Ocular measures of sleepiness are increased in night shift workers undergoing a simulated night shift near the peak time of the 6-sulfatoxymelatonin rhythm

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Ocular measures of sleepiness are increased in night shift workers undergoing a simulated night shift near the peak time of the 6-sulfatoxymelatonin rhythm

Running head: Ocular measures of sleepiness assessed in night shift workers

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Conflicts of interest
In the last 3 years Dr Lockley has received consulting fees from consulting fees from Blackrock; Cowen & Co; Endurant Capital Management; Far West Capital Management; Fidelity; Frankel Group; Impax Laboratories; Kearney Venture Partners; Lazard Capital Markets; Naturebright; New Horizon Capital; Perceptive Advisors; Polar Capital; ResearchWorks Inc.; Thomas Jefferson University; Wyvern Funds; and has current consulting contracts with Carbon Limiting Technologies
Ltd; Environmental Light Sciences, LLC; Headwaters Inc.; PlanLED; Delos Living LLC; Pegasus Capital Advisors LP; Wyle Integrated Science and Engineering; has received unrestricted equipment gifts from Bioilluminations LLC; Bionetics Corporation; and Philips Lighting; a fellowship gift from Optalert™, Pty Ltd; advance author payment and royalties from Oxford University Press; payment for editing a textbook section from Elsevier; honoraria from the National Sleep Foundation; and for an article in the Wall Street Journal; honoraria plus travel, accommodation or meals for invited seminars, conference presentations or teaching from Brookline Adult Education; Brown University; Estee Lauder (2013-2014); Harvard University (CME); MediCom Worldwide, Inc.(CME); 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; Cantifix; Connecticut Business & Industry Association Health and Safety Conference; Emergency Services Steering Committee; Harvard University; Hintsa Performance AG; Illuminating Engineering Society; Massachusetts General Hospital; Midwest Lighting Institute; New England College of Occupational and Environmental Medicine; Ontario Association of Fire Chiefs; Rio Tinto; UMass Memorial; University of Manchester; University of Texas Medical Branch; Woolcock Institute of Medical Research; ongoing investigator-initiated research grants from Biological Illuminations LLC; Respironics Inc.; and Vanda Pharmaceuticals Inc.; completed service agreements with Rio Tinto Iron Ore and Vanda Pharmaceuticals Inc.; two completed and one ongoing sponsor-initiated clinical research contracts with Vanda Pharmaceuticals Inc. Dr Lockley also 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 and has received revenue from a patent on the use of short-wavelength light, which is assigned to the University of Surrey. Dr Lockley has also served as a paid expert witness on behalf of 8 public bodies and one union for arbitration and cases related to sleep, circadian rhythms, and work hours. Dr Lockley also serves as a Theme Leader in the Cooperative Research Centre for Alertness, Safety and Productivity.

Dr Rajaratnam reports that he has served as a consultant through his institution to Vanda Pharmaceuticals, Philips Respironics, EdanSafe, The Australian Workers’ Union, National Transport Commission, and Transport Accident Commission, and has through his institution received research grants and/or unrestricted educational grants from Vanda Pharmaceuticals, Takeda Pharmaceuticals North America, Philips Lighting, Philips Respironics, Cephalon, and ResMed Foundation, and reimbursements for conference travel expenses from Vanda Pharmaceuticals. His institution has received equipment donations or other support from Optalert™, Compumedics, and Tyco Healthcare. He has also served as an expert witness and/or consultant to shift work organizations. Dr Rajaratnam also serves as a Program Leader in the Cooperative Research Centre for Alertness, Safety and Productivity.

Dr Ftouni and Dr Sletten serve as a Project Leaders in the Cooperative Research Centre for Alertness, Safety and Productivity.

Dr Nicholas and Dr Kennaway have no conflicts to declare.
Abstract

**Study objectives:** The study examined the relationship between the circadian rhythm of 6-sulphatoxymelatonin (aMT6s) and ocular measures of sleepiness and neurobehavioural performance in shift workers undergoing a simulated night shift.

**Methods:** Twenty-two shift workers (mean age = 33.4, SD = 11.8 years) were tested at approximately the beginning (2000 h) and the end (0555 h) of a simulated night shift in the laboratory. At the time point corresponding to the end of the simulated shift, 14 participants were classified as being within range of 6-sulphatoxymelatonin (aMT6s) acrophase - defined as three hours before or after aMT6s peak - and eight were classified as outside aMT6s acrophase range. Participants completed the Karolinska Sleepiness Scale (KSS) and the auditory psychomotor vigilance task (aPVT). Waking electroencephalography (EEG) was recorded and infrared reflectance oculography was used to collect ocular measures of sleepiness; positive and negative amplitude/velocity ratio (PosAVR, NegAVR), mean blink total duration (BTD), the percentage of eye closure (%TEC), and a composite score of sleepiness levels (Johns Drowsiness Scale; JDS).

**Results:** Participants who were tested within aMT6s acrophase range displayed higher levels of sleepiness on ocular measures (%TEC, BTD, PosAVR, JDS), objective sleepiness (EEG delta power frequency band), subjective ratings of sleepiness, and neurobehavioural performance, compared to those who were outside aMT6s acrophase range.

**Conclusions:** The study demonstrated that objective ocular measures of sleepiness are sensitive to circadian rhythm misalignment in shift workers.

