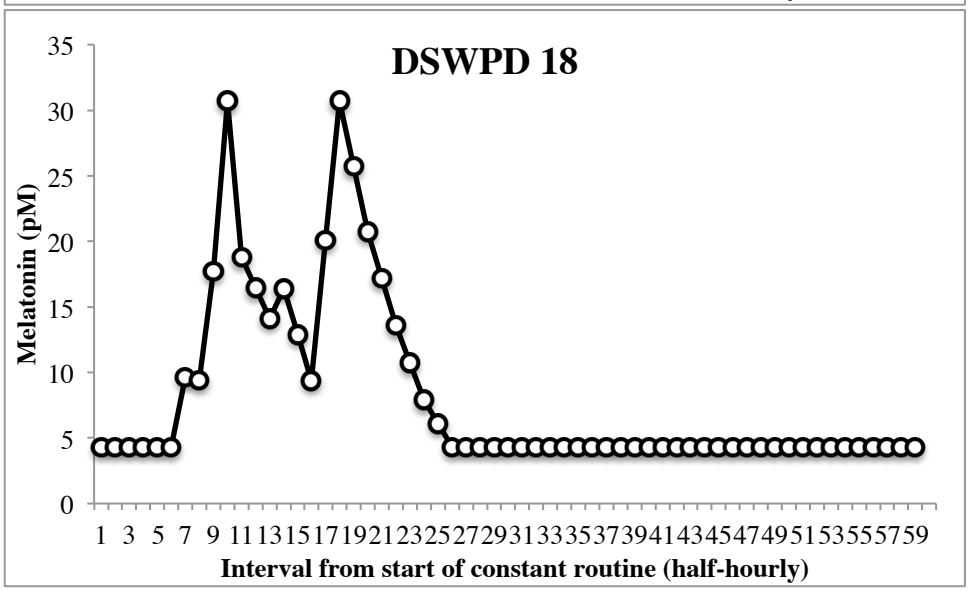

DSWPD 13
Melatonin (pM)
0
20
40
60
80
100
120
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)

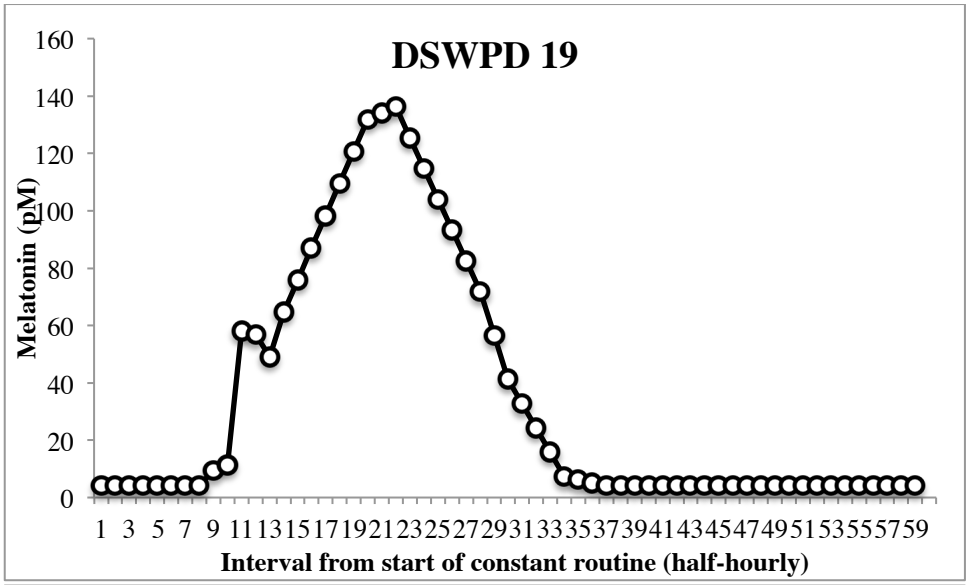

DSWPD 14
Melatonin (pM)
0
10
20
30
40
50
60
70
80
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)

