Temporal dynamics of circadian phase shifting response to consecutive night shifts in healthcare workers: Role of light-dark exposure

Julia E. Stone[1,2], Tracey L. Sletten[1,2], Michelle Magee[1,2], Saranea Ganesan[1,2], Megan D. Mulhall[1,2], Allison Collins[3], Mark Howard[1,2,3], Steven W. Lockley[1,2,4,5], Shantha M.W. Rajaratnam[1,2,4,5]

[1]Cooperative Research Centre for Alertness, Safety and Productivity, Melbourne, Victoria, Australia

[2]School of Psychological Sciences and Monash Institute of Cognitive and Clinical Neurosciences, Faculty of Medicine, Nursing & Health Sciences, Monash University, Clayton, Victoria, Australia

[3]Institute for Breathing and Sleep, Austin Health, Victoria, Australia

[4]Division of Sleep and Circadian Disorders, Departments of Medicine and Neurology, Brigham and Women's Hospital, Boston, Massachusetts, USA

[5]Division of Sleep Medicine, Harvard Medical School, Boston, Massachusetts, USA

Keywords: shift work, circadian, light, melatonin, phase shift

Corresponding author: Shantha M.W. Rajaratnam, School of Psychological Sciences and Monash Institute of Cognitive and Clinical Neurosciences, Monash University, Building 17, Wellington Road, Clayton VIC, 3800, Australia. [Email: shantha.rajaratnam@monash.edu]

Julia Stone is a PhD candidate in the Alertness CRC; School of Psychological Sciences at Monash University, Australia; and Monash Institute of Cognitive and Clinical Neurosciences.

Her research interests lie in circadian rhythms and shift work, particularly measuring and predicting circadian response to changes in light/dark cycle. Julia has a Bachelor of Arts with Honours (Psychology) from the University of Melbourne.

Key Points Summary

- Shift work is highly prevalent and is associated with significant adverse health impact.
- There is substantial inter-individual variability in the way the circadian clock responds to changing shift cycles. The mechanisms underlying this variability are not well understood.
- We tested the hypothesis that light-dark exposure is a significant contributor to this variability; when combined with diurnal preference, relative timing of light exposure accounted for 71% of individual variability in circadian phase response to night shift work.
- These results will drive development of personalised approaches to manage circadian disruption among shift workers and other vulnerable populations to potentially reduce the increased risk of disease in these populations.

Abstract

Night shift workers show highly variable rates of circadian adaptation. This study examined the relationship between light exposure patterns and magnitude of circadian phase resetting in response to night shift work. In 21 participants (nursing and medical staff in an Intensive Care Unit [ICU]) circadian phase was measured using 6-sulphatoxymelatonin (aMT6s) at baseline (day/evening shifts of days off) and after 3 to 4 consecutive night shifts. Daily light exposure was examined relative to individual circadian phase to quantify light intensity in the phase delay and phase advance portions of the light phase response curve (PRC). There was substantial inter-individual variability in the direction and magnitude of phase shift after 3 or 4 consecutive night shifts (mean phase delay -1:08 ± 1:31 h; range -3:43 h delay to +3:07 h phase advance). The relative difference in the distribution of light relative to the PRC combined with diurnal preference accounted for 71% of the variability in phase shift. Regression analysis incorporating these factors estimated phase shift to within ±60 minutes in 85% of participants. No participants met criteria for partial adaptation to night work after 3 or 4 consecutive night shifts. Our findings provide evidence the phase resetting that does occur is based on individual light exposure patterns relative to an individual's baseline circadian phase. Thus, a 'one size fits all' approach to promoting adaptation to shift work using light therapy, implemented without knowledge of circadian phase, may not be efficacious for all individuals.

Introduction

Night shift work causes misalignment between endogenous circadian rhythms and the imposed work-rest schedule, with negative consequences for daytime sleep and alertness on shift (Rajaratnam & Arendt, 2001; Jensen *et al.*, 2015). Theoretically, the circadian system is able to adapt to night schedules by shifting the timing of the sleep propensity rhythm such that the peak occurs during the latter half of the daytime rest period (Eastman & Martin, 1999). Re-aligning the circadian pacemaker with the imposed sleep-wake cycle leads to improved sleep and neurobehavioural outcomes (Baehr *et al.*, 1999; Crowley *et al.*, 2004; Boivin *et al.*, 2012; Chapdelaine *et al.*, 2012; Boudreau *et al.*, 2013). Under some circumstances, such as the isolated environments of Antarctica

(Ross *et al.*, 1995) and North Sea oil platforms (Barnes *et al.*, 1998a), complete circadian adaptation to shiftwork has been observed. Circadian adaptation, however, is reported to be rare in most typical field settings, even after numerous consecutive night shifts (Folkard, 2008; Ferguson *et al.*, 2012; Jensen *et al.*, 2015).

Preliminary findings in laboratory and field settings indicate there is substantial inter-individual variability in the circadian phase shifting response to night shift work in terms of both the magnitude (Barnes *et al.*, 1998a; Hansen *et al.*, 2010) and direction of the shift (Dumont *et al.*, 2001; Gibbs *et al.*, 2007). Variation in the magnitude of phase shift is observed in offshore settings; even when all workers phase delay over multiple consecutive night shifts, some delay more than others (Hansen *et al.*, 2010). At an individual level, phase advance and phase delay shifts can occur in response to the same abrupt change from night to day schedules (Deacon & Arendt, 1996; Gibbs *et al.*, 2007). Factors that determine the direction and magnitude of phase resetting in shift workers have not been systematically examined.

The light-dark cycle is the primary determinant of resetting circadian phase (Duffy & Wright, 2005), capable of inducing phase shifts either by advance or delay depending on the timing of exposure relative to current circadian phase (Czeisler & Gooley, 2007). Light phase response curves (PRC) describe the circadian response to light relative to the circadian phase at which light exposure occurs (Khalsa *et al.*, 2003; St Hilaire *et al.*, 2012). By abruptly shifting the timing of the sleep-wake cycle, night workers are exposed to more light during the biological night compared to day workers, as well as exposure to bright light during the morning commute home (Dumont *et al.*, 2001; Dumont *et al.*, 2012). Seasonal differences in the direction and magnitude of phase shift are also observed in offshore shift work settings, suggesting differences in the timing of natural light exposure may influence the circadian response to night work (Barnes *et al.*, 1998b). Dumont and colleagues (2001) examined 24-h light exposure patterns in night workers, finding that workers with a later circadian phase were exposed to higher intensity light in the evening and darker sleeping environments during the day compared to those with advanced phase. This study did not assess circadian phase prior to the night shifts, however, so the possible influences of baseline phase differences were not considered.