**Key words:** Oculometrics, circadian misalignment, shift work, performance, sleepiness

**Study Rationale:** The research was conducted to examine the relationship between circadian phase and ocular measures of sleepiness and neurobehavioural performance in a sample of shift workers.

**Study Impact:** The research demonstrates that ocular measures of sleepiness are significantly elevated when shift workers are tested near the peak of the urinary melatonin metabolite (6-sulphatoxymelatonin; aMT6s) rhythm, compared to when tested outside the aMT6s peak range. These findings suggest that ocular measures of sleepiness may support alertness monitoring in shift workers.
Introduction

Shift work has become a ubiquitous part of labour practices due to the increasing pressure for 24-hour provision of goods and services. This drive for round-the-clock productivity, however, has resulted in negative consequences to health, safety, and performance\(^1\). Such consequences have been well-documented in a number of occupational sectors in which peak functioning during the night shift is critical, for example commercial transport, mining, emergency services and health-care\(^2\).

The impairment to alertness that occurs during night shift work is mainly attributed to misalignment of the imposed sleep-wake cycle from the internally-generated circadian (~24-hour) rhythm of sleepiness\(^3\), often against a background of chronic sleep deficiency and prolonged periods of wakefulness. Alertness and neurobehavioural performance peak when the circadian pacemaker promotes wakefulness during the daytime, and decline markedly during the night, particularly between 0300h and 0600 h\(^4\), as the circadian pacemaker strongly promotes sleep\(^5\). The deleterious effect of the circadian peak in sleep propensity occurring during the night shift is further exacerbated by accumulated (homeostatic) sleep pressure that results from poor sleep quality and inadequate sleep duration. These are caused by the sleep episode occurring at an inappropriate circadian phase\(^6\), and can often prolong the duration of the wake episode. The interaction between circadian and homeostatic factors, particularly at the end of a night shift, amplifies the risk of accidents and injuries\(^7\). Increased sleepiness at the end of a night shift, and the consequent increase in attentional lapses and likelihood of falling asleep\(^8\), is a major risk factor for motor vehicle crashes\(^8,9\). The risk of motor vehicle crash is substantially elevated between 2am to 7am\(^9\).

Sleepiness monitoring systems are often recommended as a component of comprehensive Fatigue Risk Management Systems\(^10\), although validation studies are limited. The most common technologies assessing sleepiness in real-time utilise ocular metrics.
Recent studies have assessed the efficacy of ocular metrics as practical tools in providing accurate, real-time, continuous assessment of alertness level. Ocular measures, such as blink durations, are associated with the onset of sleep or microsleeps, and are observed in sleep-deprived individuals. Such blinks exhibit unique properties, including slowing of the closing and reopening phases of a blink, which reflect the sleep/wake-related changes by the central nervous system (CNS). These ocular parameters have also been found to be sensitive to variations in circadian phase and increased homeostatic sleepiness over time, suggesting potential utility as a real-time sleepiness monitoring system in occupational and on-road settings. Hence, the current study employed the use of an ocular-based sleepiness detection system (Optalert™) to assess sleepiness levels in a shift working population during a simulated night shift, following a series of night shifts worked in the ‘real-world’. The study aimed to examine the relationship between ocular measures of sleepiness and circadian phase in shift workers. Specifically, sleepiness and neurobehavioural performance were examined in those who were within range of the acrophase of the urinary metabolite of melatonin 6-sulphatoxymelatonin (aMT6s) at the end of the simulated night shift, compared to those who outside aMT6s acrophase range.
Materials and methods

Ethical approval

The study was approved by the Monash University Human Research Ethics Committee. All participants provided written informed consent. The data presented are part of a larger, multi-centre study examining the effects of a novel light intervention on alertness and neurobehavioral performance in night shift workers.

Participants and pre-study conditions

A total of 27 healthy shift workers (9M, 18F) aged 31.8 ± 11.2 years (mean ± SD, range 18 – 64) completed the study at the Monash University Sleep and Circadian Medicine Laboratory. Participants were required to be regular night shift workers, working at least five night shifts per month including at least two consecutive night shifts. Night shifts were defined as shifts with at least six hours on duty between 2200 and 0800 h, and with a maximum scheduled shift length of 12 hours. All participants were initially screened for use of medications or drugs of abuse, and any history of medical or psychiatric disorders. Participants were screened for sleep apnea using the Berlin Questionnaire and the Multivariate Apnea Prediction (MAP) Questionnaire. Participants were deemed as high risk of sleep apnea if they screened positive on ≥ 2 categories on the Berlin Questionnaire and positive on the MAP Apnea Index. Participants were ineligible if they reported transmeridian travel in the past month. Participants completed a number of subjective questionnaires including the Epworth Sleepiness Scale (ESS); Pittsburgh Sleep Quality Index (PSQI); and the Morningness/Eveningness Questionnaire (MEQ). The laboratory-controlled simulated night shift was scheduled immediately following a sequence of at least two consecutive night shifts of each individual’s normal shift work schedule.
For one to three weeks before entering the laboratory for the simulated night shift, participants completed a daily sleep/work hours log and wore a wrist actigraphy device (Actiwatch-L, Mini-mitter, Inc., Bend, OR). Sleep diary data were used to define the time in bed interval for actigraphic sleep analysis, whereby, if self-reported bed time was 30 minutes or more prior to or post a sustained substantial reduction in activity, then bed time was modified to the start of the sustained substantial reduction in activity; if self-reported wake time was 30 minutes or more prior to or post a sustained substantial increase in activity, then wake time was modified to the start of the sustained substantial increase in activity.

