Title: Prevalence of Circadian Misalignment and Its Association with Depressive Symptoms in Delayed Sleep Phase Disorder

Short Title: Circadian Misalignment in DSPD

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ABSTRACT

Study objective: To examine the prevalence of circadian misalignment in clinically diagnosed DSPD, and compare mood and daytime functioning in those with and without a circadian basis for the disorder.

Methods: 182 DSPD patients aged 16 to 64 years, engaged in regular employment or school, underwent sleep-wake monitoring in the home, followed by a sleep laboratory visit for assessment of salivary dim light melatonin onset (DLMO). Based on the DLMO assessments, patients were classified into two groups: circadian DSPD, defined as DLMO occurring at or after desired bedtime, or non-circadian DSPD, defined as DLMO occurring before desired bedtime.

Results: 103 patients (57%) were classified as circadian DSPD and 79 (43%) as non-circadian DSPD. DLMO occurred 1.66 h later in circadian DSPD compared to non-circadian DSPD ($p<0.001$). Moderate-severe depressive symptoms (Beck Depression Inventory-II) were more prevalent in circadian DSPD (14.0%) than in non-circadian DSPD (3.8%; $p<0.05$). Relative to non-circadian DSPD patients, circadian DSPD patients had 4.31 times increased odds of at least mild depressive symptoms (95% CI 1.75 to 10.64, $p<0.01$). No group differences were found for daytime sleepiness or function, but DSPD symptoms were rated by clinicians to be more severe in those with circadian DSPD.

Conclusions: Almost half of patients clinically diagnosed with DSPD did not show misalignment between the circadian pacemaker and the desired bedtime, suggesting that the reported difficulties initiating sleep at the desired bedtime are unlikely to be explained by the (mis)timing of the circadian rhythm of sleep propensity. Circadian misalignment in DSPD is associated with increased depressive symptoms and DSPD symptom severity.

Keywords: delayed sleep, depression, circadian misalignment

SIGNIFICANCE

Delayed Sleep Phase Disorder (DSPD) is a highly prevalent, often undiagnosed primary circadian sleep disorder, caused by a delay in the endogenous circadian clock. Here we demonstrated that almost 50% of patients clinically diagnosed with DSPD did not show evidence of having a delay in circadian clock relative to desired bedtime, suggesting a non-circadian etiology for their sleep complaints. Additionally, we found that those with circadian misalignment showed significantly elevated depressive symptoms, providing further evidence for a circadian basis for mood disturbances. These findings support the measurement of the melatonin rhythm to distinguish patients with and without circadian misalignment, as a part of the routine diagnostic process. Furthermore, measurement of the melatonin rhythm may improve treatment outcomes by more accurately phenotyping patients to identify those who are likely to benefit from chronobiological treatments, and to optimise the timing of chronobiological treatments such as light-dark exposure and melatonin.

INTRODUCTION

Delayed Sleep Phase Disorder (DSPD) is a circadian rhythm sleep disorder (CRSD) characterized by a delay in the major sleep episode relative to the desired or required sleep-wake cycle, resulting in difficulty both initiating sleep and subsequently awakening at the desired or required clock times[1]. DSPD is more prevalent in teenagers and young adults (7-16%)[1] than in middle-aged adults (3.1%)[2].

DSPD is associated with significant functional impairments. Adult DSPD patients report significant impairments to job performance, financial difficulties and marital problems[3], and also greater medication use, particularly hypnotics[4]. Adolescents with DSPD show poorer school performance[5,6], dysfunctional school behaviors, underachievement[7], and are more likely to engage in negative health behaviors such as smoking[8], and excessive alcohol[6] and caffeine use[9]. Depressive symptoms are commonly reported in DSPD patients[10]. Abe and colleagues showed that 64% of patients with DSPD had a comorbid diagnosis of depression[11]. In a survey of 917 consecutive sleep clinic patients, 41% of DSPD patients showed evidence of depressive symptoms[12]. Preliminary findings suggest that treatment of DSPD with exogenous melatonin significantly improved depressive symptoms[13].

The higher rate of depression in the DSPD population is perhaps not surprising, as circadian misalignment appears to play a role in the pathophysiology of mood disorders[14], particularly depression[15,16], although the precise mechanisms underlying the associations are unclear. Disruption of the circadian system increases vulnerability to major depression[17,18], and specific polymorphisms in the core circadian clock genes *CLOCK*, *BMAL1*, *PERIOD3* and *TIMELESS* are associated with increased risk of mood disorders[19]. Furthermore, short sleep duration, which is common in DSPD patients, may interact with a vulnerability generated by a polymorphism in the serotonin transporter gene (5-HTTLPR in *SLC6A4*) to increase the risk of depression in DSPD[20].

In clinical practice, DSPD as a circadian disorder is often not easily differentiated from sleep initiation insomnia, particularly in the absence of an objective and reliable diagnostic test for DSPD. Therefore, some patients classified as having DSPD based on current diagnostic criteria, which rely predominantly on self-reported symptoms[21,22], may not show a delay in the timing of the circadian pacemaker[23-26] and be incorrectly diagnosed. Separate from the circadian mechanisms that underlie DSPD, DSPD-like symptoms may be attributed to reduced homeostatic sleep pressure leading to increased evening alertness[27], poor sleep hygiene[28], and pre-sleep hyperarousal[29,30]. Different treatment approaches are likely needed to effectively manage DSPD symptoms depending on the specific etiology.

We investigated the timing of the melatonin rhythm relative to desired sleep time in patients with clinically diagnosed DSPD[1], to determine the proportion of DSPD attributable to circadian misalignment. Patients were classified into circadian and non-circadian DSPD types based on a laboratory-based circadian phase assessment using salivary Dim Light Melatonin Onset (DLMO). We then compared mood and daytime functioning in those with and without delayed circadian timing.

