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Original Article

ORIGINAL ARTICLE

Circadian tau differences and rhythm associations in delayed sleep–wake phase disorder and sighted non-24-hour sleep–wake rhythm disorder

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Abstract

Study Objectives: We investigated biological and behavioral rhythm period lengths (i.e. *taus*) of delayed sleep–wake phase disorder (DSWPD) and non-24-hour sleep–wake rhythm disorder (N24SWD). Based on circadian phase timing (temperature and dim light melatonin onset), DSWPD participants were dichotomized into a circadian-delayed and a circadian non-delayed group to investigate etiological differences.

Methods: Participants with DSWPD (n = 26, 17 m, age: 21.85 ± 4.97 years), full-sighted N24SWD (n = 4, 3 m, age: 25.75 ± 4.99 years) and 18 controls (10 m, age: 23.72 ± 5.10 years) participated in an 80-h modified constant routine. An ultradian protocol of 1-h "days" in dim light, controlled conditions alternated 20-min sleep/dark periods with 40-min enforced wakefulness/light. Subjective sleepiness ratings were recorded prior to every sleep/dark opportunity and median reaction time (vigilance) was measured hourly. Obtained sleep (sleep propensity) was derived from 20-min sleep/dark 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 two-component cosine model.

Results: Patients with DSWPD and N24SWD had significantly longer melatonin and temperature *taus* compared to controls. Circadian non-delayed DSWPD had normally timed temperature and melatonin rhythms but were typically sleeping at relatively late circadian phases compared to those with circadian-delayed DSWPD.

Conclusions: People with DSWPD and N24SWD exhibit significantly longer biological circadian rhythm period lengths compared to controls. Approximately half of those diagnosed with DSWPD do not have abnormally delayed circadian rhythm timings suggesting abnormal phase relationship between biological rhythms and behavioral sleep period or potentially conditioned sleep-onset insomnia.

Statement of Significance

Patients with delayed sleep–wake phase disorder (DSWPD) have delayed but stable rhythms while those with non-24-hour sleep–wake rhythm disorder (N24SWD) have an unrelenting tendency to systematically delay their sleep–wake cycles. In both disorders, it is unclear how the sleep–wake patterns become stubbornly delayed and difficult to treat. This research suggests that several etiologies probably contribute to a diagnosis of DSWPD. People with DSWPD and N24SWD exhibit significantly longer biological circadian rhythm period lengths compared to controls, thus any late sleep-in will delay their rhythms more than normal. However, approximately half of patients diagnosed with DSWPD do not have abnormally delayed circadian rhythms while exhibiting all other symptoms necessary for a clinical diagnosis. The findings also suggest that conditioned sleep onset insomnia might be a further etiological contributor in some cases of DSWPD.

Key words: circadian; phase; rhythms; delayed sleep–wake phase disorder; non-24-hour sleep–wake disorder; entrainment phase angle; oscillator

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Introduction

The International Classification for Sleep Disorders-3 (ICSD-3) [1] and the Diagnostic and Statistical Manual of Mental Health Disorders-5 [2] categorize delayed sleep–wake phase disorder (DSWPD) and non-24-hour sleep–wake disorder (N24SWD) as circadian-based disorders. Patients with DSWPD exhibit circadian rhythms that are timed approximately 3 or more hours later than normal [3, 4]. Circadian rhythms in patients with N24SWD cannot be synchronized to the 24-h light–dark cycle thus resulting in non-24-hour sleep–wake cycles that are usually significantly longer than the 24-h period [1]. Empirical studies indicate that Circadian Rhythm Sleep–Wake Disorders (CRSWD) originate from multi-facetted etiologies [5–7]. DSWPD is the most common CRSWD whose etiology has been assumed to be a delay of underlying biological circadian rhythms [1].

In addition to circadian rhythm delays, abnormal relationships between the timing of circadian rhythms and sleep/wake cycles have also been hypothesized to underpin 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 of the PAE in patients with DSWPD and N24SWD compared to controls. Patients with DSWPD have been suggested by some to have a longer PAE interval (i.e. greater delay) between their circadian rhythm timing (e.g. temperature nadir) and typical sleep offset [4, 8–10]. The PAE in patients with N24SWD appears to be even longer [11]. Sleep timed later relative to circadian rhythm timing extends the period of "eyes closed darkness" following the temperature nadir and blocks retinal light stimulation during the phase-advancing period of the phase response curve to light stimulation of the circadian system [12]. Hence the late timing of patients with DSWPD and N24SWD sleep periods relative to their biological clock could further exacerbate their circadian and sleep/wake rhythm delay. However, one study using a free-running methodology contradicts these findings, showing a shorter PAE interval (i.e. less delay) between circadian rhythm timing (e.g. temperature nadir) and typical sleep offset in DSWPD relative to controls [13].

Ambulatory findings in patients with DSWPD suggest that the PAE does not differ between DSWPD and controls [14, 15]. 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 the sleep–wake cycle timing and the environmental changes, such as the social context or external lifestyle pressures on the measured circadian rhythm timing. One aim of the present study is to evaluate the PAE of patients with DSWPD in a well-controlled laboratory protocol.

Like DSWPD, evening-type individuals exhibit ~2–3-h differences in their circadian timing relative to morning-chronotypes [16–19]. However, one of these studies also shows that the phase of minimum subjective sleepiness (SS) in evening-types was timed 9 h later compared to morning-types [17]. The evening-types' maximum SS phase was timed 6 h later compared to morning-types', suggesting that the delay in sleepiness rhythms could be due to longer SS rhythm periods. 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 [20]. A longer SS period could result in delays of individuals' sleep timing. This would result in greater exposure to evening light and decrease the phase-advancing effects of morning light, thus delaying individuals' endogenous circadian rhythms. Given patients with DSWPD exhibit extreme evening-type preference, these findings could help to elucidate factors that lead to DSWPD, specifically the PAE. It would be suggested that patients with DSWPD become sleepy and then alert at significantly later phases of their circadian cycle than normally entrained sleepers.