Participants were asked to refrain from use of any prescription or non-prescription medications throughout the pre-study monitoring and simulated shift, and abstain from any recreational drugs for at least a month prior to the study. Participants were also required to abstain from alcohol for at least 24 hours, and abstain from caffeine and nicotine for 12 hours, prior to sleep laboratory entry. Compliance was verified by urine toxicology upon admission to the laboratory.

For 48 h prior to the beginning of the simulated night-shift, participants collected approximately four-hourly sequential urine samples (eight-hourly during sleep episodes) for measurement of the major urinary metabolite of melatonin, 6-sulphatoxymelatonin (aMT6s). The final urine sample was taken in the laboratory at the beginning of the simulated night shift. After each collection, participants recorded the volume of the sample and the time of urine collection. A 5-mL urine aliquot was frozen (-20°C) and subsequently analysed by aMT6s radioimmunoassay at the Adelaide Research Assay Facility, University of Adelaide using reagents purchased from Stockgrand, Ltd., University of Surrey (Guildford, UK). The aMT6s intra-assay Coefficient of Variation (CV) was 7.2% and the inter-assay CVs were 23%, 7% and 11% at 3.6 ng/ml, 15.6 ng/ml and 29.6 ng/ml, respectively. The minimum detectable concentration was 0.5 ng/ml. Participants were asked to continue their own
schedule in the 48 hours prior to the shift, and no instructions were given to restrict light exposure.

Shift work occupation categories were as follows; Health Professionals (n=7, 26%); Machine and Stationary Plant Operators (4, 18%); Hospitality workers (3, 11%); Hospitality, Retail and Service Managers (3, 11%); Business, Human Resource and Marketing Professionals (2, 9%); Other (5, 18%); and three (14%) participants’ occupations were unknown.

*Simulated night-shift protocol*

The simulated night shift occurred in a sleep laboratory free of time cues: participants had no access to windows, clocks, television, radio, internet, or telephone, and were continually supervised by trained staff. Participants were instructed to arrive at the laboratory at 1730 h. Testing began at 1900 h and ended at 0815 h the following day and participants were required to remain awake while supervised. Between 1900 h and 2300 h, and from 0700 to 0815 h, participants were exposed to standard room lighting at eye level (vertical plane). Between 2300 h and 0700 h, participants were randomly allocated to one of two fluorescent lighting conditions: 4,000 K or 17,000 K (Philips Bright Light Devices, HF3305, The Netherlands). Lighting illuminance levels were maintained between 100 to 150 lux across the simulated shift. Only data collected from the 4,000 K light group are reported herein.

For the duration of the simulated shift participants were asked to remain seated in the centre of the room in front of a computer which was used to administer each test battery, except for scheduled brief toilet breaks provided every two hours. Participants completed hourly cognitive test batteries from 1900 h to 0100 h, a test at 0300 h, and then hourly tests from 0500 h to 0800 h. In between test batteries participants were permitted to read or watch selected movies.
Data presented here were collected during the beginning (2000 h) and end (0555 h) of the simulated night shift.

**Infrared Oculography**

Participants’ eye and eyelid movements were monitored by infrared reflectance (IR) oculography (Optalert™) to record drowsiness levels continuously during the simulated night shift. IR transducers fitted to spectacle frames were positioned towards the top eye lid to measure the relative velocity of the opening and closing phase of the eye lid, and duration of each blink. Optalert™ glasses can be worn with contact lenses, and also support prescription spectacles. However, none of the participants in our study required prescription lenses. The system provides thirteen ocular measures sampled each minute, of which the following were selected based on previous work (detailed description of each measure provided in Table 1): positive and negative amplitude/velocity ratio of each blink (PosAVR, NegAVR); Johns Drowsiness Scale (JDS) score; the percentage of time with eyes closed (%TEC); and mean blink total durations (BTD). For all measures, higher values indicate higher levels of sleepiness. The commercially available system is designed to emit auditory warnings; however, these warnings were disabled in the present research study, such that the system was used purely to monitor alertness state.

**Sleepiness and Performance Assessments**

Subjective sleepiness was rated using the Karolinska Sleepiness Scale (KSS; 9-point scale from 1-“very alert” to 9-“very sleepy, fighting sleep”) by pressing the appropriate number on a computer keyboard when prompted.

Sustained attention was assessed using a 10-minute auditory psychomotor vigilance task (aPVT). An auditory stimulus was presented at random intervals (1-9 seconds); the participant was asked to respond to the sound by pressing a button as quickly as possible. No
simultaneous visual stimulus was presented. The auditory version of the PVT was used as it was more appropriate for administration during a period of light exposure within the larger protocol (data not included here), and to maintain consistency with our previous studies. The aPVT is a validated measure of sustained attention and a reliable index of objective sleepiness, and correlates with visual PVT during sleep deprivation.