METHOD

This multi-center study was approved by the Monash University Human Research Ethics Committee, The University of Sydney Human Research and Ethics Committee, Southern Adelaide Clinical Human Research Ethics Committee, and The University of Adelaide Human Research Ethics Committee. All participants provided written informed consent and were reimbursed for study-related expenses. The current study was part of a larger randomized controlled trial testing the efficacy of exogenous melatonin for DSPD, Delayed Sleep on Melatonin (DelSoM) Study Group (ACTRN12612000425897).

Participants

One hundred and eighty-two participants (89 M, 93 F) aged between 16 to 64 years, with a body mass index (BMI) between 18 and 35 kg/m^2, were recruited at three study sites: Melbourne (Monash University), Sydney (Woolcock Institute of Medical Research) and Adelaide (Flinders University). Participants were recruited from the community via radio, newspaper, television and poster advertisements, and referrals from sleep physicians, general practitioners and psychologists. Participants met diagnostic criteria for DSPD[1] based on clinical interview by a sleep physician. Additionally, we did not specifically exclude participants with sleep initiation insomnia as this type of insomnia is not easily differentiated from the symptoms of DSPD. See Figure 1 and supplemental material for detailed inclusion/exclusion criteria.

Circadian and non-circadian DSPD phenotypes

Participants were classified into phenotype groups based on the relationship between DLMO and desired bedtime (DBT). A circadian DSPD phenotype was defined as having a DLMO time at or

after DBT. To allow for potential measurement error in DLMO and/or DBT, those with DLMO occurring up to 30 minutes before DBT were also classified as circadian DSPD. The non-circadian DSPD phenotype was defined as having a DLMO time > 30 min before DBT. DBT was derived from the following question: "On the night before school or work, what time would you need to go to bed in order to feel fully rested in the morning?"[31].

Given that the diagnostic criteria for DSPD refer to required or desired bedtime (Criterion A)[32], our definition of circadian DSPD phenotype was based on the timing of the onset of melatonin synthesis relative to *desired* or *required* bedtime, rather than actual bedtime, which may be driven by social demands or constraints. In healthy individuals, melatonin onset occurs on average ~2 hours before sleep onset[33]. In our patient population, we first determined whether or not desired bedtime differed from habitual bedtime. If it did, we then examined whether or not DLMO was appropriately timed relative to desired sleep time. We assumed that if desired bedtime occurred at least 30 minutes after DLMO, the timing of DLMO (and, by extrapolation, the circadian rhythm of sleep propensity) was unlikely to be the primary cause of the reported difficulty initiating sleep at the desired bedtime (i.e. the hallmark symptom of DSPD).

Screening

Participants completed: online questionnaires assessing DSPD symptoms[31], sleep-wake habits, sleep impairments, daytime functioning and diurnal preference[34] (see supplemental material for details); further screening by telephone; formal screening visit after providing written informed consent; and a clinical consultation with a physician specializing in sleep medicine. In addition to confirming diagnosis of DSPD[1], the physician rated symptom severity and duration using the Clinical Global Impressions of Severity (CGI-S)[35] (see supplemental material).

Sleep-wake assessment

Participants were asked to maintain their usual sleep-wake patterns and were monitored for at least 7 days immediately prior to a laboratory visit using a sleep diary, verified by wrist actigraphy (Actiwatch Spectrum, Philips Respironics, Bend, OR, USA). In cases of actigraphy malfunction or non-compliance (n=13, 7.03%), participants were required to repeat the sleep-wake monitoring. Participants also completed a daytime activity diary to record daytime work, school or study commitments.

The Morningness-Eveningness Questionnaire was used to determine diurnal preference based on established cut-off scores for these categories[34]. To increase probability of DSPD, at preliminary screening, those with a composite score greater than 49, indicating that they were either intermediate or morning types, were excluded from the study.

Mean self-reported habitual bedtime, habitual sleep time, and total sleep time were calculated from sleep diaries over 7 consecutive days.

Daytime sleepiness and functioning

Prior to the laboratory visit, participants completed an online questionnaire including the following: Epworth Sleepiness Scale (ESS)[36], Sheehan Disability Scale (SDS)[37], and Beck Depression Inventory, Second Edition (BDI-II)[38] (see supplemental material). The ESS was collapsed into two categorical variables based on the recommended reference range of 0-10 (normal) and greater than 10 (excessive daytime sleepiness)[39].

Circadian phase assessment

After the sleep-wake assessment, participants attended the laboratory for measurement of circadian phase. Participants arrived 6 hours before their self-reported habitual sleep onset time, and remained in a light-proof, sound attenuated and temperature-controlled suite until at least 2 hours after their habitual sleep onset time. Ambient light levels were maintained at <10 lux (measured in the direction of gaze at standard height of seated position; vertical plane, 137 cm from floor[40-42]) and saliva samples (~2 mL) were collected hourly from 5 hours before to 2 hours after habitual sleep onset time. Participants were instructed to remain seated for 20 minutes and were not permitted to consume food or beverages for 10 minutes before each sample. They were permitted to watch television (<10 lux), read and engage in quiet activities between samples, but were required to remain awake for the duration of the visit, monitored by direct observation. Samples were collected (Salivette, Sarstedt, Numbrecht, Germany) as previously described[43], centrifuged and stored immediately at -20°C. After the final sample collection, participants were transported to their homes.

Dim Light Melatonin Onset (DLMO): Saliva (200µl) was assayed in duplicate for melatonin by radioimmunoassay[44] within 1 week of collection, using procedures developed by University of Adelaide and licensed to Buhlmann Laboratories (Allschwil, Switzerland). Limit of detection of the assay was 1pg/mL and the inter-assay CVs were 7.4% at 4.41pg/mL and 10.7% at 48.14pg/mL. DLMO for each participant was determined as the time that melatonin concentrations crossed and remained above a threshold of 2.3pg/mL, calculated from linear interpolation between the samples immediately before and after the threshold[45].