To control confounding factors related to PAE, the present study investigated SS, objective sleepiness (i.e. amount of sleep obtained in fixed duration opportunities), and vigilance rhythms (i.e. median reaction time) in patients with DSWPD, N24SWD, and controls in a time-free environment. This is the third study of its kind to investigate circadian timing and phase angles in N24SWD [21, 22]. By removing external factors that contribute to circadian and sleep timing (e.g. light and time cues), as well as controlling homeostatic sleep pressure by giving equal length sleep opportunities at equal intervals across the full 24-h period such that any measured variable is equally and minimally perturbed, we aimed to investigate circadian sleepiness and vigilance rhythms in patients with DSWPD, N24SWD, and controls relative to their biological rhythms (i.e. core body temperature and melatonin). These outcomes will inform whether the Lack *et al.* [17] findings regarding evening types could be extended to the clinical sample of patients with DSWPD and N24SWD. It was hypothesized that patients with DSWPD and N24SWD would show significantly later maxima of alertness, as measured by behavioral 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*, patients with DSWPD and N24SWD were predicted to have significantly longer behavioral *taus* compared to their endogenous circadian *taus* and compared to controls.

In good sleepers, different zones of alertness and sleepiness have been identified with respect to certain biological rhythm phases, this PAE timing relationship may vary between individuals. Behavioral rhythms may oscillate independent of the biological rhythms as in the spontaneous internal desynchronization studies [23, 24]. Forced desynchrony experiments are based on this notion that the sleep/wake pattern is forced but they may be able to occur spontaneously under some free running circumstances.

Moreover, although patients with DSWPD on the average show significant delays in circadian rhythms, there is great individual variability in the extent of delay of these circadian rhythms. DSWPD patients with delayed and without delayed circadian rhythm timing (circadian non-delayed) have been recently identified [25–27]. It is suggested that circadian non-delayed DSWPD patients may have abnormalities in behavioral circadian rhythms (i.e. SS, sleep propensity [SP], and vigilance rhythms). The important clinical implication of such a finding would be that patients with circadian non-delayed DSWPD would not benefit from chronobiologic treatment [26]. In Micic *et al.* [25], we described a dichotomized sample of patients with DSWPD in which some exhibited circadian timing closely comparable to the cluster of controls (i.e. circadian entrained) and those who exhibited notably later circadian phase markers of core body temperature and melatonin (i.e. circadian delayed). A secondary aim of the present study is to examine and statistically compare the PAE in these sub-groups of patients with

DSWPD. Hence, the primary aim of the present study was to investigate the extent to which intrinsic behavioral circadian rhythms of objective and SS contribute to DSWPD and N24SWD. The secondary aim was to further investigate the etiological differences and contributors in circadian delayed- versus circadian non-delayed DSWPD.

Methods

Procedure details pertaining to the same sample and protocol procedures have been previously published [28].

Participants

A total of 26 participants who met the ICSD-3 criteria for DSWPD (age $M = 21.85 \pm 4.97$, 17m, 9f) and 18 healthy control sleepers (age 23.72 ± 5.10 years, 10m, 8f) participated in a modified constant routine in the Flinders University Sleep and Circadian Research Laboratory. During the screening process, four full-sighted patients (3m, 1f, age: 25.75 ± 4.99 years) met diagnostic criteria for N24SWD and were 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 entire study. 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. Four controls were taking contraceptive medications, one was taking Thyroxine and one using Ventalin and Symbicort. Three participants with DSWPD were on contraceptives, two on amoxicillin, two on Allopurinol, one each on Rosuvastatin, Olanzapine, Sodium Valproate, and Insulin. Two participants with N24SWD were each taking Olanzapine and Thyroxine.

Inclusion/exclusion criteria

To meet DSWPD criteria, candidates had to report evening-type scores on the Morningness-Eveningness Questionnaire (MEQ) [29], a minimum of 2-h discrepancy between their preferred and current sleep pattern, sleep onset that was later than 1:00 am but the quality of sleep that was otherwise sound according to the Pittsburgh Sleep Quality Index (PSQI) [30] when sleeping at their habitual sleep time. Furthermore, they had to report significant daytime impairment on the Sheehan Disability Scale [31, 32] that was associated with the delay in their sleeping pattern. Control sleepers were individuals who displayed normal entrainment to a 24-h day and thus scored intermediate scores on the MEQ and showed no preference to adjust their sleeping patterns earlier or later than 30 min from their current sleep time. They reported good sleep quality (PSQI < 6) 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 comorbid sleep disorders, using drugs of abuse, concurrent medication likely to affect sleep/alertness, circadian rhythms, or melatonin (e.g. selective serotonin reuptake inhibitors)—without approved discontinuation prior to enrollment (including over the counter medicines or herbal substances) for one month prior. Further exclusion criteria included smoking >1 cigarette/day and on average, consuming >250 mg/day caffeine and/or >14 standard alcoholic drinks per week, or being outside the extended normal body mass index range of <18 and >32 kg/m^2 [33]. Exclusion occurred if participants had a history of psychiatric disorders or substance abuse in the past 12 months, were pregnant/lactating, traveled >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 10:00 pm and 8:00 am). 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).

Measures

Core body temperature

Core body temperature was measured every minute during the ultradian protocol using Jonah ingestible core body temperature capsules (Philips/Respironics, California). VitalSense monitors (Philips/Respironics) 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. The Jonah capsule passes through the gastrointestinal tract without affecting bodily functions and 4 temperature readings per minute are transmitted at 15-s intervals to capture 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 measure of core body temperature [34]. Capsules were ingested 2-h prior to protocol commencement to allow stabilization 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 80-h protocol and, in rare cases, two capsules were required. To ensure signals were recorded, the monitor remained near the participant and was placed on their bed, within 50 cm of their body. Hourly measurements were determined by averaging minute recordings.

Salivary melatonin

Saliva samples were collected hourly immediately upon awakening from each 20-min 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 dim light melatonin onsets (DLMO) on the first and final evening of the ultradian protocol. Participants whose DLMO was estimated to occur later than 2:00 am on the final evening (i.e. after the cessation of the experiment), also took samples at half-hourly intervals on the second

to last evening to ensure the final DLMO time could be accurately captured. Samples were labeled and immediately frozen at −20°C after collection. The frozen samples were later analyzed 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; [35]) were added to measure melatonin in the saliva. Sensitivity of the direct radioimmunoassay was <4.3 pM and the intra-assay coefficient of variation was always <10%. The inter-assay coefficient of variation was 15% at 100 pM.