**Waking EEG Recordings**

EEG and electro-oculogram (EOG) recordings were made continuously throughout the simulated night shift using a portable, wireless, ambulatory, polysomnographic recorder (Siesta, Compumedics Ltd., Victoria, Australia). EEG electrodes were positioned according to the International 10/20 System and recorded at eight sites (F3, F4, C3, C4, P3, P4, O1 and O2) against a Cz reference and subsequently digitally re-referenced to linked mastoids (M1 and M2). Only data from C3 are reported here. EOG was recorded with electrodes placed 2 cm above and below lateral canthus of the left and right eye, respectively. EEG and EOG data were sampled at 512 Hz and electrode impedances were < 10 kΩ. Raw EEG time series were imported as European Data Format (EDF) files into Curry 7 software (Compumedics Neuroscan, Victoria, Australia) and were digitally band-pass filtered (0.3 – 30 Hz, 24 dB/octave). EEG data were sampled during the 3-minute Karolinska Drowsiness Test (KDT), during which participants were instructed to fixate on a 5 cm black dot on a computer screen and remain as still as possible.

**Data analysis**

Cosinor analysis of aMT6s values were used to determine acrophase time or peak of aMT6s. Participants were classified into groups based on whether or not they were within range of
the aMT6s acrophase, which coincides approximately with the peak in the circadian rhythm of sleep propensity \textsuperscript{33}. The range of aMT6s acrophase was defined based on a review by Arendt \textsuperscript{34} describing the biological night as the hours between aMT6s onset and offset and refined based on Lockley, Skene, Arendt, Tabandeh, Bird and Defrance \textsuperscript{35}, who reported the mean and 2-SD range of normal aMT6s acrophases as $4.2 \pm 2.9$ h. Therefore, in the current study, participants were classified as being within aMT6s acrophase range if the final testing session (0555 h) occurred $\pm$ 3 hours from the time of their aMT6s acrophase as measured in the 24 hours prior to entering the laboratory. The conservative estimate used here is aimed at isolating the six hour time interval in which sleepiness is expected to be highest.

For aPVT measures, reaction times less than 100 ms and $>10,000$ ms were excluded as false starts and distractions, respectively\textsuperscript{30, 36}. Mean aPVT reaction time (RT) and number of lapses (responses $>500$ms) for each 10-minute test were calculated. aPVT RT scores were transformed with an inverse ($1/\text{RT}$) function, and square root was applied to aPVT lapses \textsuperscript{37}.

Ten minute recordings of each ocular measure were extracted coincident with the 10-minute aPVT. Ocular measures recorded during aPVT were selected for analysis to reduce variance across participants as they were instructed to remain still with eyes directed at the screen ahead and concentrate on the task. Ocular measures recorded during the KDT could not be used in the analysis as participants were instructed to blink as little as possible during the three minute task. As ocular measures are derived from eye blinks, ocular measures recorded during this task may be an inaccurate representation of the participants’ sleepiness level. Ocular data were cleaned based on signal quality \textsuperscript{11}; data were retained when more than 50\% of each one minute epoch displayed a clear blink signal.

Waking EEG signals were derived from C3 referenced to linked mastoids during each KDT. ECG artefact were removed using subtraction of a single component derived from a Principle Components Analysis of an average ECG artefact across all electrodes. Two-second
epochs containing muscle or eye blink artefacts were discarded from further analysis (<5% of all epochs). Artefact-free two-second epochs were subjected to off-line spectral analysis using a fast-Fourier transformation and a 5% hanning window (Curry 7 software, Compumedics Neuroscan, Victoria, Australia). Power spectral data were analysed between 0.5 and 30 Hz to remove any high and low frequency artefacts with outliers greater/less than two standard deviations from the mean being removed. EEG power values were summed across consecutive frequency bins (0.5 Hz resolution) within four frequency bands (delta [0.5–4.0 Hz], theta [4.1–8.0 Hz], alpha [8.1–12.0 Hz], and beta [14.1–30 Hz]). Summed frequency band values were then averaged across 2 second epochs within each KDT for final analysis.

IBM SPSS Statistics 20.0 (IBM Corp., Somers, NY, USA) was used for statistical analysis. A mixed design ANCOVA was conducted to assess the interaction of circadian group (participants ± 3 hours of aMT6s acrophase range and those who were tested outside aMT6s acrophase range) across two time points (baseline 2000 h, and end of shift 0555 h) on participants’ ocular measures, aPVT performance, subjective sleepiness, and EEG. Participants’ age, body mass index (BMI), and time in bed (TIB) in the 24 hours prior to the simulated shift were used as a priori covariates in the analysis: age-related changes in the timing of circadian drive have been found; BMI increases the risk of obstructive sleep apnea; and time in bed in the past 24 hours was included to control for variation in homeostatic sleep pressure while assessing differences in circadian phase. To confirm that the assumption of equal variance was not violated, Levene’s test for Equality of Variances was set at > 0.05 for all analyses.

Results
*Data retention*

Five out of 27 participants had poor quality aMT6s rhythm as determined by cosinor analysis of the 24-hour rhythms across the 48-hour collection period, and visual inspection of the consistency in the pattern of the 24 hour rhythm; data were consequently excluded from analyses. The remaining 22 participants exhibited a significant cosinor fit (α set 0.1040; 91% were $p<0.05$). As expected, no participants were within aMT6s acrophase range at the baseline testing session (see Figure 1). The end-shift testing session occurred ± 3 hours of aMT6s acrophase for 14/22 participants (hours relative to aMT6s acrophase: mean = 0.17 ± SD 0.59 h; range = -2.83 to 2.73 h), while eight participants were tested outside of this range (hours relative to aMT6s acrophase: mean = -6.08 ± SD 3.00 h; range =-17.92 to 5.35 h).