Data analysis

SPSS Statistics Version 20.0 (IBM, Armonk, New York) was used for all data analysis. Data are expressed as mean ± standard deviation (SD) unless otherwise stated. Significance level was 0.05. Mood, as assessed by BDI-II, was defined as the primary outcome. BDI-II scores were categorized as follows: minimal depression (0-13); mild (14-19); and moderate-severe (20-63)[38]. Variables were compared between circadian and non-circadian DSPD phenotypes using chi-squared (goodness of fit) test or independent samples t-test.

A direct logistic regression was conducted to assess the association between DSPD phenotypes and depression symptom severity (minimal vs. mild-severe depression symptoms). Age, sex, total sleep time, and habitual sleep time were included as potential confounders in the model.

RESULTS

Participant characteristics

Participants were 29.8 ± 10.6 years (range 16 to 64 years) with BMI 24.7 ± 4.0 kg/m^2. Despite being clinically diagnosed with DSPD, 79 participants (43%) did not show an abnormal phase relationship between DLMO time and DBT, and were thus classified as non-circadian DSPD phenotype. The remaining 103 participants (57%) were classified as circadian DSPD phenotype.

No significant differences were found between DSPD phenotype groups in age, BMI, sex or nature of work or study commitments, although we observed a trend for circadian DSPD phenotype to be younger (see Table 1).

Circadian and non-circadian DSPD phenotypes did not show differences in desired or habitual bedtimes on nights before work and non-work days, and wake times on work days (see Table 2). Wake time on non-work days, however, was significantly later in the circadian DSPD group compared to the non-circadian DSPD group (mean difference 0.80 hours; 95% confidence interval [CI] 0.30 to 1.30; $p<0.01$). Circadian and non-circadian DSPD phenotypes did not show differences in diurnal preference (Table 2).

Circadian phase relationships with bedtime and sleep time

DLMO clock time for the circadian DSPD phenotype participants was on average 1.66 h later than the non-circadian DSPD phenotype participants ($p<0.001$; 95% CI 1.30 to 2.02). On average, those with a circadian DSPD phenotype had a DLMO time that occurred 0.65 ± 0.90 hours *after* DBT (range: DLMO occurred 0.50 hours before to 3.22 hours after DBT), compared with those with a non-circadian DSPD phenotype in whom DLMO occurred 1.48 ± 0.78 hours *before* DBT (range:

DLMO occurred 3.43 hours to 0.52 hours before DBT; $p<0.001$; 95% CI 1.88 to 2.38) (Figure 2A).

DLMO occurred on average 1.90 ± 1.07 hours before habitual sleep time (range: 4.88 to 0.73 hours) in the circadian DSPD phenotype group, and 3.20 ± 1.06 hours before habitual sleep time (range 5.97 to 1.73 hours) in the non-circadian DSPD phenotype group ($p<0.001$; 95% CI 0.98 to 1.61).

Depressive symptoms

Overall, 24.2% of the sample showed mild-severe depression symptoms (BDI-II score ≥14), and 9.6% showed moderate-severe depression symptoms (BDI-II score ≥ 20). Of those reporting mild-severe depression symptoms, 81% had a circadian DSPD phenotype (Figure 2B). Mean BDI-II score was higher in the circadian DSPD phenotype (10.55 ± 10.20) than the non-circadian phenotype (7.24 ± 6.02) ($p<0.01$). A significantly greater proportion of participants in the circadian DSPD group reported mild-severe depression symptoms (35.0%) compared with the non-circadian DSPD participants (10.3%; $p<0.001$; Figure 3). Likewise, a significantly greater proportion of participants in the circadian DSPD group reported moderate-severe depression symptoms (14.0%) compared with the non-circadian DSPD participants (3.8%; $p<0.05$).

In post-hoc analyses, we excluded 11 participants who reported current antidepressant medication use (6.0% of sample) and found that depressive symptoms remained significantly higher in those with a circadian DSPD phenotype (10.44 ± 10.36, n = 95) compared to those with the non-circadian phenotype (7.17 ± 5.01, n = 76, $p<0.05$).

We assessed the association between DSPD phenotype and depression symptoms (minimal depressive symptoms vs. mild-severe depressive symptoms), controlling for age, sex, total sleep

time and habitual sleep time. Those with the circadian DSPD phenotype were found to have greater odds of reporting mild-severe depressive symptoms (adjusted odds ratio [AOR] 4.31, 95% CI 1.75 to 10.64, $p<0.01$). Current anti-depressant medication use was not significant in the model and therefore was not included in the final analysis.

To further examine the relationship between circadian misalignment and depression, we compared the phase difference between DLMO and DBT in those with mild-severe depressive symptoms to those with minimal depressive symptoms. In those with mild-severe depressive symptoms, DLMO occurred 0.49 ± 1.30 hours after DBT (range: DLMO 2.80 hours before to 3.13 hours after DBT), whereas DLMO occurred 0.51 ± 1.31 hours before DBT (DLMO; range: DLMO 3.43 before to 3.22 hours after DBT) in those with minimal depressive symptoms (95% CI 1.45 to -0.56; $p<0.001$).

Sleepiness and Daytime Function

There were no significant group differences in the questionnaire-based assessments for sleepiness ($\chi^2(1)=0.393$, $p=0.531$), or daytime functioning ($t(177)=0.704$, $p=0.482$; Table 2).

Clinical Severity Assessment

On the clinician-rated CGI-S, completed before the assessment of circadian phase, more participants in the circadian DSPD phenotype group were classified as moderately-severely ill (n=63; 61.2%) compared with the non-circadian phenotype group (n=33, 42.9%; $\chi^2(1)=5.934$, $p<0.05$; Table 2).