Sleep propensity

SP (i.e. objective sleepiness) is the inverse measure of sleep onset latency, or how long participants slept during each fixed sleep opportunity. Therefore, higher SP values indicate more objective sleepiness/less alertness. SP was assessed at hourly intervals, during the 20-min sleep opportunity using the Compumedics enhance Somte portable recorders (Compumedics, Melbourne, Australia). Prior to the commencement of the ultradian protocol, participants were fitted with electroencephalogram (EEG), electrooculography (EOG), and electromyography (EMG) electrodes attached to a portable Compumedics Somte recorder (Compumedics). 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 80 h constant routine and kept at <8K Ohms. A trained sleep technician determined the onset of sleep using conventional criteria [36], and was blind to condition allocation. PSG 3.0 software (Compumedics), was used to measure SP as the amount of sleep obtained in each 20-min 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-min sleep opportunity. This helped to dissipate homeostatic sleep drive and avoid accumulation of excessive drive across the 80-h laboratory session.

Subjective sleepiness

Prior to each sleep opportunity, participants were asked to indicate their perceived level of sleepiness using the Stanford Sleepiness Scale (SSS) [37, 38]. 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. This scale has been identified as a "gold standard" measure of SS at any moment in time and is amongst the most widely used assessments of SS [38, 39]. It is a strong measure of SS that has been validated and showed convergent validity with other objective and subjective measures of sleepiness such as the Visual Analogue Scale ($r = 0.60$) [37, 40, 41].

Psychomotor vigilance

Psychomotor vigilance was assessed using a 5-min Palm version of the task on a Zire71 hand-held device (PalmOne Inc.) [42–44]. Participants were instructed to press the response button as quickly as possible when a target stimulus appeared on the psychomotor vigilance task (PVT) screen. Participants used the thumb of their dominant hand to respond to a visual stimulus. They were instructed that speed and accuracy of performance were equally important on all tasks. The interstimulus intervals varied between 2 and 10 s with the duration of a single Palm PVT session lasting for 5 min 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. The outcome measure of "vigilance" was measured by the reciprocal of RT, such that faster RT indicated greater vigilance and longer RT indicated lower vigilance. The PVT has a negligible learning curve and within one testing session, participants typically reach asymptotic responding capability [45]. The device has an approximately 10 ms uncertainty in accurately measuring reaction times [46]. The 5-min PVT has been established as a reliable measure that is sensitive to circadian modulation of neurobehavioral functions [15, 43, 44].

Ultradian protocol

After a rigorous screening process, participants underwent an 80-h constant ultradian routine with 1-h "days" consisting of 20-min sleep opportunities alternating with 40-min of enforced wakefulness [28]. Participants resided in temporal isolation (i.e. 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 always available 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, and listening to music). They consumed 200 kCalorie equi-caloric snacks at 2-h intervals, 15-min prior to administration of the PVT task, with 200 mL water. Participants were continuously monitored via real-time PSG, infrared cameras and regular bedside visits during and between frequent and regular testing.

Participants were familiarized with the protocol and tasks prior to the commencement of the 80-h constant ultradian routine. The protocol formally commenced on Thursdays at 6:00 pm and concluded on Mondays, 02:00 am. The VitalSense device was activated with core temperature data recorded at minute intervals. Every "hour-day" commenced on the hour, with a 20-min sleep opportunity. Participants gave their SS ratings using the SSS, the PSG recorder was turned on and lights were turned off to <1 lux during sleep opportunities. SP was measured as the number of minutes participants slept during each sleep opportunity. At the end of the 20-min sleep opportunity, lights were turned back on to <10 lux for a 40-min period of enforced wakefulness. 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-min into the hour, or 20 min 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 the experiment.

Data analysis

The resultant 80-h core body temperature (T), SS, SP, and vigilance (V) values were plotted using KaleidaGraph version 4.1.3 for Mac. Circadian rhythms of core temperature were established using 2-component (24-h and 12-h components) cosine curves that best accounted for the plotted values [47]. Uniform methodology was used to generate two-component cosine curves for established 80-point values of SP, 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, and V in the first 24-h period of the protocol for their baseline circadian phase measures. These were compared using SPSS Statistics software to assess time associations between maximum objective and SS, and lowest vigilance.

Assayed salivary melatonin values were also charted using KaleidaGraph software. DLMO were estimated using an absolute threshold of 10 pM, with at least two consecutive samples thereafter remaining above the 10 pM threshold, 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). Please refer to Micic *et al.* [25] for a detailed outline of methods that were used to dichotomize circadian delayed DSWPD (n = 14) and circadian non-delayed DSWPD (n = 13).

Results

Circadian timing and tau lengths

The timing of patients' with DSWPD sleeping patterns one week immediately prior to commencement of the laboratory protocol and the times of their minimum and maximum alertness in the first 24 h of the constant ultradian routine are presented in Table 1. These were compared to healthy control sleepers and are further illustrated in Figure 1. A series of independent samples *t*-tests confirm that the timing of patients with DSWPD sleeping patterns and circadian rhythm measures were significantly delayed by 2–3 h compared to controls. Based on effect sizes (i.e. Cohen's d), the greatest between-group differences were observed for mid-sleep timing, DLMO, maximum SP, and both minimum vigilance times. Due to the unstable nature of patients' with N24SWD sleeping patterns, central tendency data would not be meaningful and thus is not presented for this clinical subgroup in Table 1 nor Figure 1.

We explored circadian biological versus behavioral *tau* differences between patients with DSWPD, N24SWD, and controls. The group means are shown in Figure 2. It shows generally longer core temperature and melatonin rhythm taus for the groups with DSWPD and N24SWD than controls. A 3 by 5 repeated measures analysis of variance (ANOVA) was used to investigate between- and within-groups *taus* of core body temperature, melatonin, SS, SP, and vigilance. There were significant main effects of both groups ($F(4,4) = 2.95$, $p = 0.03$, $\eta^2 = 0.23$) and different rhythms ($F(4,4) = 3.89$, $p = 0.023$, $\eta^2 = 0.17$) but no significant overall interaction effect. Follow-up post hoc independent *t*-tests presented in Figure 2 indicate that patients with DSWPD in general exhibited longer *taus* relative to controls, as a result of longer biological *taus* rather than behavioral rhythms. Patients with N24SWD portrayed significantly longer *taus* of core body temperature and melatonin compared to both controls and patients with DSWPD. Paired samples *t*-test analyses (Table 2) indicate that the period lengths of melatonin and core body temperature, melatonin and vigilance, SS and SP, and SS and vigilance were significantly correlated within-subjects. The within-subjects analyses also indicate that most circadian rhythm period lengths were statistically different from one another (see Table 2).