Few studies have examined light exposure relative to individual circadian phase in night workers (e.g., Dumont *et al.,* 2001). Large variability in phase is observed between individuals in both healthy controls (Sack *et al.*, 1992; Duffy & Czeisler, 2002; Martin & Eastman, 2002; Burgess *et*

al., 2003b; Crowley *et al.*, 2003; Wright *et al.*, 2005; Sletten *et al.*, 2010), and night shift workers (Deacon & Arendt, 1996; Dumont *et al.*, 2001; Hansen *et al.*, 2010; Ftouni *et al.*, 2015). As the circadian response to light is phase-dependent, the substantial inter-individual variability that exists in circadian phase in night workers means that circadian phase adjustment in response to night shift work is likely to differ between individuals irrespective of exposure to the same light-dark cycle.

The current study aimed to examine the temporal dynamics of circadian phase shifting from baseline, over multiple consecutive night shifts in nursing and medical staff, and to identify factors that predict individual phase shifting response, in particular the circadian timing of light exposure.

Methods:

Ethical Approval

The study protocol was approved by the Austin Health and Monash University Human Research Ethics Committees and conformed to the standards set by the latest revision of the *Declaration of Helsinki*. All participants provided written informed consent prior to enrolment and received $100 payment upon completion of the study.

Participants

Nursing and medical staff were recruited from an Intensive Care Unit (ICU) at Austin Health, Heidelberg, Australia. Nurses worked variable patterns of day (07:00 – 15:30 h), evening (15:00 – 21:30 h) and night (21:00 – 07:30 h) shifts. Medical staff worked a consistent rotating roster of 7 consecutive day shifts (08:00 – 20:30 h), 7 days off, and 7 consecutive night shifts (20:00 – 08:30 h). Participants were recruited via scheduled in-service presentations, email advertising and targeted recruitment during shifts. Staff members were enrolled if their roster contained at least 4 days or evening shifts or days off followed by at least 3 or 4 consecutive night shifts during the study period. Work schedules were prospectively confirmed (detailed below): 5 participants had a documented night shift in the 3-4 weeks prior to participation. Of them, one worked a night shift 8 days prior to baseline, two had night shifts 14 days prior, one 16 days prior and another 28 days prior to baseline

phase collection. Due to the rotating roster, doctors had three weeks in between night shift rotations.

Questionnaires

Participants completed an online sleep-health questionnaire including demographic information (age, sex, body mass index (BMI) and shift work history) and subjective measures of general health and sleep. The Pittsburgh Sleep Quality Index (PSQI; Cronbach's alpha = 0.80) is a 19-item self-rated questionnaire, and was used to assess sleep quality and disturbances over the past month (Buysse *et al.*, 1989; Carpenter & Andrykowski, 1998). The Epworth Sleepiness Scale (ESS; Cronbach's alpha = 0.88) was used to assess daytime sleepiness, where participants were asked to rate on a 4-point scale the chances that they would fall asleep in 8 common daily situations (Johns, 1991; Johns, 1992). The Morningness-Eveningness Composite Questionnaire (MEQ; Cronbach's alpha = 0.87) is a 13-item questionnaire that was used to assess individual preferences associated with morning or evening activities (Smith *et al.*, 1989). Participants maintained a work log for the duration of their involvement where they recorded the start and end times (including breaks) for all shifts. Shift types were prospectively confirmed by timesheet records maintained by hospital administration. Of the 346 shifts recorded by participants, 314 shifts matched timesheet records (level of agreement 90.8%; work diaries were used for 92.8% of shifts (321 entries), timesheet records were used for 6.4% of shifts (22 entries), scheduled roster for 0.29% (1 entry) and 0.58% could not be used (2 diary entries from separate participants).

Individual light exposure

For the duration of the study, participants wore a wrist activity monitor (Actiwatch Spectrum or Spectrum Plus, Philips Respironics, Bend, OR, USA) on the non-dominant wrist to monitor light exposure continuously in 1-minute epochs (medium sensitivity; 40 activity counts per epoch). Participants were asked to wear the device at all times, except when the device could be damaged (e.g. while showering) or would interfere with the hospital operational requirements, and to ensure that the light sensor remained uncovered at all times (e.g. by sleeves).

Sleep-wake activity

Participants maintained daily sleep diaries throughout the study, recording sleep and wake times for each sleep episode, which were used to determine the actigraphy analysis interval for each sleep episode (Actiware 6 software, Philips Respironics, Bend, OR, USA). If there was a substantial reduction in activity ≥ 30 minutes prior to or after self-reported bedtime, bedtime was adjusted to occur at the start of the period of reduced activity. Similarly if there was a substantial increase in activity ≥ 30 minutes prior to or after self-reported wake time, wake time was adjusted to the start of the increase in activity (Ftouni *et al.,* 2015). Actigraphy analysis was used to determine sleep and wake times when diary information was not available (10%, 12 sleep entries). Sleep and wake times were used to clean light data for artefacts (described below).