DISCUSSION

This study examined the phase of the circadian melatonin rhythm relative to the desired sleep-wake cycle in a sample of DSPD patients, and compared mood and daytime function in patients with and without a circadian phase misalignment. There are two key findings. First, we showed that almost half (43%) of patients with a clinical diagnosis of DSPD did not have misaligned timing of the melatonin rhythm, and were therefore misdiagnosed with a *circadian rhythm* sleep disorder. Second, those with misaligned timing of the melatonin rhythm relative to the desired sleep-wake cycle reported a higher prevalence and severity of depressive symptoms than those who did not have misaligned rhythms. Clinician assessments, made prior to circadian phase assessment, showed that a higher proportion of the patients who have misaligned timing of the melatonin rhythm have moderate-severe illness severity compared with those without circadian misalignment. These findings demonstrate that in patients with DSPD symptoms, those who have objectively-assessed circadian misalignment report greater depressive symptoms and illness severity.

To our knowledge, this is the first study to examine circadian phase relative to desired bedtime in a clinically diagnosed DSPD sample, showing that a high proportion of patients are misdiagnosed with a circadian rhythm sleep disorder. Interestingly, despite its recognized circadian etiology, current diagnostic criteria do not include assessment of circadian phase, using markers such as DLMO[32,46]. In the current study, 43% of patients who were clinically diagnosed with DSPD were found to have no evidence of circadian misalignment (i.e., DLMO occurred at 30 min or more before desired bedtime). This finding supports the contention[22,47,48] that circadian phase assessment should become part of the standard diagnostic evaluation for DSPD to better differentiate phenotypes and optimize the treatment regime based on the underlying etiology. Cost effectiveness and reimbursement options for DLMO as a diagnostic tool remain to be evaluated.

One reason for determining the objective presence of circadian misalignment is that individual DLMO information may have implications for therapy. While those with circadian misalignment may benefit from treatments that specifically target the circadian system, such as light-dark exposure and/or melatonin (agonist) therapy[49-52], as well as other treatments such as cognitive behavioral therapy for insomnia[53], those without circadian misalignment are not likely to benefit from the targeted circadian interventions. Therefore, DLMO assessment may provide information that enables a more targeted therapeutic approach to patients with symptoms of DSPD.

Based on subjective sleep-wake assessments, which form the basis of current diagnostic criteria for the disorder, those with and without circadian misalignment were relatively indistinguishable. This is unsurprising, given that there can be considerable inter-individual variability in phase relationships between the melatonin rhythm and the sleep-wake cycle, despite similar sleep-wake schedules[33,43,54,55]. There is also substantial intra-individual variability observed in the current study, as evidenced by the difference in phase relationships between the melatonin rhythm and habitual bedtime on work and non-work nights. Habitual bedtime and wake time and DBT were not significantly different between groups on work nights, but habitual wake time on non-work days was significantly later in the circadian DSPD phenotype group compared to the non-circadian DSPD group, consistent with the significantly later timing of the melatonin rhythm in this group.

Previous studies have reported associations between circadian rhythm misalignment and depressed mood[56,57]. In seasonal depression, depressive symptom severity is strongly related to the degree of circadian rhythm misalignment[58]. High rates of depression have also been reported in DSPD patients[10,11]. A previous study (n=20) showed abnormal timing of the urinary melatonin metabolite rhythm in DSPD patients with depressive symptoms compared to those without depressive symptoms[13]. Our study shows that *circadian misalignment* in patients clinically diagnosed with

DSPD is strongly associated with depressive symptoms, even after controlling for total sleep time and other potential confounding variables; those in the circadian DSPD group had 4.31 times increased odds of reporting at least mild depressive symptoms. The relationship between circadian misalignment and depressive symptoms was further supported by the finding that in those with at least mild depressive symptoms, DLMO occurred 0.5 hours after DBT, while DLMO occurred 0.5 h before DBT in those with minimal depressive symptoms.

Whilst the exact mechanisms linking circadian misalignment and mood disturbances are not yet fully understood, there is evidence that the genes associated with circadian misalignment may contribute to susceptibility to mood disorders[19]. Polymorphisms of circadian clock genes such as CLOCK, PER2 and PER3 are reported to be associated with extreme evening preference[59-62]; although not all studies agree[63-66]. Some of these genes are also associated with an increased susceptibility to mood disorders[67,68], providing a possible genetic basis for the observed associations. In our study, DLMO occurred almost 2 hours later in the circadian DSPD phenotype group in conjunction with higher depressive symptoms. The circadian DSPD phenotype on average wakes up closer to the time of the nadir of the body temperature rhythm, which is also the nadir of positive affect[69]. Frequent experience of low positive affect in the mornings may contribute to the increased depressive symptoms of the circadian DSPD group.

Chronic sleep deficiency and delayed sleep timing[70,71] are both associated with increased risk of depression, and are commonly experienced by DSPD patients[72]. In our analysis, however, by including both the duration and timing of sleep as potential confounders, we showed that misaligned timing of the melatonin rhythm relative to the desired sleep-wake cycle is independently associated with depressive symptoms. Finally a missense variant (P10L) of the

melanopsin gene is associated with predisposition to seasonal depression[73], indicating that alteration in circadian light sensitivity may contribute to mood disturbances.

In those individuals diagnosed with DSPD but found to have no circadian misalignment, factors that may contribute to the DSPD-like sleep complaints include decreased rate of accumulation of homeostatic sleep pressure[27] and psychophysiological factors that increase pre-sleep arousal, such as anxiety[29]. Hiller and colleagues[30] recently reported that sleep initiation in adolescents is impeded by cognitive processes such as catastrophizing as well as anxiety, although their study did not measure circadian phase. While our study did not include participants with a current diagnosis of an anxiety disorder, because we did not specifically screen for such disorders, it is possible that those with undiagnosed anxiety disorders were included. These factors could help explain why sleep initiation is difficult in the non-circadian DSPD phenotype, despite having a normal circadian phase.