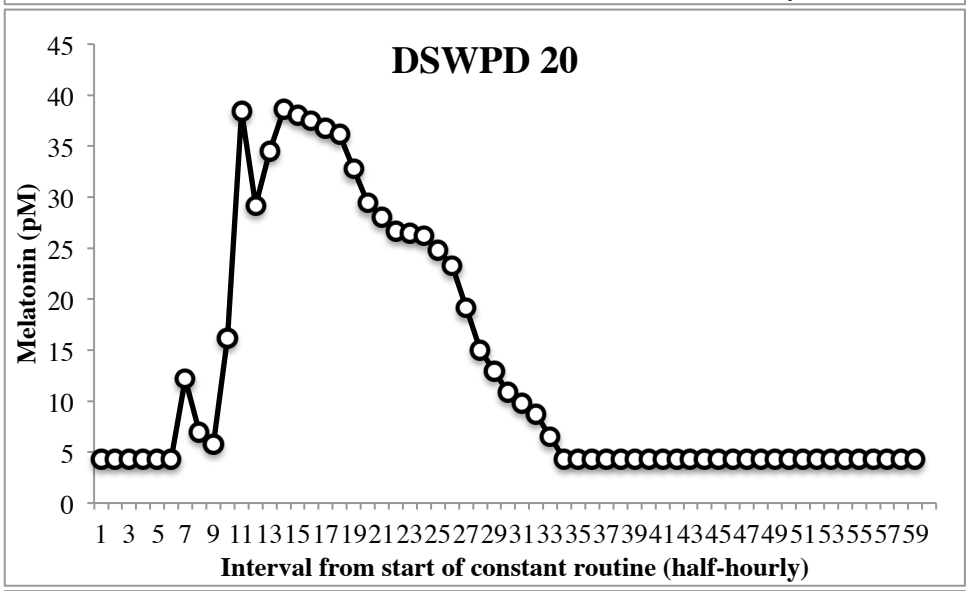

DSWPD 15
Melatonin (pM)
0
5
10
15
20
25
30
35
40
45
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)

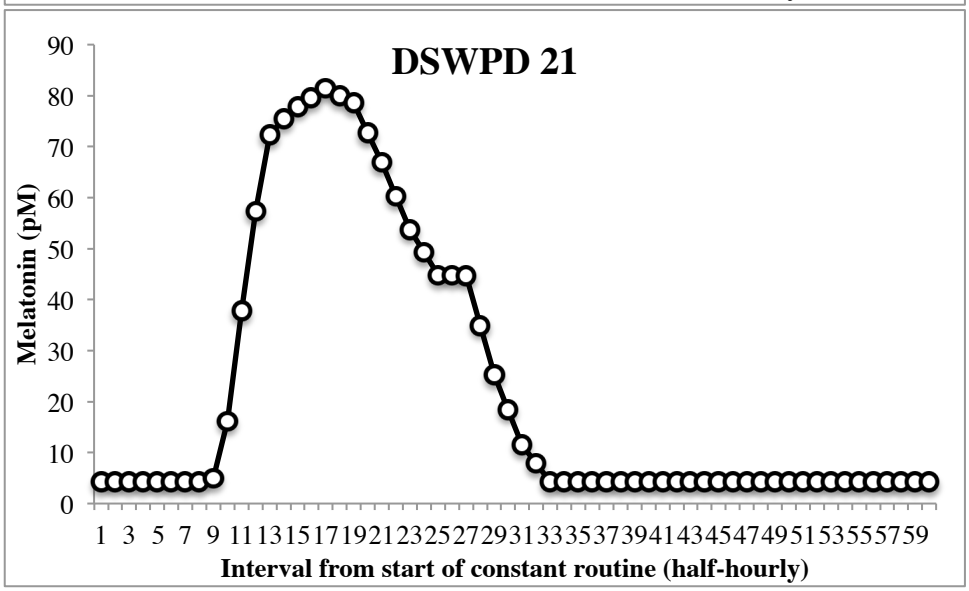

DSWPD 16
Melatonin (pM)
0 10 20 30 40 50 60 70 80 90 100
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)