![Figure 1](image)

Of the 22 participants with aMT6s data, one had insufficient ocular data at the baseline test due to poor signal quality and the 2100 h test was subsequently substituted as the baseline. Three additional participants had missing ocular data at the 0555 h scheduled test and therefore data from the 0500 test were substituted. A further six participants were excluded from ocular analyses; one participant had a weak signal throughout testing and, as a result, an insufficient amount of blinks were recorded; two participants did not have sufficient baseline ocular data for comparison; and three participants had a large amount of noise at all time points causing blinks to be unclear and data unreliable. A total of 16 participants were therefore included in the ocular analyses (within aMT6s acrophase range, n = 9; outside aMT6s acrophase range, n = 7).

One participant did not have EEG recorded during the 2000 h scheduled test; EEG data during the next KDT (2100 h) test was substituted as the baseline time point for all
measures. One participant did not have sufficient EEG data collected throughout simulated shift KDTs and thus excluded from EEG analyses.

Overall, data during the baseline test occurred at a mean time of 2012 h (SD ± 0018 h), while the mean end-of-shift test occurred at 0550 h (SD ± 0019 h). Participants’ work shifts prior to the simulated shift started on average at 2103 h (SD ± 0150 h) and ended at 0608 h (SD ± 0116 h). Table 2 displays participant details.

Table 2

Ocular measures and sleepiness

All ocular measures (%TEC, BTD, PosAVR, JDS), except NegAVR, displayed a significant time by circadian group interaction (see Table 3). At the completion of the simulated shift, the mean %TEC values of participants ± 3 hours of aMT6s acrophase range increased more than 10-fold from 0.73 % (SD±1.0) to 8.01% (±1.8) (Figure 2; See Supplementary Online Material [SOM] Figure S1), whereas participants outside aMT6s acrophase range had only a minor increase from baseline to the end-of-shift ($F_{1,11}=6.00, p = .032$). JDS scores of participants ± 3 hours of aMT6s acrophase range significantly increased to nearly double their baseline value, from 2.5 (±0.7) to 4.9 (±0.6), at the end-of-shift in comparison to baseline, while participants outside aMT6s acrophase range displayed only a slight increase across time points ($F_{1,11}=7.50, p = .019$). A similar result was observed in BTD between the two groups ($F_{1,11}=9.86, p = .009$). A significant increase in PosAVR values from baseline levels was also observed in participants ± 3 hours of aMT6s acrophase range ($F_{1,11}=20.36, p = .001$). NegAVR did not show significant interaction or main effects.
Performance and subjective sleepiness

KSS displayed a significant time by circadian group interaction ($F_{1,17}=14.74$, $p = .001$; Figure 3). Mean subjective sleepiness scores of participants ± 3 hours of aMT6s acrophase range increased from 4.3 (SD ± 0.6) to 7.6 (± 0.5) across the simulated shift, while subjective sleepiness of participants who were outside aMT6s acrophase range decreased marginally at the end of the shift from 5.2 (± 0.8) to 5.0 (± 0.7).

Similar results were observed in the aPVT RT scores, which showed a significant time by circadian group interaction ($F_{1,17}=7.86$, $p = .012$; Figure 3; See [SOM] Figure S1). RT scores decreased over time in participants tested outside the aMT6s acrophase range, while RT scores increased at the end of the simulated shift for participants who were tested within aMT6s acrophase range. aPVT lapses followed a similar trend ($F_{1,17}=3.49$, $p = .079$).

EEG assessment of alertness

Significant interaction of time and circadian group was detected only in the EEG delta frequency band (0.5–4.0 Hz) ($F_{1,13}=4.77$, $p = .048$). Participants within aMT6s acrophase range showed an increase in the delta frequency from baseline to the end-of-shift test, compared to those outside aMT6s acrophase range (Figure 4).
Discussion

This study demonstrated that ocular measures of sleepiness (%TEC, BTD, PosAVR, JDS) show predictable increases across a simulated night shift when shift workers are assessed ± 3 hours of aMT6s acrophase range, compared to those outside the aMT6s acrophase range. Similar results were observed in measures of subjective sleepiness (KSS), objective sleepiness (EEG delta activity), and attentional performance (aPVT).

We observed considerable inter-individual variability in circadian phase, as assessed by the timing of the peak in the aMT6s rhythm; the end-of-shift assessment of sleepiness and performance occurred at a time ranging from 7.2 h before to 6.0 hours after the aMT6s peak. This variability in circadian phase across the simulated night shift may be the result of behavioural, biological, and/or environmental influences. Participants had worked a variable number of night shifts before entering the laboratory, therefore resulting in variability in sleep-wake history and light-dark exposure. We did not, however, observe significant differences in shift or sleep-wake history between participants in the two circadian phase groups, or between shift workers with an earlier phase (i.e. aMT6s acrophase before end of shift test; n = 10) versus those with a later phase (i.e. aMT6s acrophase after end of shift test; n = 12; p>0.05), suggesting that these factors are unlikely to account for the observed differences in circadian phase.