Table 1. Mean mid-sleep times on free days, commitment days, and overall for patients with DSWPD relative to controls

Circadian parameter	Controls	DSWPD	Mean Diff. ± SE	Cohen's *d**	*t*-value	*P* value
Habitual mid-sleep timing†						
Mid-sleep average	03:43 am ± 37 m	06:10 am ±1 h 27 m	2.44 ± 0.32	2.09	7.54	<0.001
Mid-sleep free days	04:04 am ± 32 m	07:18 am ± 1 h 26 m	3.23 ± 0.31	5.91	10.50	<0.001
Mid-sleep work days	03:17 am ± 32 m	05:06 am ± 1 h 7 m	1.82 ± 0.25	1.96	7.15	<0.001
Core body temperature (*T*)						
Tmin	04:55 am ± 1 h 56 m	07:00 am ± 2 h 24 m	2.09 ± 0.68	0.94	3.06	0.004
Tmax	6:20 pm ± 2 h 26 m	8:18 pm ± 2 h 25 m	1.95 ± 0.74	0.81	2.63	0.012
Melatonin (DLMO)						
DLMOff‡	08:17 am ± 1 h 07 m	10:41 am ± 3 h 00 m	2.41 ± 0.76	1.00	4.04	<0.001
DLMO‡	8:56 pm ± 1 h 26 m	11:12 pm ± 1 h 58 m	2.27 ± 0.56	1.27	3.16	0.003
Subjective sleepiness (SS)						
SS Maximum‡	06:11 am ± 2 h 25 m	08:46 am ± 2 h 44 m	2.13 ± 0.84	0.78	2.53	0.015
SS Minimum‡	5:16 pm ± 3 h 20 m	8:26 pm ± 4 h 13 m	3.17 ± 1.14	0.82	2.66	0.011
Sleep propensity (SP)						
SP Maximum	05:52 am ± 1 h 20 m	08:46 am ± 2 h 30 m	2.90 ± 0.73	1.11	4.00	0.001
SP Minimum	8:45 pm ± 1 h 46 m	10:47 am ± 2 h 01 m	2.35 ± 0.63	0.82	3.71	0.001
Vigilance (V)						
V Minimum‡	05:53 am ± 2 h 20 m	08:54 am ± 2 h 09 m	2.98 ± 0.69	1.27	4.43	<0.001
V Maximum‡	6:38 pm ± 3 h 32 m	9:58 pm ± 3 h 49 m	3.50 ± 1.13	0.90	3.09	0.005

Minima and maxima of alertness measured by core body temperature, melatonin, subjective sleepiness, sleep propensity, and vigilance during the first 24-h phase markers of the ultradian routine are also shown.

T, core body temperature; DLMO, dim light melatonin onset; DLMOff, dim light melatonin offset; SS, subjective sleepiness; SP, sleep propensity; V, vigilance.

*Cohen's d Size of effect: $d > 0.20$ = small; $d > 0.50$ = medium; $d > 0.80$ = large.

†Actigraphy data confirmed by sleep–wake diaries.

‡Mean clock time ± standard deviation (*SD*) in hours and minutes (h:mm ± h:mm).

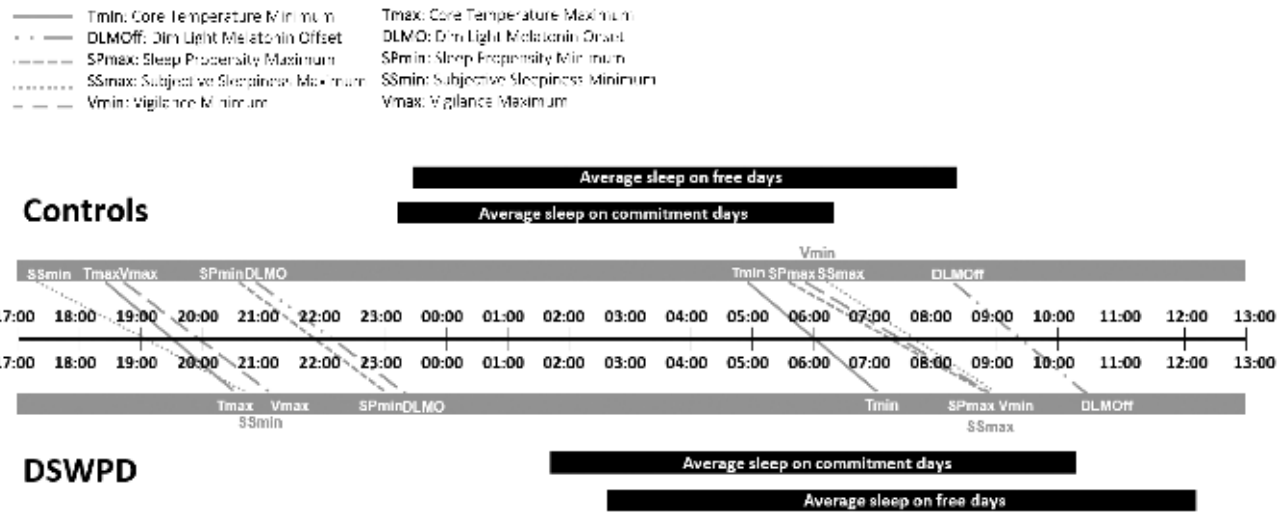

Figure 1. Minima and maxima of alertness measured by core body temperature (T_{min} and T_{max}), melatonin (DLMO and DLMOff), SS (SS_{min} and SS_{max}), SP (SP_{min} and SP_{max}), and vigilance (V_{min} and V_{max}) in DSWPD (bottom) relative to controls (top), across the first 24-h cycle of the ultradian routine. Patients' with DSWPD and controls' average sleep periods on workdays versus free days are superimposed.