Circadian phase

The rhythm of the urinary melatonin metabolite, 6-sulphatoxymelatonin (aMT6s), was used as a marker of circadian phase (Bojkowski *et al.*, 1987; Lockley *et al.*, 1997). Participants collected sequential urine samples at approximately 4-hour intervals (8-hours during the sleep episode) for 48 hours as previously described (Ftouni *et al.*, 2015). Phase assessments were timed to occur at last rostered day shift (baseline), on their first night shift (night 1), and over the final consecutive night shift (night 3 or 4). In a subset of participants, circadian phase was also assessed on a 6^{th} or 7^{th} consecutive night shift. For each sample, participants recorded void times, sample collection time, and the total volume. A 5 ml aliquot was stored frozen at -20°C and subsequently analysed for aMT6s concentration using radioimmunoassay (Aldhous & Arendt, 1988) at the Adelaide Research Assay Facility (University of Adelaide, Australia), using reagents purchased from Stockgrand Ltd (University of Surrey, Guildford, UK). Samples were assayed in two batches; The intra-assay coefficients of variation (CV) were 7.4% and 6.7%, respectively and the inter-assay CVs were 8.7%, 6.3%, 7.7% at 2.7 ng/mL, 11.5 ng/mL and 19.7 ng/mL, respectively (batch 1, 30% n=376 samples) and 14.9%, 3.5%, 5.4% at 5.7 ng/mL, 24.1 ng/mL and 41.9 ng/mL, respectively (batch 2, 70% n=881 samples). The minimum detectable concentration was 0.5 ng/mL.

Data analysis

The concentration of aMT6s in each sample in ng/mL was multiplied by the volume of the sample and divided by duration of the collection interval to obtain aMT6s concentration per hour (ng/h), and then plotted relative to the midpoint of the time between the sample and the previous sample. Cosinor analysis of aMT6s values was conducted to determine the acrophase (peak) time of the aMT6s rhythm (Nelson *et al.*, 1979). Circadian phase measured on day shift was taken as baseline phase, and assumed to be the same immediately prior to night shifts: sleep and work patterns in between collection and night shifts were checked for major changes in behaviour that would be inconsistent with this assumption. The degree of phase shift was calculated as the difference in clock time between aMT6s acrophase at baseline and the final night shift.

Bright light exposure data were extracted from wrist actigraphy devices. To account for artefact data due to coverage of the sensor by clothing, wake time values < 1 lux were excluded from analysis (Obayashi *et al.*, 2016). Light data were also excluded when Actiware software indicated the device was off-wrist. Light data were then log transformed as previously described (Dumont *et al.*, 2001).

Given that the circadian response to light depends on an individual's phase at the time of exposure, light patterns were examined relative to individual circadian phase during the major advance and delay regions of the human PRC to light (Khalsa *et al.,* 2003; Figure 1). We assume aMT6s acrophase as the approximate 'crossover point' for the PRC, because previous studies have reported an average time difference of approximately 2 hours between plasma melatonin and aMT6s acrophase (Nowak *et al.*, 1987; Arendt, 1995; Ross *et al.*, 1995; Benloucif *et al.*, 2008), and the reported time difference of 1.8 hours between plasma melatonin peak and core body temperature minimum (Shanahan & Czeisler, 1991). To examine light relative to circadian phase over each night shift, a daily aMT6s acrophase was estimated using linear interpolation between the clock time of acrophase on baseline and final night shifts. In participants with large differences in phase (>1 h) between baseline assessment (day shifts) and night one assessment, sleep and wake times were inspected to confirm whether baseline phase could be used as an accurate estimate of acrophase prior to commencing night shifts.

To determine the amount of light exposure during the delay and advance zones of the PRC, light data were averaged in 90 degree bins for the interval before ('delay' zone) and after ('advance'

zone) acrophase for the day immediately prior to, and over each night shift. Each 90 degree bin was calculated individually for each subject to account for variability in phase shift over night shifts, such that 360 degrees equalled 24 h plus the individual's phase shift per day. The difference in exposure to 'delaying' *versus* 'advancing' light (the difference in average lux 90 degrees before versus 90 degrees after acrophase) was examined at assumed baseline, over each night shift (night 1, night 2, night 3, night 4), and averaged across all night shifts. If more than 50% of data for a bin was missing, data for that bin were excluded from the analyses.

Statistical analyses were conducted using SPSS version 23 (SPSS Inc., Chicago, IL, USA). Independent t-tests were used to compare acrophase time at baseline and final night shift, magnitude of phase shift, and number of days between baseline phase assessment and first night shift between participants who worked 3 *versus* 4 night shifts. Pearson correlations were used to examine the relationship between phase shift and potential explanatory factors (baseline phase, age, MEQ, BMI and difference in light between 'delay' and 'advance' zones on each night shift).

Based on the outcome of the correlation analysis, multiple linear regression was used to examine the proportion of variance in phase shift explained by the difference between delay and advance light exposure (at baseline and each night shift) in combination with morningness-eveningness score, BMI, age and acrophase at baseline.

Results

Data retention

Of 41 participants who completed data collection, 6 (15%) did not have adequate aMT6s data (incomplete collection or poor quality aMT6s rhythm determined by cosinor analysis), 8 (20%) participants did not collect sufficient urine samples for the final night shift, and 2 (5%) had documented use of melatonin during the urine collection period. Therefore, data from 25 participants (21 nurses (16 female); 4 doctors (3 female)), aged 33.4 ± 8.4 years (mean ± SD) were included in the analyses. Demographics, subjective sleep quality, shift work history and shift schedules immediately prior to baseline phase assessment are reported in Table 1. An additional 3 participants had aMT6s data at baseline and on a 6^{th} or 7^{th} night shift, plus 1 of the previous data set

who had a repeated phase assessment on the 7th night shift; these participants were also included as a separate subset of circadian phase data, but were excluded from subsequent light analysis.

For 3 participants (nurses), phase on day shifts was unavailable so the 48-h urine collection including the first night shift was used as the baseline. For 7 participants, one or both urine collection periods were shortened due to operational constraints, with urine collected over 24-30 hours. In another 7 participants one or both collections were shortened due to collection errors. Included collections (50 collections; 25 participants) had a significant cosinor fit (α set 0.10; 94% were $p < 0.05$, 98% were $p < 0.10$, one collection was $p = 0.11$).

Of the 25 participants with adequate aMT6s data, 4 had insufficient light data due to actiwatch malfunction or noncompliance; one subject had no 'delay' light data at baseline, so could not be included in analyses of relative difference in amount of delaying and advancing light at baseline. Subsequently 20 participants were included in light analyses at baseline and the regression analysis.