Measures of blink duration (BTD), amplitude/velocity ratio (PosAVR), percentage of time with eyes closed (%TEC), and a composite measure of these variables (JDS) displayed larger increases in sleepiness across the simulated night shift in those who were tested ± 3 hours of aMT6s acrophase range, compared to those tested outside this range. The increased rate of accumulation of sleepiness across the night shift in those tested within aMT6s acrophase range likely reflects the interactive effects of circadian and homeostatic influences on sleepiness. For example, sleep efficiency was relatively low in our participants, most
likely because sleep is scheduled during the daytime when the circadian pacemaker is promoting wakefulness\textsuperscript{41}, thus resulting in chronic sleep deficiency. Ocular metrics have previously demonstrated their sensitivity to the fluctuations in sleepiness due to homeostatic and circadian processes\textsuperscript{11,23}.

In line with the oculometric measures of sleepiness, subjective sleepiness (KSS), neurobehavioural impairment (aPVT), and objective sleepiness (EEG) also increased at the end of the simulated night shift in participants ± 3 hours of aMT6s acrophase range. All EEG power frequency bands showed a trend towards increasing across the simulated night shift in both circadian groups, suggesting overall general accumulation of sleepiness across the shift in all shift workers. However, participants who ended their shift within the range of aMT6s acrophase displayed significantly higher delta power compared to those who were outside aMT6s acrophase range. This suggests a higher sleep propensity when tested around the aMT6s peak, over and above the effects of elevated homeostatic sleep pressure caused by chronic sleep deficiency and prolonged wakefulness. Similar findings were observed in a study by Torsvall and Åkerstedt \textsuperscript{42}, who assessed subjective and objective sleepiness in night working train drivers. The study showed more rapid increase in self-reported sleepiness and EEG alpha, theta, and delta activity during the night journey\textsuperscript{42}. The current study extended these findings by showing that working near the aMT6s peak substantially influences the degree of sleepiness observed at the end of a night shift.

The findings of the study provide further evidence of the utility of oculometrics as objective measures of sleepiness in shift workers. It is acknowledged, however, that the assessment of neurobehavioural impairment (aPVT) occurred under controlled laboratory conditions and hence the extent to which findings may be extrapolated to field settings is
unknown. It should be noted that 18% (5/27) of the initial sample had missing phase data. Furthermore, six of the 22 participants with circadian data (59% of the initial sample) had ocular data deemed to be of poor quality and were excluded, further limiting the sample size. It should be noted, however, that while the collection procedure meant that ocular measures were sometimes limited by signal quality, the measures themselves remain sensitive to circadian and homeostatic variations in alertness\textsuperscript{11, 12, 27}. Careful consideration should be given to how oculometric data are collected in future studies. Nonetheless, several aspects of the study were ecologically valid. First, night shift workers were recruited to the study, and assessed in this protocol following a sequence of night shifts in the real-world. Second, the oculographic system (Optalert\textsuperscript{TM}) we utilised is marketed and increasingly used as a technology to support fatigue risk management in occupational settings. A major limitation in the assessment of driver sleepiness is the lack of widely-accepted, real-time methods. Measures that rely on offline processing or repeated neurocognitive testing are not practical in this context. While subjective assessments of sleepiness have been found to be accurate in the laboratory, their relationship to objective sleepiness measures has not been shown reliably in applied settings \textsuperscript{43, 44}. Hence self-ratings of sleepiness have limited value in the workplace compared to objective sleepiness measures, which do not rely upon the accuracy of an individual’s introspection. In-vehicle sleepiness-detection systems provide the driver with immediate notification of their current alertness state and risk of performance impairment and attentional failure in the future, potentially preventing sleepiness-related motor vehicle crashes. The present study adds to the growing literature showing the utility of oculometrics in sleepiness measurement technologies, specifically by showing the sensitivity of these metrics to reduced alertness due to the circadian nadir.
aMT6s profiles in the field may be impacted by light suppression of melatonin synthesis. While this may be a potential limitation to the research, the method is well-established in the literature as a suitable real-world assessment of circadian phase, of which the techniques and protocols used here have been applied successfully in several prior studies of shift workers on North Sea oil rigs 45-49, in shift working nurses 50-52, in shift workers living in Antarctica 53, 54, and in many clinical populations and experimental protocols (e.g. 35, 55-58). To assess the potential impact of light on aMT6s, in a previous study we compared aMT6s with urinary cortisol (which is much less confounded by light) in a subset of sighted individuals (n=7) and found a strong relationship between the phase estimates (average Pearson r ± SD, 0.98 ± 0.01, p<0.05)40. The study is limited by the short time lag between the estimation of phase and performance assessment in the laboratory (up to 24 hours).

The findings of the study highlight the importance of effective alertness management strategies during the night shift work and the drive home at around the circadian nadir. Assessing whether or not an individual is working near the circadian peak during a night shift appears to be an important component of safety risk management. Crowley, Lee, Tseng, Fogg and Eastman 59 found that performance, sleepiness, and mood are improved in participants who show circadian entrainment to a simulated night shift, compared to those who do not show entrainment, and thus are working at an adverse circadian phase. The authors reported significant associations between the magnitude of circadian entrainment and performance levels59. The current study supports the findings of Crowley and colleagues, and further demonstrates that in a sample of shift workers performing a simulated night shift after a series of night shifts in their normal environment, performance is impaired and sleepiness increased when assessments occur near the circadian nadir of alertness.
While the study used a relatively small sample, and thus limiting statistical power and the ability to generalise across the population, the study conducted in a sample of actual shift workers contributes to the body of knowledge examining the consequences of working near the aMT6s acrophase. Targeted interventions are warranted to manage the negative impact on occupational safety and performance due to circadian disruption, and the consequential sleep deficiency, associated with shift work \(^1\),\(^7\). The mismatch between the circadian pacemaker, the sleep-wake cycle, and environmental cues has been found to have serious impacts on performance and safety \(^2\) particularly when there is a need to perform during the biological night. Future studies assessing ocular measures of sleepiness should investigate inter- and intra-individual variation in these measures, to provide greater insight into their utility and validity.