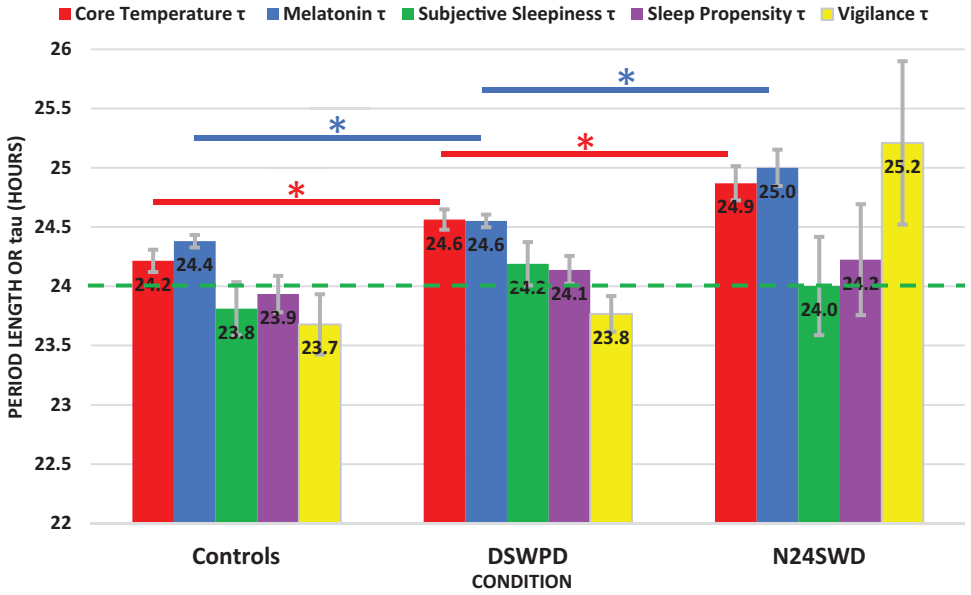

Figure 2. Group means in rhythm lengths (*taus* or τ) of core body temperature τ, melatonin τ, SS τ, SP τ, and vigilance τ during the 80-h ultradian routine. *$p < 0.05$ difference relative to controls. Vertical bars indicate standard error and horizontal line at 24 h indicates a typical solar day.

PAE (assessment of the first 24 ultradian hours only)

The differences in identified temperature circadian nadirs and behavioral phases (i.e. vigilance minimum and sleepiness/SP maximums) during the first 24-h of the experiment, were used to test the assumption that there could be greater phase angle differences between core temperature and behavioral sleepiness in patients with DSWPD relative to controls. The phase differences between Tmin and the behavioral phase markers are shown for the two groups in Figure 2. Generally, patients with DSWPD showed a greater delay of maximum SP relative to core temperature phase marker, when compared with controls(Supplementary Table S1).

In addition to testing differences in specific rhythm phase markers, cross-correlations were calculated within each individual to test associations between biological and behavioral rhythms. Since there were differences in *taus* between groups for the rhythm variables, the cross-correlations across all 80 h may be reduced by differential phase change between different rhythms arising from the different *taus*. Therefore, correlation coefficients were calculated from the first 24-h data to investigate spontaneous associations between rhythms for patients with DSWPD and N24SWD, relative to controls. Correlations between two variables were calculated at the same time point and at one-hour phase lagged time points for several hours after and before the same time point to determine the phase lag of the highest correlation. This method of determining phase angles between variables considers all 24 h of the data rather than only the one phase marker for the whole rhythm. The maximum correlations were all highly significant and varied between 0.51 and 0.78 for the two large groups (Supplementary Table S2). The group means for phase lag of

Table 2. Within-subject associations of biological and behavioral circadian period lengths and mean difference comparisons in biological and behavioral circadian period lengths

Period length comparisons	*N*	*r* value	Sig.	Mean diff ± *SD* (h)	*t*-value	*P* value
Melatonin and core temperature	49	0.38	0.009*	0.07 ± 0.44	1.012	0.317
Melatonin and subjective Sleepiness	49	0.11	0.474	0.29 ± 0.98	1.993	0.052
Melatonin and sleep Propensity	49	0.02	0.892	0.42 ± 0.69	4.142	<0.001*
Melatonin and vigilance	49	0.33	0.025*	0.64 ± 0.91	4.820	<0.001*
Core temperature and subjective sleepiness	49	0.21	0.147	0.30 ± 0.96	2.135	0.038*
Core temperature and sleep propensity	49	0.16	0.283	0.39 ± 0.73	3.724	0.001*
Core temperature and vigilance	49	0.14	0.343	0.61 ± 1.02	4.124	<0.001*
Subjective sleepiness and sleep propensity	49	0.53	<0.001*	0.16 ± 0.85	1.311	0.196
Subjective sleepiness and vigilance	49	0.54	<0.001*	0.40 ± 0.94	2.975	0.005*
Sleep propensity and vigilance	49	0.44	0.002*	0.24 ± 0.91	1.850	0.071

*$p < 0.05$.

Table 3. Group means of phase lag between variables producing the highest cross-correlation. Also shown are the mean differences between groups and Cohen's d effect size.

	Phase Lags (hours)			Phase Lag Differences (hours)		
	Means ± Standard Deviation			Mean Difference ± Standard Error Cohen's *d*		
Rhythms	Controls	DSWPD	N24SWD	DSWPD–Controls	N24SWD–Controls	N24SWD–DSWPD
SS and Core Temp	1.06 ± 2.13 h	0.81 ± 2.00 h	3.67 ± 8.14 h	−0.24 ± 0.63 h *d*= 0.12	2.61 ± 2.07 h *d*= 0.78	**−2.85 ± 1.77 h** ***d*=0.98***
SP and Core temp	1.12 ± 1.32 h	2.81 ± 1.98 h	2.33 ± 1.53 h	**1.48 ± 0.56 h*** ***d*= 0.79**	1.00 ± 1.00 h *d*= 0.62	0.48 ± 1.19 h *d*=0.25
V and Core temp	1.56 ± 2.43 h	0.96 ± 2.27 h	1.33 ± 1.53 h	0.59 ± 0.72 h *d*= 0.25	0.222 ± 1.47 h *d*= 0.09	.37 ± 1.36 h *d*= 0.17
Temp and Mel	1.00 ± 2.72	1.80 ± 2.68	0.75 ± 2.50	0.80 ± 0.85 *d*= 0.30	0.25 ± 1.49 *d*= 0.09	1.05 ± 1.43 *d*= 0.39
SS and Mel	2.71 ± 2.69	2.64 ± 2.36	8.75 ± 9.5	0.07 ± 0.78 *d*= 0.03	6.04 ± 2.51 *d*= 1.34	**−6.11 ± 4.77** ***d*= 1.58***
SP and Mel	3.00 ± 1.50	2.80 ± 2.79	3.00 ± 3.37	0.20 ± 0.55 *d*= 0.08	0.00 ± 1.07 *d*= 0.00	−0.20 ± 1.13 *d*= 0.07
V and Mel	3.59 ± 2.79	2.88 ± 1.99	1.67 ± 2.52	0.71 ± .74 *d*= 0.30	1.92 ± 1.73 *d*= 0.70	1.21 ± 1.24 *d*= 0.59
SP and SS	0.33 ± 1.41 h	0.63 ± 1.34 h	4.67 ± 8.08 h	0.26 ± 0.42 h *d*= 0.19	4.33 ± 4.68 h *d*= 1.48	**4.07 ± 4.67 h** ***d*= 1.62***
V and SS	−0.39 ± 1.09 h	−0.58 ± 1.53 h	1.33 ± 2.08 h	0.19 ± 0.42 h *d*= 0.14	1.72 ± 0.77 h *d*= 1.40	1.91 ± 0.96 h *d*= 0.30
V and SP	−0.11 ± 1.57 h	−0.62 ± 1.02 h	−1.00 ± 1.00 h	0.50 ± 0.39 h *d*= 0.77	0.89 ± 0.95 h *d*= 0.73	0.38 ± 0.62 h *d*= 1.59