No group differences were observed for self-reported measures of daytime sleepiness or functional impairments. Notably, however, DSPD patients in both groups reported a relatively high level of absenteeism and lost productivity, consistent with our previous report[31], and other studies reporting poorer work/academic outcomes associated with DSPD[1,3]. We found that, on average, patients reported 1.5 days of missed work or school and 1.1 days of lost productivity in the past week, translating annually to approximately 75 days and 55 days of lost work and lost productivity, respectively. Notwithstanding the likely selection bias in our study, the absenteeism rate is markedly higher than the rate observed in the general population, which is reported to be 4.4 days per year in the United Kingdom[74] and 8.9 days per year in Australia[75].

Limitations of the study are noted. Healthy volunteers were not included as a comparison group, because the primary aim was to assess melatonin rhythm phase and compare clinical characteristics

of DSPD patients with and without circadian misalignment. Although we used validated questionnaires for assessing depressive symptoms and daytime function, we did not include diagnostic interviews or objective functional assessments.

The current findings have implications for both diagnosis and treatment of DSPD. Current diagnostic criteria rely almost exclusively on self-reported symptoms, which do not reliably distinguish patients with and without an underlying circadian basis for their disorder. We show that assessment of circadian phase would greatly improve the diagnosis of DSPD. Better clinical assessments will also allow better selection and timing of treatment, potentially leading to improved outcomes. Our finding that DSPD illness severity and depression symptoms are elevated in DSPD patients with circadian misalignment highlights the importance of circadian phase assessment in the diagnosis and optimal management of DSPD, and the potential contribution of disturbances of the circadian system to mood disorders.

AUTHOR CONTRIBUTIONS

Ms Murray and Dr Rajaratnam had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Murray, Sletten, Magee, Gordon, Lovato, Bartlett, Kennaway, Lack, Grunstein, Lockley, Rajaratnam

Acquisition of data: Murray, Sletten, Magee, Gordon, Lovato, Bartlett, Kennaway, Lack, Grunstein, Lockley, Rajaratnam

Analysis and interpretation of data: Murray, Sletten, Magee, Kennaway, Lack, Grunstein, Lockley, Rajaratnam

Drafting of the manuscript: Murray, Rajaratnam

Critical revision of the manuscript for important intellectual content: Murray, Sletten, Magee, Gordon, Lovato, Bartlett, Kennaway, Lack, Grunstein, Lockley, Rajaratnam

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Study supervision: Murray, Sletten, Magee, Rajaratnam

CONFLICTS OF INTEREST

Ms Murray reports no conflicts of interest.

Dr Sletten reports her institution has received equipment donations or other support from Philips Lighting, Philips Respironics, Optalert and Compumedics. Dr Sletten serves as a Project Leader in the Cooperative Research Centre for Alertness, Safety and Productivity.

Dr Magee reports no conflicts of interest.

Dr Gordon serves as a Project Leader in the Cooperative Research Centre for Alertness, Safety and Productivity.

Dr Lovato reports no conflicts of interest.

Dr Bartlett reports no conflicts of interest.

Dr Kennaway reports no conflicts of interest.

Dr Lack is shareholder in Re-Time Pty Ltd.

Dr Grunstein serves as a consultant to, and is a Program Leader for, the Cooperative Research Centre for Alertness, Safety and Productivity. He has provided non-remunerated advice to Merck and has been a medico-legal expert witness for Queensland Health, NSW Nurses Federation, NSW Health and NSW Director of Public Prosecutions.

Dr Lockley holds current consulting contracts with Delos Living LLC; Environmental Light Sciences LLC; Headwaters Inc.; Hintsa Performance AG; Pegasus Capital Advisors LP; PlanLED; Focal Point LLC; and Wyle Integrated Science and Engineering. In the past 5 years, he has received consulting fees from Carbon Limiting Technologies Ltd for work conducted with PhotonStar LED. He has also received consulting fees from Naturebright; Thomas Jefferson University; and minor consulting fees from 15 financial companies related to non-24-hour sleep-wake disorder in the blind and the publicly-available clinical trial results. He has received unrestricted equipment gifts from Biological Illuminations LLC; Bionetics Corporation; Philips Lighting; an unrestricted monetary gift to support research from Swinburne University of Technology, Australia; a fellowship gift from Optalert, Pty, Melbourne, Australia; and holds equity in iSLEEP, Pty, Melbourne, Australia. SWL receives royalties from Oxford University Press; and received honoraria for editing a textbook section from Elsevier and for drafting website

text for the National Sleep Foundation; and for an article in the Wall Street Journal. Dr Lockley has received honoraria plus support for travel, accommodation or meals for invited seminars, conference presentations or teaching from American Society for Photobiology; Bassett Research Institute; Brookline Adult Education; Brown University; Emergency Social Services Association Conference; Estee Lauder; Harvard University (CME); MediCom Worldwide, Inc (CME); North East Sleep Society; and Portland General Electric. He has received support for travel, accommodation and/or meals only (no honoraria) for invited seminars, conference presentations or teaching from 8th International Conference on Managing Fatigue; 14th Annual Tennessee Perfusion Conference; American Academy of Sleep Medicine; Bar Harbor Chamber of Commerce; Cantifix; Connecticut Business & Industry Association Health and Safety Conference; Emergency Services Steering Committee; Ferrari; Harvard University; Hintsa Performance AG; Illinois Coalition for Responsible Outdoor Lighting; Illuminating Engineering Society; Lighting Science Group Corp; Massachusetts General Hospital; Midwest Lighting Institute; National Research Council Canada; New England College of Occupational and Environmental Medicine; Ontario Association of Fire Chiefs; Rio Tinto; Sleep HealthCenters; University of Connecticut Health Center; UMass Memorial; University of Manchester; University of Texas Medical Branch; Vanda Pharmaceuticals Inc.; Warwick Medical School; Woolcock Institute of Medical Research; Wyle Integrated Science and Engineering (NASA). SWL has completed investigator-initiated research grants from Alcon Inc. and Vanda Pharmaceuticals Inc., and has ongoing investigator-initiated research grants from Biological Illumination LLC, Philips Lighting, and Philips Respironics Inc. He has completed service agreements with Rio Tinto Iron Ore and Vanda Pharmaceuticals Inc., and has completed three sponsor-initiated clinical research contracts with Vanda Pharmaceuticals Inc. Dr Lockley holds a process patent for the use of short-wavelength