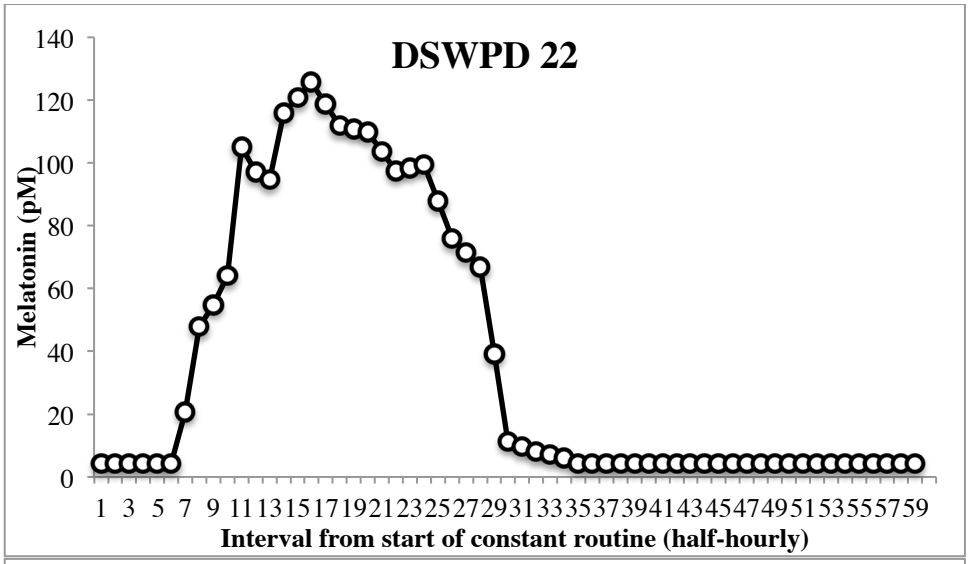

DSWPD 17
Melatonin (pM)
0 20 40 60 80 100 120 140
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)

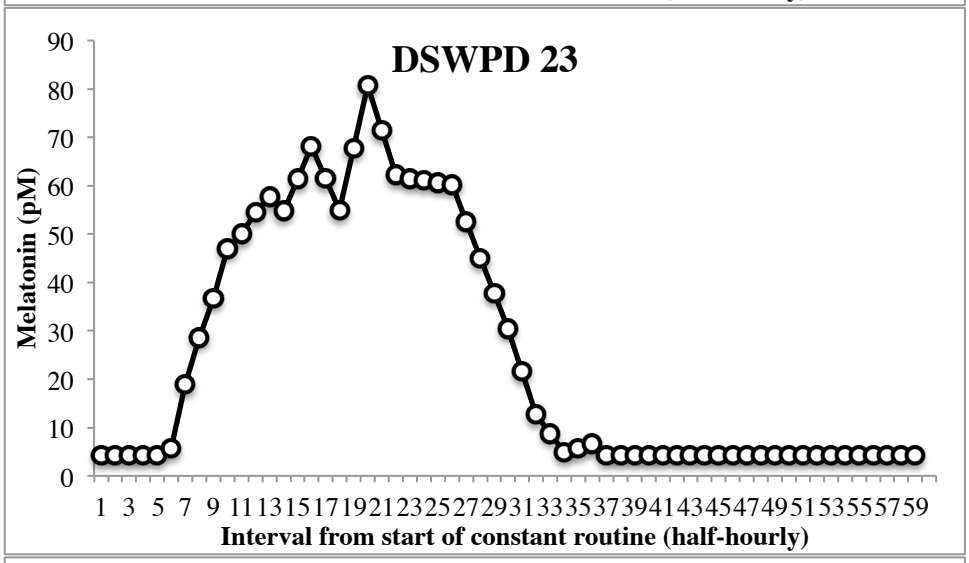

DSWPD 18
Melatonin (pM)
0 5 10 15 20 25 30 35
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)