Circadian phase

At baseline, the mean aMT6s acrophase time was 4:21 $\pm$ 1:05 h (range 2:18 – 6:45 h). After 3 or 4 consecutive night shifts, mean acrophase time was 5:18 $\pm$ 1:37 h (range 1:51 to 8:24 h; Figure 2A). We observed a greater range in acrophase time in workers after four night shifts compared to three (6:33 h and 3:08 h, respectively). There were no significant differences in acrophase at baseline or final night shift between participants working 3 or 4 night shifts ($t(23) = -0.74$, $p = 0.850$ and $t(23) = 0.09$, $p = 0.112$, respectively; Table 1). There was no relationship between baseline acrophase and MEQ score ($r = -0.05$, $p = 0.798$), although there was a trend towards a relationship between acrophase on the final night shift and MEQ scores ($r = -0.39$, $p = 0.052$), indicating that individuals with greater eveningness scores had a later final night acrophase time.

Figures 2B and 2C show the variability in direction and magnitude of aMT6s phase shift between individuals. From baseline to the final night shift, 19 participants phase delayed (shift -1:40 $\pm$ 1:00 h, range -0:04 to -3:32 h), and 6 participants phase advanced (shift 1:20 $\pm$ 1:02 h, range 0:15 – 3:07 h). Sex differences in the direction of phase shift were observed, with no men phase advancing compared to a third (32%) of the women (male range: -0:04 h to -3:18 h delay; female range: +3:07 h

advance to -3:32 h delay). The magnitude of the phase shift did not differ significantly between participants working 3 or 4 consecutive night shifts ($t(23) = -0.58$, $p = 0.165$).

Due to the variability in shift schedules, there were a different number of days between the baseline phase assessment and first night shift (2.6 $\pm$ 2.4 days (mean $\pm$SD), range 0-7 days). There was no relationship between number of days between baseline and night shift measurements and the magnitude of phase shift ($r = -0.11$, $p = 0.613$).

In a small subset of participants who worked 6 (n = 1) or 7 (n = 3) consecutive night shifts the mean acrophase at baseline was 4:24 $\pm$ 2:22 h (range 2:46 to 7:54 h). After 6 or 7 night shifts there was mean acrophase time of 9:16 $\pm$ 4:15 h (range 5:04 to 14:54 h). All 4 participants phase delayed, with a large range in the magnitude of observed phase shift (range -1:57 to -7:14 h).

Variables associated with phase shift

Figure 3 illustrates light exposure in key phase shifting zones for each individual. A significant positive association was found between phase shift and light exposure relative to circadian phase, with those receiving a greater proportion of light in the advance zone more likely to phase advance, and those with more delay zone light were more likely to phase delay (Figure 4). This relationship was observed when measuring light exposure at baseline ($r^2 = 0.27$, $p = 0.019$), during the second night shift ($r^2 = 0.41$, $p = 0.003$), third night shift ($r^2 = 0.24$, $p = 0.032$), and light averaged over all night shifts ($r^2 = 0.33$, $p = 0.007$). There was no statistical difference in the amount of light (difference in average delaying and advancing light, relative to individual phase) over baseline ($t(18) = -0.44$, $p = 0.663$) or the first 3 night shifts (night 1, $t(18) = -0.49$, $p = 0.628$; night 2, $t(18) = -0.68$, $p = 0.507$; night 3, $t(17) = -0.10$, $p = 0.926$) between those who worked 3 or 4 night shifts. The magnitude of phase shift was not significantly associated with the acrophase time at baseline ($r^2 = 0.12$, $p = 0.084$), age ($r^2 = 0.02$, $p = 0.533$), BMI ($r^2 = 0.13$, $p = 0.073$) or diurnal preference (MEQ; $r^2 = 0.12$, $p = 0.084$). Acrophase on the final night shift was strongly associated with phase shift ($r^2 = 0.61$, $p < 0.0001$), indicating the individuals with later acrophase had responded to night shift with larger phase delays.

Regression analysis

Multiple linear regression demonstrated that the difference between advance and delay zone light exposure at baseline, and averaged across all night shifts, accounted for 47% of the variance in phase shift between individuals (adj r^2= 0.54, p = 0.001). Adding MEQ score improved the model, accounting for 71% of the variability in phase shift (adj r^2 = 0.71, p = <0.001; Figure 5). The regression model predicted a phase shift within ±30 minutes in 45% of individuals, and within ±60 minutes in 85%. For additional regression models fitted, see Supplementary Table 1.

Discussion

This study demonstrates significant inter-individual variability in the direction and magnitude of circadian phase shift in response to multiple consecutive night shifts in nursing and medical staff working rotating shift schedules in the ICU. Furthermore, we showed that this variability can largely be explained by individual differences in the amount of light exposure in key phase shifting times, predicted by the human PRC to light. Finally, we found that 71% of the observed variability in the phase shift response to night shift work can be explained by a combination of the distribution of light exposure relative to individual circadian phase and diurnal preference. Using these variables, we are able to predict magnitude of phase shift to within ±60 mins in 85% of individuals (which is substantial given the 6.5h range in phase shifts observed). These findings support the concept that differences in light exposure relative to circadian phase strongly contribute to inter-individual variability in circadian response to night shift work.

We observed a range of ~4.5 hours in the timing of aMT6s acrophase at baseline, which is consistent with previous findings in individuals on regular diurnal schedules assessed in the laboratory (Wright *et al.*, 2005; Sletten *et al.*, 2010). While it is difficult to determine a true *baseline* phase in shift workers, all workers had at least 7 days of confirmed day-active schedules prior to their baseline phase assessment. This variability at baseline may reflect differences in underlying circadian physiology such as intrinsic period (Duffy *et al.,* 2001; Wright *et al.,* 2001), differing light-dark exposure on day shifts or longer term differences in work schedules not captured in the work rosters studied.

The range in acrophase time after night shifts we observed is similar to that reported in previous simulated night shift and field assessments of phase in shift workers (Barnes *et al.*, 1998a; Crowley *et al.*, 2003; Hansen *et al.*, 2010; Ftouni *et al.*, 2015). While we saw a larger range in acrophase time after 4 night shifts compared to 3 nights, this was not explained by group differences in average phase shift, or light exposure patterns. After 6 or 7 night shifts, the range in acrophase was even larger (9.8 h), despite the small subset sample size. Previous work in operational settings has observed larger phase shifts with increased number of consecutive night shifts (Barnes *et al.*, 1998a; Jensen *et al.*, 2015). It may be that the additional time on the changed light-dark cycle allowed for some of our participants to continue phase shifting over the fourth night shift. Together these findings provide evidence that the timing of aMT6s acrophase varies considerably in night shift workers, both on day and night shift schedules.