Differences in bold indicate significance ($p < 0.05$). Positive values suggest that the maximum cross-correlation is obtained when the first variable follows or precedes the second by that number of hours. Increasing values of the SS and SP variables indicate greater physiological sleepiness. Lower values of the V and Temp measures indicate greater physiological sleepiness.
Mel, melatonin; DLMOff, dim light melatonin offset; SS, subjective sleepiness; SP, sleep propensity; V, vigilance. $n = 1$ DSWPD and $n = 1$ control lacked sufficient saliva to determine full melatonin profiles and were excluded from these analyses.
*$p < 0.05$.

maximum cross-correlations are presented in Table 3. The results confirmed the previous analysis that patients with DSWPD had a greater delay of SPmax after Tmin than the control group.

The cross-correlation analyses indicate that melatonin and temperature rhythms were highly correlated in all three groups (r = 0.72–0.83). The maximum cross-correlation between the two rhythms was obtained when the core body temperature rhythm occurred approximately an hour (M = 0.75–1.8 h) after the salivary melatonin rhythm. Irrespective of their significantly longer biological *taus*, patients with DSWPD had significantly greater lags of SP following the temperature rhythm compared to controls on the first 24 h of the ultradian routine.

Circadian delayed and circadian non-delayed DSWPD

Figure 3 shows the relative timings of circadian rhythms phase markers relative to habitual sleep timing on commitment and free days for the two DSWPD subgroups. On both commitment and free days, the circadian non-delayed sub-group slept at later circadian phases for biological and behavioral rhythms compared to the circadian delayed groups.

Individuals with circadian delayed and circadian non-delayed DSWPD were also compared to healthy controls in terms of circadian (e.g. MEQ, mid-sleep factor, circadian taus, total sleep obtained across 80-h as a surrogate for homeostatic sleep

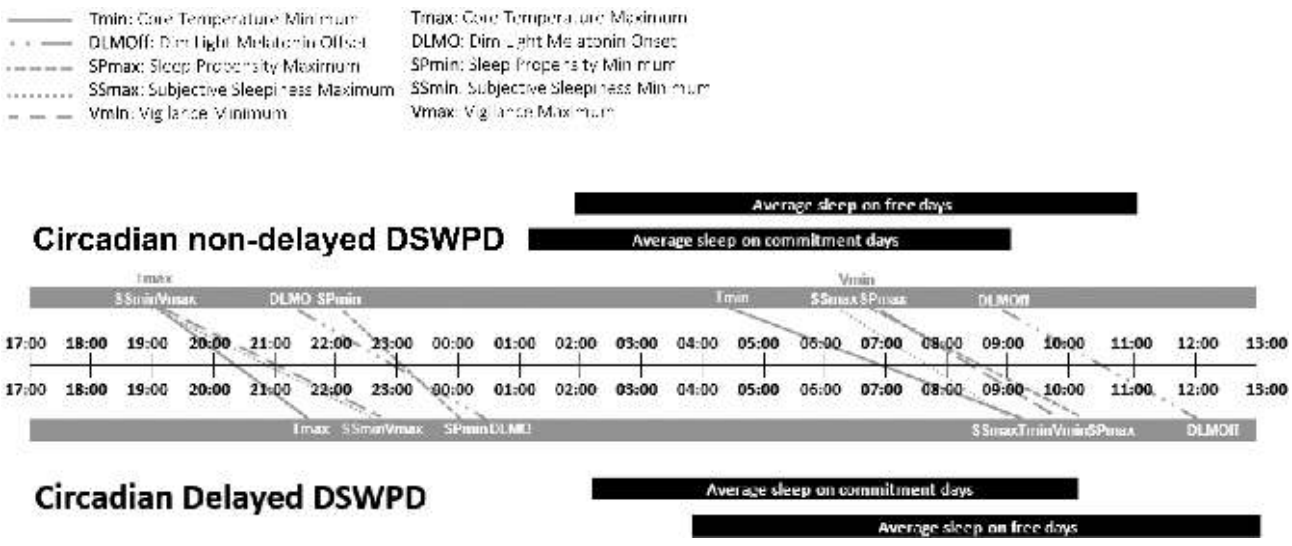

Figure 3. Minima and maxima of alertness measured by core body temperature (T_{min} and T_{max}), melatonin (DLMO and DLMOff), SS (SS_{min} and SS_{max}), SP (SP_{min} and SP_{max}), and vigilance (V_{min} and V_{max}) in circadian delayed DSWPD (bottom) compared to circadian non-delayed DSWPD (top), across the first 24-h cycle of the ultradian routine. Circadian non-delayed versus circadian delayed average sleep periods on commitment days versus free days are superimposed.

pressure), psychological (e.g. habitual sleep latency, depression, anxiety, stress, motivation to change sleep) and lifestyle (e.g. work/school/family/social life disruption) outcomes to examine whether there were other differences between these sub-groups that may be associated with the different PAEs. These are shown in Supplementary Table S3. None of the comparisons between the sub-groups were significant. However, effect sizes indicate that the circadian delayed DSWPD were greater evening-types, had poorer sleep quality, greater social life disruption, more days of absence, lower motivation to advance sleep timing, greater anxiety, depression and stress, and had slightly longer circadian *taus* than the circadian non-delayed DSWPD.