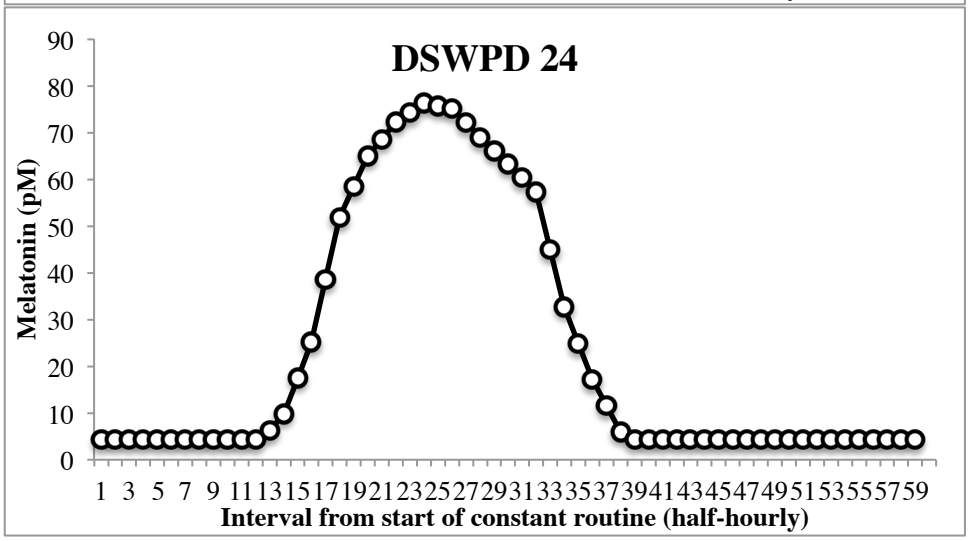

DSWPD 19
Melatonin (pM)
0 20 40 60 80 100 120 140 160
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)

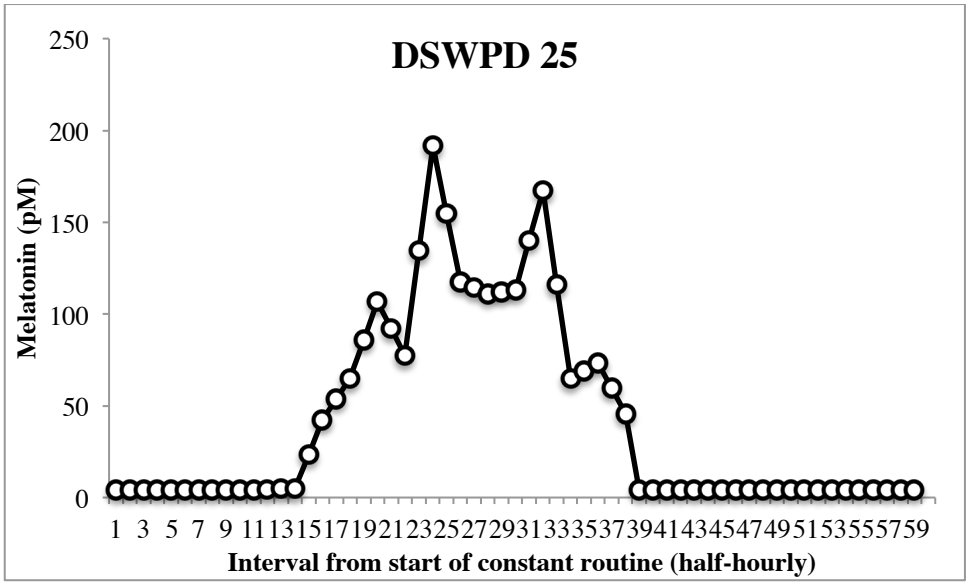

DSWPD 20
Melatonin (pM)
0 5 10 15 20 25 30 35 40 45
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)