A large range in both the direction and magnitude of phase shift was observed over 3 to 4 night shifts, indicating considerable inter-individual variability in the circadian response to night shifts. While previous findings indicate there is inter-individual variability in circadian response to changes in light-dark schedules in highly controlled laboratory (Deacon & Arendt, 1996) or geographically isolated field settings (Barnes, Deacon, *et al*., 1998; Barnes, Forbes, *et al.,* 1998; Gibbs *et al.,* 2007), this study extends previous work to demonstrate considerable variation between individuals in the direction and magnitude of phase shift response to night work in a naturalistic setting. The variability in phase shifts observed in this study highlights the importance of investigating individual level data when assessing circadian response to shift schedules and the subsequent implications for safety on shift. Significant performance impairments have been observed in night workers tested within ±3 h either side of aMT6s acrophase (Ftouni *et al.*, 2015). Based on these findings, most workers in our sample would therefore have been at work or commuting home at a biologically high-risk time, particularly if the aMT6s acrophase had shifted to occur in the first hours of the daytime sleep. Conversely, individuals who phase advanced would be shifting the nadir in performance earlier in the evening, and thus be at work or possibly commuting to work at a similarly high-risk biological time.

Eastman and Martin (1999) propose that partial adaptation (i.e. shifting melatonin peak into the first half of the daytime sleep episode) is sufficient to see improvements in performance and safety on shift. After 3 or 4 consecutive night shifts we observed one subject with acrophase occurring after 8:00 am, and two later than 7:00 am; none of the participants started their daytime

sleep prior to aMT6s acrophase time. Therefore, by this criterion, none of our subjects could be considered even partially adapted to night work after 3 or 4 nights. In the subset of participants who worked 6 or 7 night shifts, two met criteria for partial adaptation to night shift, with acrophase time occurring within the first half of the daytime sleep episode. One of these participants showed a clear gradual delay in sleep timing in the 7 days prior to night shift, which is indicative of a delay in the timing of light exposure conducive to phase delays. Previous findings of partial adaptation to night shift were observed after a larger number of night shifts (>7 nights) and/or followed interventions specifically designed to promote phase shifts by manipulating light exposure (Eastman & Martin, 1999; Boivin & James, 2002; Crowley *et al.*, 2003), or were in remote offshore settings with limited exposure to natural sunlight (Barnes *et al.*, 1998a; Barnes *et al.*, 1998b; Gibbs *et al.*, 2007; Hansen *et al.*, 2010). Our findings indicate that it is unlikely that workers in a hospital setting will adapt to night schedules within 3 or 4 nights without a substantial targeted intervention that modifies light-dark exposure. This may have implications for the wellbeing of such night shift workers. Gumenyuk *et al.* (2012) found that circadian phase in workers who met criteria for Shift Work Disorder was within the range of that observed for day-active workers after being exposed to at least 3 consecutive night shifts. In contrast, those without Shift Work Disorder symptoms showed markedly delayed circadian phase in response to the same exposure to night shifts. These findings suggest that lack of circadian adaptation to night shift may contribute to shift work intolerance and clinical manifestations such as sleepiness and insomnia, which are the hallmark symptoms of Shift Work Disorder.

We did not observe any phase advances in the 6 males included in the analysis, compared to a range in phase shift direction in females (6 phase advanced, 13 phase delayed). This apparent sex difference was not explained by differences in the number of night shifts, occupation or diurnal preference, nor by differing light exposure patterns. Phase advances have been previously observed in males in response to light pulses in the laboratory (Warman *et al.*, 2003) and in offshore shift work settings (Gibbs *et al.*, 2007). It is not clear whether the difference in direction of phase shift between sexes is due to differences in underlying physiology (e.g. sex differences in length of intrinsic period (Duffy *et al.*, 2011; Eastman *et al.*, 2017) or sensitivity to phase shifting effects of light (Goel & Lee, 1995; Karatsoreos *et al.*, 2011)), or whether we did not see phase advances in males due to the small sample size.

The difference between amount of phase delaying and phase advancing light exposure showed a strong relationship with the direction and magnitude of phase shift across the schedule,

supporting the hypothesis that the individual pattern of light exposure relative to circadian phase is a major determinant of circadian response to shift work. Previous work has documented the 24-hour profile of light exposure in night workers, and compared the light profiles with circadian phase position at the end of a series of night shifts (Dumont *et al.*, 2001). This study found that workers with a delayed circadian phase had greater light exposure during the evening compared to those with advanced phase. We have extended this work to examine light exposure patterns relative to individual circadian phase, over a measured phase shift across consecutive night shifts. In doing so we quantified the pattern of light exposure, based on a PRC established in the laboratory (Khalsa *et al.*, 2003) to make predictions about when the circadian pacemaker is likely to be most sensitive to the phase shifting effects of light. By examining the difference between the amount of phase delaying and phase advancing light, we were able to estimate the net phase shifting signal of light exposure for each individual. This finding suggests that, as expected, light exposure plays a critical role in determining circadian response to shift work in a field setting, in a manner that is predictable from a phase response curve.

By extension, these results indicate that manipulation of light exposure is an important component to any intervention attempting to promote circadian phase shifts to reduce the negative consequences of night shift work. Given the large variability in circadian phase at baseline, generalised recommendations of light exposure based on clock time is not necessarily a suitable approach for all workers, and may actually be counterproductive. For example, an individual with aMT6s acrophase occurring early in the night shift (e.g., 3 am) being advised to seek light during the night and avoid bright morning light exposure on the commute home (which can be difficult to achieve in practice; (Lockley, 2005)) will not be reducing the phase advancing effects of light as intended. Instead they will likely be exposed to phase advancing light in the latter part of the night shift, after the aMT6s acrophase, and avoiding light during the commute home would have minimal impact in counteracting these effects. Accordingly, interventions aimed at promoting circadian adaptation to night work would likely have greater efficacy if light exposure recommendations are based on the timing of an individual's circadian phase rather than generalised management based on clock time. Conversely, an intervention approach that has been proposed to account for individual variability in circadian phase is to systematically shift the timing of light exposure so as to facilitate maximal phase shifting in individuals across a range of circadian phases (Mitchell *et al.*, 1997; Crowley *et al.*, 2003). Notwithstanding the potential utility of this approach, we suggest that knowledge of circadian phase can inform improved personalised intervention strategies for shift

work, to either promote adaptation (followed by re-adaptation) or non-adaptation, depending on the specific shift rotations.