In conclusion, shift workers who are working within range of the aMT6s acrophase display increased sleepiness and performance impairment during a simulated night shift. The study is the first to demonstrate sleepiness-related ocular responses to the circadian nadir of alertness in a sample of shift workers, and contributes to the ongoing effort to develop real-time sleepiness measures as a part of occupational risk management systems.

**Acknowledgements**

We would like to thank the research staff at the Sleep and Circadian Medicine Laboratory for their recruitment and data collection efforts, particularly, Ms Jade Murray and Dr Michelle Magee.

**Figure Legends**

**Figure 1. Relationships between testing times and circadian phase in each participant.**

Melatonin urinary metabolite aMT6s acrophase of each participant (open diamond symbols)
overlayed with start of shift baseline test times (open circle symbols), and end of shift test time: those tested outside aMT6s acrophase range (open square symbols) and those tested ± 3 hours of aMT6s acrophase range (closed square symbols). Note: error bars displayed represent ±3 hours from the time of aMT6s acrophase, which according to previous work\textsuperscript{34, 35}, is the expected time of the circadian nadir of alertness.

**Figure 2. Ocular measures assessed across a simulated night shift, between circadian groups.** Circadian group was categorised based on whether each participant was tested ± 3 hours of the melatonin urinary metabolite (aMT6s) acrophase. A: Mean scores at baseline 2000 h time point, and end of simulated shift time point 0555 h. B: Mean change from baseline scores. Asterisks indicate significant interaction ($p < 0.05$). Error bars represent standard deviation. JDS, Johns Drowsiness Scale; PosAVR, positive amplitude/velocity ratio; TEC\%, percentage of time with eyes closed; NegAVR, negative amplitude/velocity ratio; BTD, blink total duration.

**Figure 3. Self-rated sleepiness and auditory PVT (aPVT) assessed across simulated night shift, between circadian groups.** Circadian group was categorised based on whether each participant was tested ± 3 hours of the melatonin urinary metabolite (aMT6s) acrophase. A: Mean scores at baseline 2000 h time point, and end of simulated shift time point 0555 h. B: Mean change from baseline scores. Error bars represent standard deviation. Asterisks indicate significant interaction ($p < 0.05$). KSS, Karolinska Sleepiness Score; aPVT, auditory psychomotor vigilance task; RT, reaction time.
Figure 4. Change EEG power from baseline test to end of shift test between circadian groups. Circadian group was categorised based on whether each participant was tested ± 3 hours of the melatonin urinary metabolite (aMT6s) acrophase (Mean ± SD). EEG was grouped into the following frequency bands: delta (0.5–4.0 Hz), theta (4.1–8.0 Hz), alpha (8.1–12.0 Hz), and beta (14.1–30 Hz). Asterisks indicate significant interaction ($p < 0.05$). Error bars represent standard deviation.
**Tables**

**Table 1. Ocular measures derived from Optalert™ sleepiness technology**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Johns Drowsiness Scale Score (JDS)</strong></td>
<td>JDS is a composite score of ocular measures used to calculate an individual’s level of sleepiness in real time, providing a minute-to-minute Johns Drowsiness Scale (JDS) rating (see Johns et al.(^{21})). JDS is a continuous scale with scores ranging from 0 to 10 (very alert to very drowsy, respectively). The commercially-available system is designed to emit auditory warnings when individuals reach a JDS score of 4.5 to 4.9 (cautionary level of sleepiness), and a score of 5.0 or above (critical level of sleepiness), which is associated with an increased risk of severe lane excursions on a driving simulator(^{21, 60, 61}).</td>
</tr>
<tr>
<td><strong>Positive Amplitude/Velocity Ratio (PosAVR)</strong></td>
<td>Ratio of the maximum amplitude to the maximum velocity of the closing phase of a blink(^{21}).</td>
</tr>
<tr>
<td><strong>Percentage of time with eyes closed (%TEC)</strong></td>
<td>Percentage of time that the eyes are deemed closed in each minute. The eyes are deemed closed when the velocity of the eyelid movement following the closing of the eyelid drops below the velocity threshold, and is deemed closed until the velocity increases back above this threshold indicating the beginning of the re-opening of the eyelids.</td>
</tr>
<tr>
<td><strong>Negative Amplitude/Velocity Ratio (NegAVR)</strong></td>
<td>Ratio of the maximum amplitude to the maximum velocity of the re-opening phase of a blink(^{21}).</td>
</tr>
<tr>
<td><strong>Blink total duration (BTD; seconds)</strong></td>
<td>The mean total blink duration of all blinks over the course of 1 min. Total blink duration includes the duration of the closing, closed, and re-opening phases of each blink.</td>
</tr>
</tbody>
</table>
### Table 2. Participant descriptives