light for resetting the human circadian pacemaker and improving alertness and performance which is assigned to the Brigham and Women's Hospital per Hospital policy. He has also received minor revenue from a patent on the use of short-wavelength light which is assigned to the University of Surrey. Dr. Lockley has served as a paid expert on behalf of seven public bodies and one union in arbitrations in relation to sleep, circadian rhythms and work hours.

Dr Rajaratnam reports that he has served as a consultant through his institution to Vanda Pharmaceuticals, Philips Respironics, EdanSafe, National Transport Commission, Rail, Bus and Train Union, Australian Workers' Union, Tontine Group, Transport Accident Commission, Meda Consumer Healthcare, New South Wales Department of Education & Communities, and has through his institution received research grants and from Vanda Pharmaceuticals and Philips Respironics, and reimbursements for conference travel expenses from Vanda Pharmaceuticals. He serves as a consultant to, and is a Program Leader for, the Cooperative Research Centre for Alertness, Safety and Productivity. His institution has received equipment donations or other support from Optalert, Compumedics, Philips Lighting and Tyco Healthcare. He was President and a Director of the Australasian Sleep Association. He has also served as an expert witness and/or consultant to shift work organizations.

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TRIAL REGISTRATION

Not applicable

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SUPPLEMENTAL MATERIAL

Participant Screening

Participants were included if they met the following criteria: aged 16 to 65 years (equal gender); a body mass index > 18 and < 35kg/m2; high risk of DSPD according to established criteria[1,72]; ≥ 5 consecutive days each week in which the individual participates in day work or school; self-reported willingness to go to bed at the *desired bedtime* on the night before work/school days at least 5 nights per week (for the purposes of an intervention trial that followed the present study). Participants were excluded if any of the following were reported: comorbid sleep disorder (except insomnia); drugs of abuse or concurrent medication (including over-the-counter medicines or herbal substances) likely to affect sleep (other than antidepressants) without approved discontinuation before study; history of psychiatric disorder in the past 12 months, other than depression; caffeine consumption > 300 mg per day; alcohol consumption > 14 standard drinks per week; history of substance abuse in past 12 months; investigational drug use in past 60 days; pregnancy or lactation; night shift work in past 6 months; transmeridian travel in past 2 months; allergies to any medicines, foods, preservatives or dyes; liver, kidney or autoimmune disease. As approximately 75% of DSPD patients have a past or current history of depression[72], depression was not exclusionary. Individuals who had a current diagnosis of depression were required to have shown adequate response (determined by prescribing clinician) to antidepressant treatment with at least minimum effective dose according to the medication label for 6 weeks prior to enrolment. Additionally, we did not specifically exclude participants with sleep initiation insomnia as this type of insomnia is not easily differentiated from the symptoms of DSPD.

Self-report Sleep/Daytime Function Evaluation Measures

Horne-Ostberg Morningness-Eveningness Questionnaire (MEQ)[34] consists of 19 items relating to an individual's preferences for mental and physical activity. The items are either Likert-type, with four or five options from which to choose, or time scale responses. A final composite score is calculated when scores for individual questions are combined. A higher composite score indicates greater morning preference, whilst lower scores indicate greater evening preference. Mid-range scores indicate neither morning or evening preference.

The Epworth Sleepiness Scale (ESS)[36] is a self-report assessment of daytime sleepiness, comprised of a list of 8 situations in which patients are asked to rate the likelihood of falling asleep on a scale of 0-3, with a score of 0 being "no chance" and a score of 3 being "high chance of dozing".

Sheehan Disability Scale (SDS)[37] is a self-report measure assessing functional impairment across three domains: work/school, social and family life. Each domain contains a 10-point visual analog scale, simultaneously anchored verbally, numerically and spatiovisually. Disability in each domain is rated by the participant.

Beck Depression Inventory, Second Edition (BDI-II)[38] is a self-report measure assessing the severity of depressive symptoms currently being experienced. It contains 21 items describing symptoms of depression that participants rate on a scale of 0-3. A total score is obtained by summing scores for each item. Total score ranges between 0-63 and higher total scores indicate greater severity of depressive symptoms.

Clinical Global Impressions-Severity Scale (CGI)[35] consists of one, observer-rated item, scored on a 7-point scale, ranging from 1 (normal) through to 7 (amongst the most severely ill patients).