Previous work has found relationships between diurnal preference and phase response to abrupt changes in sleep-wake (and consequently light-dark) schedules, with greater eveningness tendency associated with larger phase delays following a delay in the light-dark cycle (Mitchell *et al.*, 1997; Dumont *et al.*, 2001). While we did not find a significant association, participants who had the greatest eveningness scores showed the largest phase delays. No participants in our sample were morning-types, possibly because these individuals have self-selected out of shift work due to lower tolerance (Saksvik *et al.*, 2011). We suggest that the relationship between phase shift and diurnal preference may have been stronger with a larger range in MEQ scores. Furthermore, diurnal preference is associated with individual differences in circadian period in healthy volunteers (Duffy *et al.*, 2001). Differences in circadian period are associated with the magnitude of phase shift following a 9-hour delay in the light-dark cycle (Eastman *et al.*, 2016). In our study, including diurnal preference in a regression model with light exposure accounted for an additional 17% of variability in phase shift in our sample, a larger individual contribution than other factors such as BMI (8%) or aMT6s acrophase at baseline (5%) when each factor was tested in combination with light data. This observation suggests that diurnal preference provides important additional information in predicting circadian response to night work. and longer circadian period has been associated with larger phase delays. Further investigation is, however, required to determine whether there are differences in the phase response curve to light as a function of diurnal preference.

It is possible that shift workers' diurnal preference is related to activity-rest behaviours which influence light-dark exposure, thereby influencing circadian phase shift response to night shifts. For example, individuals with strong evening type tendency may inadvertently seek more light throughout the night, increasing the likelihood of greater exposure during phase delaying times. We did not, however, see a relationship between MEQ score and acrophase at baseline. This may be due to a number of reasons, including unstable phase relationships whilst on rotating shift schedules, inadequate sample size, and/or the influence of homeostatic processes on morningness/eveningness scores due to differences in dissipation of sleep pressure (Mongrain *et al.*, 2006).

The large proportion of variability in phase shift explained by our regression model has potential applications for individualised shift work management, with customised phase shift goals

based on initial phase position to distribute sleepiness between individuals across the night shift. For example, for evening-type individuals with late phase the goal would be a phase delay in preparation for night shift and sleep after the shift, whereas morning-type individual could adapt by phase advance and sleep before the shift. Complementary shift scheduling could utilise this approach, such that individuals with a mixture of advanced and delayed phase position are on shift together, to reduce accident risk on shift by spreading acrophase time (and thus time of reduced alertness and poor performance) across the shift schedule. Shift work interventions may also incorporate phase shifting strategies in preparation for night shifts to reduce the risk of alertness impairment in the first few shifts, similar to schedules for phase delay or advance prior to transmeridian travel to reduce jetlag (Burgess *et al.*, 2003a).

While this study has high ecological validity, there are a number of limitations. Due to the naturalistic setting we had limited control over confounding factors such as drug use, which may influence the measurement of urinary aMT6s (Arendt, 2005). Participants with self-reported use of sleep medications were excluded from analysis, however. We classified delay and advance zones based on aMT6s acrophase, relying on Khalsa's human PRC (Khalsa *et al.*, 2003) which was developed in a controlled laboratory setting, referenced to core body temperature minimum relative to the midpoint of light exposure. Given that recent work suggests that the majority of the light effect occurs toward the start of a light exposure (Chang *et al.*, 2012) we tested whether realignment of the PRC to light onset would improve our estimate of light exposure, however we did not find any significant improvement in our phase predictions. We also assumed a linear phase shift across night shifts in order to make predictions about individual delay and advance zones of the PRC. Future work collecting urine continuously across all night shifts would more accurately map the temporal dynamics of phase shifting and light exposure. Given that we include light relative to phase over each night shift, calculated using knowledge of circadian phase after successive night shifts, our regression model must be considered explanatory rather than predictive of phase shift. Additionally, we measured light using a wrist-worn device, which is a potential source of error as this is not necessarily equivalent to the light hitting the cornea, and ultimately the retina.

This study is potentially limited by the impact of light suppression of melatonin synthesis on aMT6s profiles. Prior work has, however, reported a strong relationship between circadian phase measured from urinary cortisol (less impacted by light exposure) and aMT6s (n = 7; average Pearson $r \pm SD = 0.98 \pm 0.01$, $p<0.01$) (Barger et al., 2012). Measuring circadian timing via urinary aMT6s is

well-established in the literature as a reliable assessment of phase in field settings, and has been utilized successfully in numerous prior studies of shift workers including nurses (Dumont *et al.*, 2001; Hansen *et al.*, 2006; Dumont *et al.*, 2012; Ftouni *et al.*, 2015) on North Sea oil rigs (Barnes *et al.*, 1998a; Barnes *et al.*, 1998b; Gibbs *et al.*, 2002; Gibbs *et al.*, 2007; Thorne *et al.*, 2008) and in Antarctica (Midwinter & Arendt, 1991; Ross *et al.*, 1995). This method has also been used in a number of clinical populations and laboratory settings (e.g., (Bearn *et al.*, 1989; Skene *et al.*, 1990; Deacon & Arendt, 1994; Deacon & Arendt, 1996; Lockley *et al.*, 1997)).