Discussion

In this modified ultradian constant routine, we examined the initial timing and period lengths of the circadian rhythms of core body temperature, melatonin, SS, objective SP, and vigilance in patients with DSWPD, N24SWD, and controls. In general, patients with N24SWD showed the longest overall biological circadian *taus*, followed by patients with DSWPD. Controls exhibited *taus* that were closest to the 24 h light/dark cycle and consistent with the *taus* for normal controls derived from other methodologies such as 28-h "day" enforced desynchrony studies [48]. Patients with DSWPD and N24SWD have significantly longer core temperature and melatonin *taus* relative to controls. However, contrary to the hypotheses, the behavioral rhythm *taus* as measured by SS, objective sleepiness and vigilance were no longer in the patient groups than in the controls.

While causality cannot be inferred from correlational studies, the results suggest that two mechanisms may contribute to the delayed sleep difficulty of DSWPD: (1) longer biological *taus* resulting in a greater tendency to delay the phase of the circadian rhythm given the opportunity such as a late wake-up time reducing the phase advancing effect of morning light and (2) phase angle differences whereby individuals with DSWPD have a relatively delayed sleep period that may also result in more exposure to phase delaying effects of evening light and less exposure to the phase advancing effects of morning light. These results warrant further investigation in future studies with experimental interventions to determine causality. Nevertheless, the findings suggest that tools to retime biological rhythms, such as the appropriate timing of bright light administration and avoidance, as well as melatonin administration, should be the focus of chronobiological interventions in treating people with DSWPD. Especially the sub-group with markedly delayed biological circadian rhythms who are struggling to sleep and wake at relatively early circadian phases on commitment days.

Phase angle differences in DSWPD

Patients with DSWPD exhibited greater phase lag of SP rhythms relative to the core temperature rhythm. In particular, patients with DSWPD showed a greater phase angle difference between core body temperature nadir and maximum SP (approx. 2 h) than the control group (0.7 h), suggesting that the PAE between the core body temperature rhythm and objective sleepiness is ~1.3 h greater for people with DSWPD than controls. This suggests that the SP or sleep-ability circadian rhythm is over an hour later relative to the core temperature circadian rhythm in those with DSWPD. Therefore, in addition to a delay of about 2 h in the circadian core body temperature rhythm, there is a further delay of over an hour of SP in the group with DSWPD. In the present study, patients with DSWPD were selected if they had an eveningness chronotype and controls had an intermediate chronotype. Nevertheless, the results suggest that evening-type patients with DSWPD who attempt to initiate sleep at the same phase in the temperature circadian rhythm in the evening (although it is 2 h later than normal), may be less successful than attempting to sleep later. Through experience, these patients may learn to delay bedtime for more successful initiation of sleep. Results further indicate that in the morning, maximum sleep pressure for patients with DSWPD will occur over an hour later relative to their already delayed circadian rhythm, thus making arising in the morning even more difficult.

The tendencies to delay bedtimes and wake-up times to an even later circadian phase may have feedback effects on the biological circadian phase. 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 [49, 50]. Later bedtimes relative to the circadian system will expose patients to greater phase delaying effects of evening light, and thus a stronger delaying effect according to the phase response curve to light [12, 51]. Furthermore, a delay in morning awakening will delay the onset of beneficial phase advancing effects of morning light [48–50, 52]. According to the phase response curve, this later light exposure will have a diminished phase advancing effect. Thus, these larger PAEs for people with DSWPD may exacerbate their biological circadian phase delays and along with it, the even later behavioral choices of bedtimes and wake-up times.

DSWPD subgroups?

We identified and dichotomized two subgroups of patients with DSWPD, half of whom exhibited circadian phases closely comparable to the cluster of controls (circadian-non-delayed DSWPD), while the other half showed notably later circadian timing (circadian delayed DSWPD). Figure 3 shows relatively late typical baseline sleep onset and offset times compared to Tmin in patients with circadian non-delayed DSWPD compared to the delayed group. These results show that patients with circadian non-delayed DSWPD are sleeping at a relatively late PAE. The factors that are contributing to this difference are speculative, but it may be that their sleepiness and sleep ability are intrinsically relatively delayed compared to their circadian timing as suggested in Figure 3. Circadian rhythm research has shown the relationship between SP and circadian rhythm timing (i.e. PAE) in general [53, 54] but has rarely considered an individual variation of those relationships. Objective sleepiness is an important factor in determining sleep time based on both psychological and physiological states [20]. Unlike SS 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 patients with DSWPD feel heightened arousal in the late evening and may defer their bedtime for various reasons [55–59]. The choice of a much later bedtime and wake-up time would result in the delay of morning light exposure and prolong exposure to light at night. Delayed choice of bedtime leads to later awakenings and further delays of the circadian rhythm [52, 60, 61]. It may be the case that the subgroup of circadian non-delayed DSWPD has a naturally late SP rhythm and thus late sleep pattern with respect to biological rhythm timing. The problem with the delayed DSWPD is their greater delayed biological rhythms plus a normal PAE resulting in a delayed sleep/wake pattern. These speculative explanations require further empirical investigation.

For people with circadian non-delayed DSWPD, bedtime procrastination may be an alternate explanation, perhaps coupled with conditioned insomnia and/or an aversion to attempting sleep earlier [62]. They may exhibit prior negative experiences with sleep initiation or have personality traits (e.g. perfectionism [63]) that hinder bedtime (e.g. staying up late to complete tasks).

Our results show significant differences in PAE between two seemingly distinct DSWPD subtypes, which may explain the inconsistency in the evidence to date regarding PAE differences whereby some researchers have found significant PAE differences and other research does not support these findings [14, 15]. It may be that the studies finding evidence for PAE differences predominantly sampled patients with circadian non-delayed DSWPD and those finding no PAE differences consisted mainly of patients with circadian-delayed DSWPD.