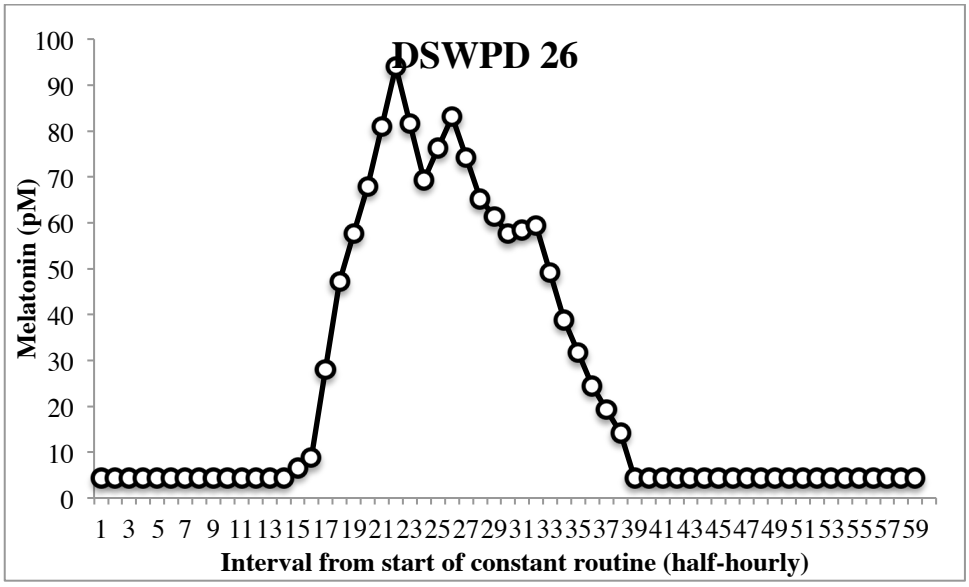

DSWPD 21
Melatonin (pM)
0 10 20 30 40 50 60 70 80 90
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)


DSWPD 22
Melatonin (pM)
0 20 40 60 80 100 120 140
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)


DSWPD 23
Melatonin (pM)
0 10 20 30 40 50 60 70 80 90
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)


DSWPD 24
Melatonin (pM)
0 10 20 30 40 50 60 70 80 90
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)


DSWPD 25
Melatonin (pM)
250
200
150
100
50
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)


DSWPD 26
Melatonin (pM)
100
90
80
70
60
50
40
30
20
10
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Interval from start of constant routine (half-hourly)

APPENDIX J

Supplementary Table 6-5 (continues on next page)

Lifestyle Factors, Subjective Evaluations of Sleep and Associated Impairments in DSWPD Patients and N24SWD Patients Relative to Controls.

	Mean ± Standard Deviation		
	Controls	DSWPD	N24SWD
Lifestyle Factors			
BMI [§]	22.3 ± 2.6	21.8 ± 3.0	23.0 ± 2.0
Caffeine intake (standard cups/day)	1.3 ± 1.2	1.2 ± 1.1	1.0 ± 1.0
Alcohol consumption (standard drinks/week)	0.9 ± 1.65	2.3 ± 4.0	0.3 ± 0.6
Weekly Commitments (number of hours)	32.0 ± 23.9	20.8 ± 10.2	8.3 ± 7.6
Weekly Commitments (number of days/week)	4.5 ± 1.7	4.3 ± 1.6	2.3 ± 2.1
MEQ score	58.5 ± 6.0	32.8 ± 6.0**	43.3 ± 15.1[††]
PSQI	1.6 ± 1.7	6.1 ± 3.8**	4.7 ± 3.1[†]
MSF^{SC} [a]	3.4 ± 0.9	6.1 ± 1.8**	3.8 ± 4.9
Self-reported sleeping pattern and sleep quality [b]			
Current body clock SOT	2230 ± 35m	0212 ± 1h 22m**	0200 ± 4h 55m
Current body clock WUT	0722 ± 43m	1105 ± 1h 40**	1510 ± 2h 22m[†]
Difficulty sleeping at CST [%]	1.08 ± 0.28	2.13 ± 0.81**	2.00 ± 1.73
Difficulty awaking at CWT %	1.46 ± 0.52	2.25 ± 1.00*	1.67 ± 1.15
Mins to fall asleep at CST	10.92 ± 6.91	30.31 ± 28.02*	26.67 ± 28.30
Total Sleep Time (h)	8.87 ± 0.81	8.63 ± 1.50	9.75 ± 0.75
Preferred sleep time	2250 ± 43m	2255 ± 57m	0030 ± 2h 17m
Preferred wake time	0739 ± 1h 36m	0805 ± 1h 3m	0900 ± 2h 0m
Difficulty sleeping at PST [%]	1.15 ± 0.38	3.94 ± 0.68**	4.67 ± 0.58[††]
Difficulty awaking at PWT [%]	1.54 ± 0.78	4.00 ± 0.97**	4.67 ± 0.58[††]
Mins to fall asleep at PST	10.85 ± 8.06	92.81 ± 49.80**	160.0 ± 12.90
Current and PST discrepancy (h)	-0.34 ± 0.74	3.28 ± 1.23**	1.50 ± 6.00
Current and PWT discrepancy (h)	-0.28 ± 0.89	3.00 ± 1.56**	6.17 ± 0.76[†]