Circadian response to light depends on multiple factors, including intensity, timing, wavelength and light history (Czeisler & Gooley, 2007). In this study we have considered intensity, timing, and to some extent light history by examining light across the night shift schedule; however, we have not factored in light wavelength. The circadian pacemaker is particularly sensitive to phase resetting by short-wavelength light (Lockley *et al.*, 2003; Gooley *et al.*, 2010). We used estimated photopic lux from the Actiwatch Spectrum rather than measurements from the blue light channel for the following reasons: (i) familiarity with photopic lux measure in the field generally; (ii) the strong correlation observed between photopic lux and data from the blue irradiance channel measured in a subset of our participants (n=8, average $\pm$ SD Pearson's r = 0.97 $\pm$ 0.01, $p < 0.0001$) and (iii) because the blue channel data from the Actiwatch spectrum are not sufficient at this stage for assessing the impact of changes in the spectra of light exposure on biological responses. We recognize that in the future it may be possible to measure melanopic lux or melanopic/photopic lux ratios (Lucas *et al.*, 2014) using wearable devices. Such devices were not available at the time that we undertook this work.

Our findings indicate that ICU workers do not show partial circadian adaptation to night shifts over 3 to 4 consecutive night shifts, although there are large individual differences in both direction and magnitude of circadian phase shift. These findings provide strong evidence that baseline circadian phase and the individual light exposure patterns over the night shifts largely determine circadian adaptation to shift schedules, and that current 'one size fits all' approach to shift work interventions may be counterproductive for some individuals.

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Additional Information

Competing Interests

Ms Stone has no conflicts to declare. Dr Sletten and Dr Magee serve as a Project Leaders in the Cooperative Research Centre (CRC) for Alertness, Safety and Productivity. Ms Ganesan has no conflicts to declare. Ms Mulhall has no conflicts to declare. Ms Collins has no conflicts to declare. Dr Howard serves as a Theme Leader in the CRC for Alertness, Safety and Productivity, which funded this work; and has received grants from Prevention Express and TEVA, which are not related to the work reported in this paper. Dr Lockley has had a number of commercial interests in the last 12 months (2016-17). He is a Program Leader for the CRC for Alertness, Safety and Productivity, Australia, which funded this work. No other interests are directly related to the research or topic reported in this paper but, in the interests of full disclosure, are outlined below. Dr Lockley has received consulting fees from the Atlanta Falcons, Atlanta Hawks, BHP Billiton and Slingshot Insights; has current consulting contracts with Akili Interactive; Consumer Sleep Solutions; Delos Living LLC; Environmental Light Sciences LLC; Headwaters Inc.; Hintsa Performance AG; Light Cognitive; Mental Workout; OpTerra Energy Services Inc.; Pegasus Capital Advisors LP; PlanLED; and Wyle Integrated Science and Engineering; has received unrestricted equipment gifts from Biological Illuminations LLC, Bionetics Corporation and F. Lux Software LLC; royalties from Oxford University Press; and has served as a paid expert in legal proceedings related to light, sleep and health. He holds a patent through Harvard University and Brigham and Women's Hospital for 'Systems and methods for determining and/or controlling sleep quality'. Dr Rajaratnam is also a Program Leader for the CRC for Alertness, Safety and Productivity, Australia, which funded this work. Dr Rajaratnam reports grants from Vanda Pharmaceuticals, Philips Respironics, Cephalon, Rio Tinto and Shell, and has received equipment support and consultancy fees through his institution from Optalert, Tyco Healthcare, Compumedics, Mental Health Professionals Network, and Teva Pharmaceuticals, which are not related to this paper.

Author contributions:

All authors contributed to the work presented and have given final approval for its publication. J.E.S contributed to the conception of design, data collection, analysed data and compiled the manuscript. T.L.S contributed to the conception of study design, coordinated study implementation, provided critical insight and comments on data analysis interpretation and manuscript draft. M.M coordinated study implementation, provided critical insight and comments on data analysis,

interpretation and manuscript draft. S.G supported data collection and provided comments on interpretation and manuscript draft. M.D.M supported data collection and provided comments on interpretation and manuscript draft. A.C facilitated data collection and study implementation and provided comments on interpretation and manuscript draft. M.H contributed to the conception of study design, facilitated data collection and study implementation and provided critical insight and comments on interpretation and manuscript draft. S.W.L contributed to the conception of study design, provided critical insight and comments on data analysis, interpretation and manuscript draft. S.W.R contributed to the conception of study design, provided critical insight and comments on data analysis, interpretation and manuscript draft.

Funding:

This research was funded by the Cooperative Research Centre for Alertness, Safety and Productivity, Melbourne, Victoria, Australia. Grant number P2.1.01-15.

Acknowledgements:

We thank Jessica Papaleo, Matthew McLaren, Aaron Johnson, Kaitlyn Crocker, Niamh McDonald, Emma Giliberto and Trisha D'Lima from the Sleep and Circadian Medicine Laboratory, School of Psychological Sciences and Monash Institute of Cognitive and Clinical Neurosciences, Monash University, Melbourne, Australia for their assistance with data collection. We thank Dr Glenn Eastwood, Helen Young, Dr Graeme Hart and the staff of the Intensive Care Unit at Austin Health, Melbourne, Australia for their support. With thanks to Mark Salkeld at the Adelaide Research Assay Facility, Adelaide, Australia for conducting the aMT6s radioimmunoassays.

Figure Legends

Figure 1. Phase response curve to light (adapted from Khalsa et al., 2003), where 0 on the abcissa represents timing of core body temperature minimum (CBTmin) in healthy subjects. Light exposure prior to CBTmin induces phase delays, while exposure after CBTmin leads to phase advance shifts. We used aMT6s acrophase as a proxy for CBTmin. Light exposure was examined in the 90 degrees before and after individual acrophase times, where light is predicted to induce the greatest phase delays or advances, depicted by the bars either side of 0. Dark grey indicates the delay zone, light grey indicates advance zone. Each 90 degree bin was approximately 6 hours. Reproduced in part from Khalsa et al. (2003).