The results of this study also indicate the possible role of behavioral variables contributing to sleep disturbance in DWSPD [55–59]. For example, a reduced ability to initiate sleep at a conventionally desired bedtime arising from both a delayed biological circadian phase and delayed phase angle of SP rhythm would lead to difficulties initiating sleep on the part of patients with DSWPD. It is certainly the case that both DSWPD subtypes had sleep onset latencies reported from their baseline week (50 and 42 min) indicative of sleep-onset insomnia. Patients could develop resultant sleep onset insomnia from repeated, frustrated attempts to initiate sleep when attempting to sleep at conventional times [57, 58, 64, 65]. The development of sleep-onset insomnia would contribute to a delay in effective sleep onset time, a decrease of total sleep time given an enforced wake-up time on workdays with commitments, an accumulation of homeostatic sleep drive across the workweek, and a greater need for more recovery sleep, obtainable with a delayed wake time on free days. This delayed wake-up time on free days will delay circadian rhythms [52] and exacerbate the sleep onset problem when resuming workdays, the following week. Our results did not show a difference in sleep onset latency between circadian non-delayed and delayed DSWPD. Furthermore, there was no difference in the amount of sleep obtained in all 80 sleep opportunities across the protocol implying no mean difference in sleep latencies between the groups. It appears that the sleep latencies measured objectively in the laboratory for the groups with DSWPD were the same as the good sleepers and therefore normal. Therefore, conditioned insomnia does not appear to be present in DSWPD nor impact one subtype more than the other.

Non-24-hour sleep–wake disorder

In the present study, the PAE findings of patients with DSWPD do not extend to patients with N24SWD who did not show significant differences in phase angles of entrainment in behavioral rhythms relative to core temperature. The patients with N24SWD showed longer vigilance rhythms in addition to their longer biological *taus* and, interestingly, their SS rhythms preceded all other rhythms during the constant routine. Hence, our results indicate that in time-free environments, significantly longer core temperature and melatonin *taus* of patients with N24SWD contrast with the near-24-hour behavioral sleepiness rhythms. This does not support the scarce circadian-based literature on N24SWD that suggests that 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 [11, 21].

The findings of the present study indicate lower circadian associations in the group of N24SWD compared to the DSWPD and control groups' significant associations in core body temperature rhythms. This suggests a weaker connection or some degree of dissociation between rhythms in the group with N24SWD may allow them greater latitude to free run their sleep/wake cycle. The non-24-hour sleep/wake cycles of these patients can be likened to permanent jetlag and constant changes to "time-zones" such that endogenous rhythms cannot

stabilize 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 potent zeitgeber. However, it has been suggested that the peripheral clocks take longer to become entrained and will adapt at different rates [66] causing some dissociation between various rhythms. Different adaptation rates of the peripheral clocks can affect individuals' mood and well-being [67]. Therefore, if the SCN in patients with N24SWD cannot synchronize with the natural light/dark cycle due to instability in the sleep/wake cycles and circadian rhythms, they may have ongoing endogenous circadian rhythm de-synchronization in their peripheral clocks. Although entirely speculative, general trends in Supplementary Table S2 suggest that these factors might in part contribute to DSWPD also.

Limitations

A primary aim was to examine the phase relationships between biological and behavioral rhythm outcomes. It is important to note that this protocol allowed sleep hence may not be optimal to assess relationships between measured outcomes as these are impacted by sleep. For example, average hourly temperature values were curve-fitted from minute values and given sleep decreases body temperature this method may increase measurement error for temperature assessment. On the other hand, sleep impacts core body temperature similarly in all groups thus enabling between-groups comparisons. There is also a potential for sleep inertia to impact measurement of reaction time. While a 20-min interval is long enough to dissipate sleep inertia after a brief 20-min nap [68], it is unclear whether sleep inertia is longer in duration or of a larger magnitude for patients with DSWPD compared to controls and warrants further investigation.

The selection of only intermediate chronotypes in the control group may have created a selection bias. Although this is speculative, the diurnal preference for later bedtimes may contribute to phase-delayed rhythms of SP relative to core body temperature in patients with DSWPD. Hence, if controls with evening-type preferences were selected for the study, then theoretically they might have also exhibited longer SP versus core body temperature PAEs. Future work is warranted to investigate PAEs in different chronotypes with similar sleep timing to elucidate this inference.

Furthermore, the controlled, dimly lit, confined nature of our ultradian study limits our ability to interpret how behavioral and external factors (e.g. social influences and choice of bedtime) affect circadian timing. Circadian rhythm timing is subject to the effect of light [69, 70]. Therefore, choices of in-bed and rise-times that largely determine individuals' exposure to visual stimulation, could contribute to circadian misalignment in both patients with DSWPD and N24SWD. 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-h sleep/wake cycle. They are simply the rhythms of sleepiness with sleep distributed across 24 sleep opportunities each day. Dijk and Schantz [53] note that the sole contribution of neither the circadian oscillator nor the homeostatic drive can predict individuals' sleep and performance—only the interaction of both. In naturalistic environments, we predict that external environmental factors such as bedtime procrastination, nighttime media use, social engagement could only exacerbate the significant SP and core temperature phase-lags.

Conclusions

The circadian rhythm disorders of DSWPD and N24SWD are associated with longer biological rhythm *taus* but not sleepiness rhythm *taus*. Therefore, it seems more likely that the delayed sleep patterns in these groups are driven mainly by a greater tendency to delay the circadian rhythm phase whenever the braking effects of morning light are absent. It seems less likely that the delayed sleep patterns in these groups are driven by endogenous behavioral sleep circadian rhythms with a greater tendency to delay in these disorders. The curious discovery of many individuals with DSWPD who do not have an abnormally delayed core temperature or melatonin rhythm may be simply a function of individual differences in endogenous phase angle differences. Patients with circadian non-delayed DSWPD typically sleep at a later biological circadian phase which may be normal for them. This group has been considered to have a delayed sleep pattern for reasons other than a delayed circadian pacemaker and therefore not treatable with chronobiologic interventions such as morning bright light and early evening melatonin administration. However, if they simply have a different circadian rhythm phase angle to their SP rhythm but just as strong a relationship between them, then they should be treated with the same tools. Whether the phase response curves to light and melatonin administration would be the same between the subgroups with respect to the biological circadian rhythm timing or sleep pattern timing is a question that needs investigation.

Supplementary material

Supplementary material is available at *SLEEP* online.

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Ethics approval

This research was approved by the Southern Adelaide Clinical Human Research Ethics Committee (019.12; South Australian Local Health Network, Flinders University of South Australia).

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