**Significant group differences indicated at <.05 (t-test with DSWPD & control group); ** Significant group differences indicated at <.001 (t-test with DSWPD & control group); [†] Significant group differences indicated at <.05 (ANOVA with DSWPD, N24SWD, control group); [††] Significant group differences indicated at <.001 (ANOVA with DSWPD, N24SWD, control group)*

[§] BMI calculation: mass (kg) / height $(m)^2$; [a] Reported on the Munich Chronotypes Questionnaire calculated using the formula $MSF_{SC} = MSF - 0.5*(SD_F-(5*SD_W+2*SD_F)/7)$. MSF: Mid sleep on free days; SD_F: Sleep duration on free days; SD_W: Sleep duration on work days; [b] Reported on the DSWPD-Sleep Timing Questionnaire; [c] Reported on the Sheehan Disability Scale; [d] Reported on the Depression Anxiety Scale – 21 short form; [%] Range: (1) Not at all – (5) Extremely

Abbreviations: DSWPD: Delayed Sleep-Wake Phase Disorder; N24SWD: Non-24-hour Sleep/Wake Rhythm Disorder; BMI: Body Mass index; MEQ: Morningness-Eveningness Questionnaire; PSQI: Pittsburg Sleep Quality Index; MSF^{SF}: Mid-sleep on free days corrected for accumulated sleep debt during the workweek; h: reported in fraction of hours; SOT: Sleep Onset Time; WUT; Wake-up Time; CST: Current Sleep Time; CWT: Current Wake Time; PST: Preferred Sleep Time; PWT: Preferred Wake Time.

Supplementary Table 6-5 (continued)

Lifestyle Factors, Subjective Evaluations of Sleep and Associated Impairments in DSWPD Patients and N24SWD Patients Relative to Controls.

	Mean ± Standard Deviation		
	Controls	DSWPD	N24SWD
Impairment due to sleeping pattern			
Work/school disruption [c]	1.4 ± 1.7	6.2 ± 2.5**	6.3 ± 3.5[††]
Social life disruption [c]	2.1 ± 2.3	4.4 ± 2.7*	5.3 ± 5.0
Family life disruption [c]	0.9 ± 1.8	4.9 ± 3.0**	6.3 ± 3.5[††]
Overall disruption [c]	4.4 ± 5.2	15.4 ± 6.1**	18.0 ± 12.0[†]
Number of days absent from commitments due to sleep pattern (subjective weekly average) [c]	0.5 ± 0.8	1.4 ± 2.1	3.3 ± 3.5
Number of days of reduced productivity due to sleep pattern (subjective weekly average) [c]	0.6 ± 0.9	3.3 ± 2.2**	2.3 ± 1.2[†]
Motivation to change current sleeping pattern to preferred sleeping pattern [b] %	1.3 ± 1.1	2.4 ± 1.1*	3.0 ± 1.0[†]
Depression [d]	4.5 ± 4.5	15.0 ± 12.61*	14.0 ± 16.4
Anxiety [d]	3.4 ± 2.9	9.5 ± 8.3*	4.7 ± 5.0
Stress [d]	6.9 ± 7.1	15.3 ± 9.2*	11.3 ± 9.9

**Significant group differences indicated at <.05 (t-test with DSWPD & control group); ** Significant group differences indicated at <.001 (t-test with DSWPD & control group); [†] Significant group differences indicated at <.05 (ANOVA with DSWPD, N24SWD, control group); [††] Significant group differences indicated at <.001 (ANOVA with DSWPD, N24SWD, control group)*