<table>
<thead>
<tr>
<th></th>
<th>End of shift test ± 3 h of aMT6s acrophase</th>
<th>End of shift test outside ± 3 h of aMT6s acrophase</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Demographics</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>14</td>
<td>8</td>
<td>32.1 ± 13.5</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (M, F)</td>
<td>9, 5</td>
<td>6, 2</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>23.7 ± 3.2</td>
<td></td>
<td>26.9 ± 1.5</td>
</tr>
<tr>
<td>Sleep-wake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESS</td>
<td>7.7 ± 4.4</td>
<td></td>
<td>11.4 ± 4.4</td>
</tr>
<tr>
<td>≥ 10</td>
<td>4</td>
<td>28.6</td>
<td>4</td>
</tr>
<tr>
<td>PSQI</td>
<td>5.9 ± 2.5</td>
<td></td>
<td>7.4 ± 1.6</td>
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<tr>
<td>≥ 5</td>
<td>8</td>
<td>57.1</td>
<td>7</td>
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<tr>
<td>MEQ</td>
<td>53.0 ± 8.6</td>
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<td>45.1 ± 11.2</td>
</tr>
<tr>
<td>Sleep History</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wake time (24 hours prior; h)</td>
<td>13:55 ± 01:56</td>
<td></td>
<td>13:46 ± 01:52</td>
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<tr>
<td>TIB 24 h (h)</td>
<td>6.6 ± 1.3</td>
<td></td>
<td>6.3 ± 1.2</td>
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<tr>
<td>Mean TIB 7 days (h)</td>
<td>6.3 ± 1.6</td>
<td></td>
<td>6.7 ± 1.9</td>
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<tr>
<td>Sleep efficiency 24 h (%)</td>
<td>79.5 ± 9.8</td>
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<td>82.6 ± 5.0</td>
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<tr>
<td>Mean sleep efficiency 7 days (%)</td>
<td>77.6 ± 7.5</td>
<td></td>
<td>74.4 ± 10.0</td>
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<tr>
<td>Hours awake at baseline (h)</td>
<td>6.3 ± 2.0</td>
<td></td>
<td>6.6 ± 1.8</td>
</tr>
<tr>
<td>Work schedule history</td>
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<tr>
<td>Mean shift start time (prior to simulated shift; h)</td>
<td>21:10 ± 1:53</td>
<td></td>
<td>20:52 ± 1:51</td>
</tr>
<tr>
<td>Mean shift end time (prior to simulated shift; h)</td>
<td>6:05 ± 1:21</td>
<td></td>
<td>6:13 ± 1:13</td>
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<tr>
<td>Number of consecutive night shifts prior</td>
<td>3.3 ± 1.7</td>
<td></td>
<td>4.6 ± 2.2</td>
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<tr>
<td>Total hours worked</td>
<td>30.7</td>
<td>16.3</td>
<td>44.7</td>
</tr>
</tbody>
</table>

*Note:* Sleep history was based on sleep logs and verified with actigraphy. No participants napped (<1 hour) in the 24 hours prior to the simulated night shift. BMI, Body Mass Index; ESS, Epworth Sleepiness Scale (range 0 – 24; higher scores indicate greater state-sleepiness); PSQI, Pittsburgh Sleep Quality Inventory (range 0 – 21; higher scores are indicative of greater sleep difficulty); MEQ, Morningness/Eveningness Questionnaire (range 16 – 86; higher scores indicate more morning chronotype); h, Hours; TIB, Time in Bed.
Table 3. Table of analyses

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Group</th>
<th>Time</th>
<th>Group x Time</th>
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<td></td>
<td></td>
<td>F</td>
<td>df</td>
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</tr>
<tr>
<td><strong>Ocular measures</strong></td>
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<td>JDS</td>
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<tr>
<td>PosAVR</td>
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<td>1,1</td>
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<td>%TEC</td>
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<td>NegAVR</td>
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<tr>
<td>BTD</td>
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<td></td>
<td>2.25</td>
<td>1,1</td>
</tr>
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<td><strong>Neurobehavioural performance</strong></td>
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<tr>
<td>PVT RT</td>
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<td></td>
<td>0.014</td>
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<td>PVT Lapses</td>
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<td><strong>Subjective Sleepiness</strong></td>
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<tr>
<td>KSS</td>
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<td><strong>EEG measures</strong></td>
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<tr>
<td>Delta power</td>
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<td>0.366</td>
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<tr>
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<tr>
<td>Beta power</td>
<td>12, 7</td>
<td></td>
<td>0.141</td>
<td>1,1</td>
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</tbody>
</table>

\( N = \) tested within MT6s acrophase range, tested outside aMT6s acrophase range; participants’ age, body mass index (BMI), and time in bed in the 24 hours prior to the simulated shift were used as a priori covariates in the analysis.

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References

8. Lyznicki JM, Doege TC, Davis RM, Williams MA. Sleepiness, driving, and motor vehicle crashes. *JAMA* 1998;279:1908-13.


49. Thorne H, Hampton S, Morgan L, Skene DJ, Arendt J. Differences in sleep, light, and circadian phase in offshore 18:00-06:00 h and 19:00-07:00 h shift workers. *Chronobiology Int* 2008;25:225-35.


61. Stephan K, Hosking S, Regan M, Verdoorn A, Young K, Haworth N. The relationship between driving performance and the Johns Drowsiness Scale as