Figure 1: Participant recruitment and study enrollment flow chart

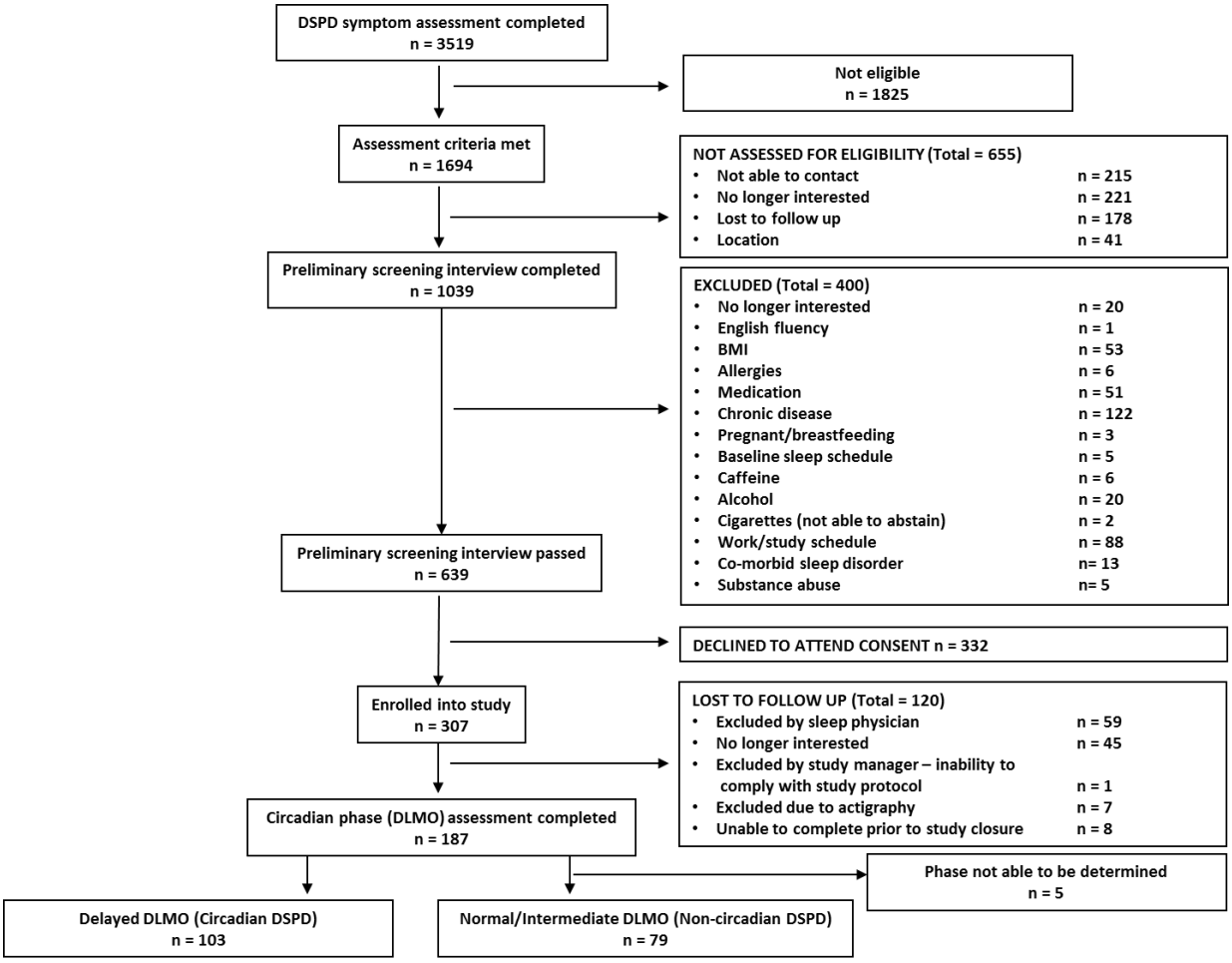
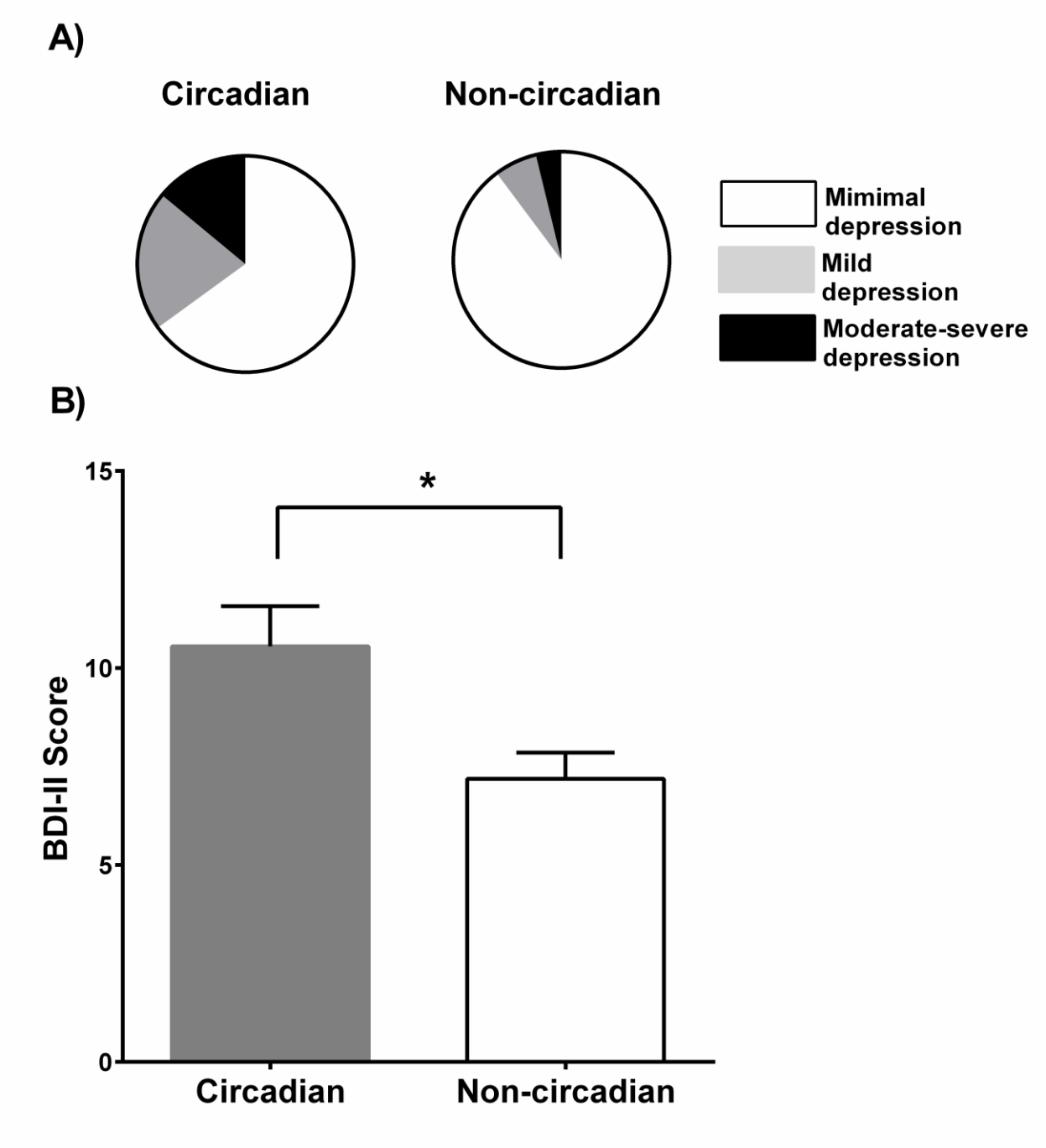