[§] BMI calculation: mass (kg) / height (m)^2; [a] Reported on the Munich Chronotypes Questionnaire calculated using the formula $MSF_{SC} = MSF\text{-}0.5*(SD_F\text{-}(5*SD_W+2*SD_F)/7)$. MSF: Mid sleep on free days; SD_F: Sleep duration on free days; SD_W: Sleep duration on work days; [b] Reported on the DSWPD-Sleep Timing Questionnaire; [c] Reported on the Sheehan Disability Scale; [d] Reported on the Depression Anxiety Scale – 21 short form; [%] Range: (1) Not at all – (5) Extremely

Abbreviations: DSWPD: Delayed Sleep-Wake Phase Disorder; N24SWD: Non-24-hour Sleep/Wake Rhythm Disorder; BMI: Body Mass index; MEQ: Morningness-Eveningness Questionnaire; PSQI: Pittsburg Sleep Quality Index; MSF^{SF}: Mid-sleep on free days corrected for accumulated sleep debt during the workweek; h: reported in fraction of hours; SOT: Sleep Onset Time; WUT; Wake-up Time; CST: Current Sleep Time; CWT: Current Wake Time; PST: Preferred Sleep Time; PWT: Preferred Wake Time.

Supplementary Table 6-6

Group Differences in circadian taus and timing of phase markers from an 80-hour ultradian routine, as well as recorded habitual sleep timing from activity monitors.

	Mean ± Standard Deviation		
	Controls	DSWPD	N24SWD
Circadian Parameters [a]			
DLMO	2040 ± 1h 24m	2327 ± 2h 13m**	2205 ± 6h 33m
Mτ (h)	24.38 ± 0.22	24.60 ± 0.26*	24.88 ± 0.23[†]
Tmin	0336 ± 1h 33m	0644 ± 2h 6m**	0349 ± 6h 10m
Tτ (h)	24.21 ± 0.45	24.50 ± 0.46	24.86 ± 0.35[†]
Average Week [b]			
Bedtime	2314 ± 38m	0211 ± 1h 21**	0159 ± 5h 31m
Get up time	0805 ± 58m	1034 ± 1h 43m**	0658 ± 4h 4m
Time in Bed (h)	8.86 ± 1.15	8.45 ± 0.82	8.98 ± 0.74
Total Sleep Time (h)	7.57 ± 1.27	7.03 ± 0.80	7.52 ± 0.66
Sleep Latency (m)	11.44 ± 11.79	16.69 ± 14.71	7.55 ± 2.92
Sleep Efficiency	84.69 ± 7.72	81.51 ± 6.94	83.60 ± 2.23
Work Days [b]			
Bedtime	2259 ± 40m	0141 ± 1h 13m**	-
Get up time	0720 ± 44m	0852 ± 1h 7m**	-
Time in Bed (h)	8.31 ± 1.21	7.31 ± 1.05*	-
Total Sleep Time (h)	7.16 ± 1.51	5.73 ± 0.87*	-
Sleep Efficiency	85.70 ± 9.57	78.78 ± 8.71	-
Free Days [b]			
Bedtime	2338 ± 1h 3m	0253 ± 1h 28m**	-
Get up time	0842 ± 53m	1155 ± 1h 35m**	-
Time in Bed (h)	9.20 ± 1.01	9.05 ± 1.08	-
Total Sleep Time (h)	7.91 ± 1.11	7.56 ± 0.81	-
Sleep Efficiency	86.15 ± 8.51	83.32 ± 6.00	-
MSF^{SC}	0355 ± 1h 0m	0644 ± 1h 21m	-

Abbreviations: DSWPD: Delayed Sleep-Wake Phase Disorder; N24SWD: Non-24-Hour Sleep-Wake Rhythm Disorder; DLMO: Dim light melatonin onset; Mτ: Melatonin tau; Tmin: Temperature minimum; Tτ: Temperature Tau; h: hours; m:minutes; MSF^{SF}*: Mid-sleep on free days corrected for accumulated sleep debt during the workweek*

** Significant group differences indicated at <.05 (t-test with DSWPD & control group)*

*** Significant group differences indicated at <.001 (t-test with DSWPD & control group)*

[†] Significant group differences indicated at <.05 (ANOVA with DSWPD, N24SWD, control group)