Figure 2: Dim light melatonin onset (DLMO) times relative to desired bedtime delayed sleep phase disorder and depressive symptom phenotypes.

Mean (± standard error) dim light melatonin onset (DLMO) times relative to desired bedtime for DSPD non-circadian (open squares) vs. circadian (solid squares) phenotypes (panel *A*), and those with minimal depressive symptoms (open circles) vs mild-severe symptoms (solid circles) (Panel *B*). Negative values indicate that DLMO time occurs before desired bedtime, and positive values indicate that DLMO occurs after desired bedtime. *p<0.001.

Figure 3: Prevalence of depressive symptoms in circadian and non-circadian delayed sleep phase disorder



Proportion of participants with minimal depression (white), mild depression (grey) and moderate-severe depression (black) in circadian and non-circadian DSPD phenotype groups (panel *A*); Mean (± standard error) depressive symptoms (BDI-II score) for circadian and non-circadian DSPD phenotype groups (panel *B*). *p<0.01.

Table 1: Participant characteristics

	Circadian	Non-circadian	p
N	103	79	
Sex n (%)	49 (47.6) M, 54 (52.4) F	40 (50.6) M, 39 (49.4) F	0.682
Age (y)	28.7 ± 9.8	31.2 ± 11.5	0.113
BMI (kg/m^2)	24.9 ± 4.3	24.4 ± 3.6	0.402
Employment n (%)			0.309
Employed, regular hours†	55 (53.4)	50 (63.3)	
Employed, variable shifts††	2 (1.9)	0 (0)	
Student, full time	45 (43.7)	29 (36.7)	
Student, part time†††	1 (1.0)	0 (0)	
Depression History			
Previous History of Diagnosis n (%)			0.659
No previous diagnosis	91 (88.3)	68 (86.1)	
Previous diagnosis	12 (11.7)	11 (13.9)	
Current Diagnosis of Depression n (%)			0.759
No current diagnosis	96 (93.2)	75 (94.9)	
Current diagnosis	7 (6.8)	4 (5.1)	

† Regular hours defined as all work hours falling between 6 am and 11 pm (includes paid, unpaid, volunteer work, homemaker, or training)
†† Variable shifts defined as work hours including at least 4 hours between 11 pm and 6 am (includes paid, unpaid, volunteer work, homemaker, or training)
††† Part time defined as enrolled at school or university ≤3 days/week

Table 2: Self-reported habitual bedtimes and wake times, desired bedtime, daytime function, clinical assessment and diurnal preference for circadian and non-circadian DSPD phenotype groups.

	Circadian	Non-circadian	p
DLMO Time (h), M ± SD	22:56 ± 1:22	21:16 ± 1:05	***<0.001***
DLMO-Habitual Bedtime Phase Angle*			
Work/school nights (h), M ± SD	-1.59 ± 1.15	-3.02 ± 1.02	***<0.001***
Non-work/school nights (h), M ± SD	-2.23 ± 1.40	-3.51 ± 1.36	***<0.001***
Desired Bedtime (Work/school nights) (h), M ± SD	22:35 ± 0:56	22:49 ± 0:49	0.093
Habitual Bedtime			
Work/school nights (h), M ± SD	23:52 ± 1:14	23:52 ± 1:16	0.972
Non-work/school nights (h), M ± SD	1:08 ± 1:27	0:47 ± 1.23	0.103
Habitual Wake time			
Work/school nights (h), M ± SD	7:57 ± 1:23	7:54 ± 1:18	0.800
Non-work/school nights (h), M ± SD	10:52 ± 1:41	10:04 ± 1:42	***0.002***
Mood			
Beck Depression Inventory-II, M ± SD	10.55 ± 10.20	7.24 ± 6.02	***0.008***
Subjective Daytime Assessment			
Epworth Sleepiness Scale n (%)			0.531
≤10	88 (85.4)	70 (88.6)	
>10	15 (14.6)	9 (11.4)	
Sheehan Disability Scale M ± SD	15.1 ± 5.4	14.5 ± 5.8	0.482
Clinical Assessment			
Clinical Global Impressions Scale n (%)			***0.015***
Mildly ill	40 (38.8)	44 (57.1)	
Moderately-severely ill	63 (61.2)	33 (42.9)	
Diurnal Preference			
Morningness-Eveningness Questionnaire n (%)			0.071
Definitely evening	93 (90.3)	64 (81.0)	
Moderately evening	10 (9.7)	15 (19.0)	

Footnote:

*Phase angle is the difference between DLMO and habitual bedtime (calculated separately for work/school nights and non-work/school nights). A negative value indicates that DLMO occurred before habitual bedtime.