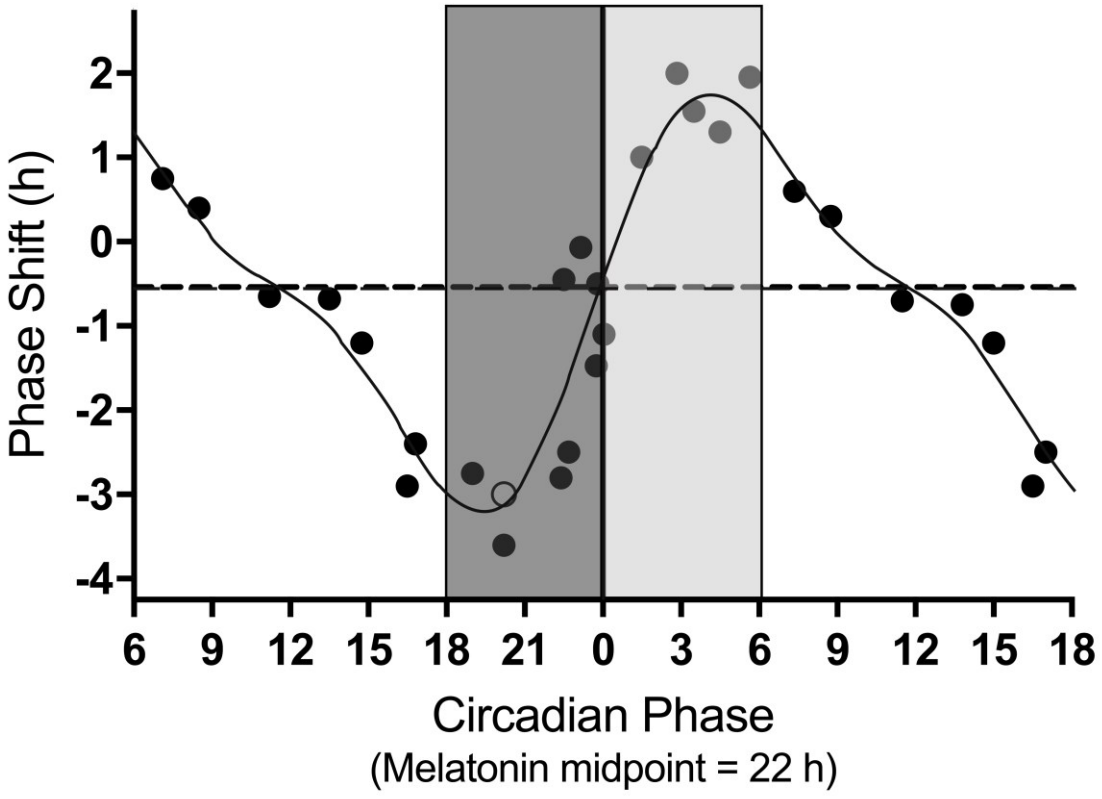

Figure 2. **A.** Boxplots of aMT6s acrophase time at baseline and at the final successive night shift. Outliers are shown as closed circles. **B.** Clock time of aMT6s acrophase at baseline (D1-D7) and on

final night shift (N3 or N4) for each participant plotted by study day. Baseline acrophase is plotted on the day aMT6s was measured, and is assumed to be acrophase immediately prior to night shifts. **C.** Change in aMT6s acrophase time from baseline to third (N3) or fourth (N4) night shift (acrophase at baseline minus acrophase at final night shift). Negative time indicates a phase delay (acrophase occurred at a later clock time after working night shifts compared to baseline), and positive indicates a phase advance (acrophase occurred at an earlier clock time after night shifts than at baseline). Men are represented as open triangles and women are shown as filled circles.

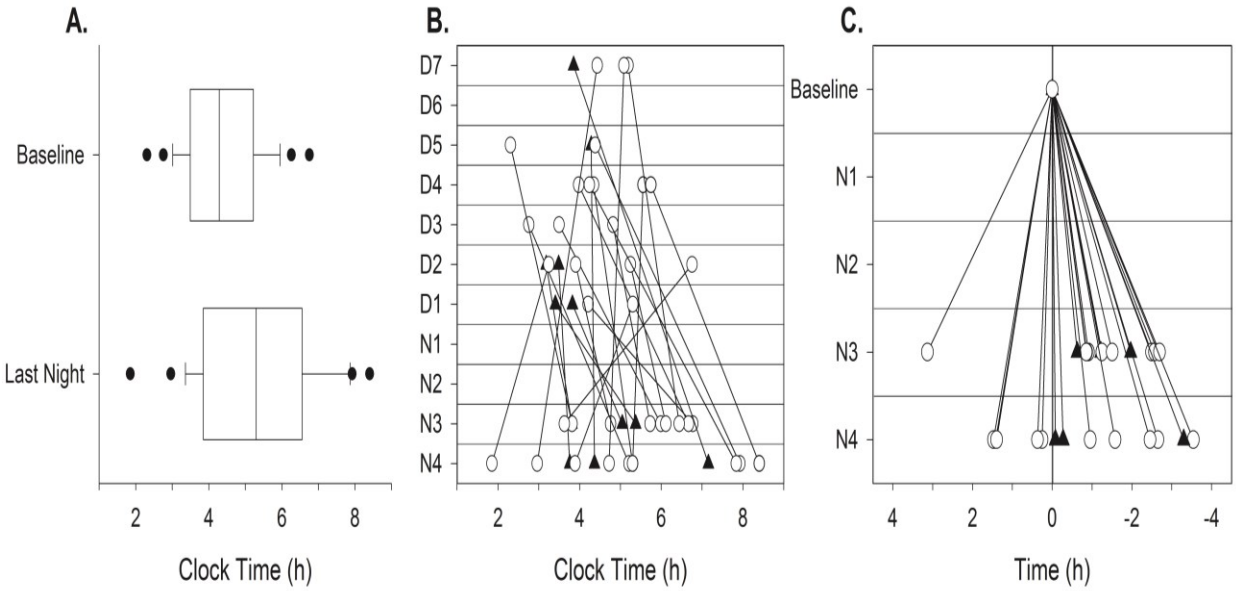

Figure 3. Light exposure relative to circadian phase for each participant. The circles represent $\log_{10}$ lux averaged in each delay (90 degrees before acrophase) and advance (90 degrees after acrophase) zone at baseline, first night shift (N1), second night shift (N2), third night shift (N3) and fourth night shift (N4). Light is averaged relative to individual circadian phase: the x axis represents circadian degrees (360 degrees = 24+daily phase shift), such that 0 indicates acrophase each day. Triangles represent aMT6s acrophase in clock time at baseline and final night shift (error bars show 95% confidence intervals). The difference between light in delay compared to advance zones was

examined at baseline and each night shift to examine the relationship with the direction of phase shift for each participant.

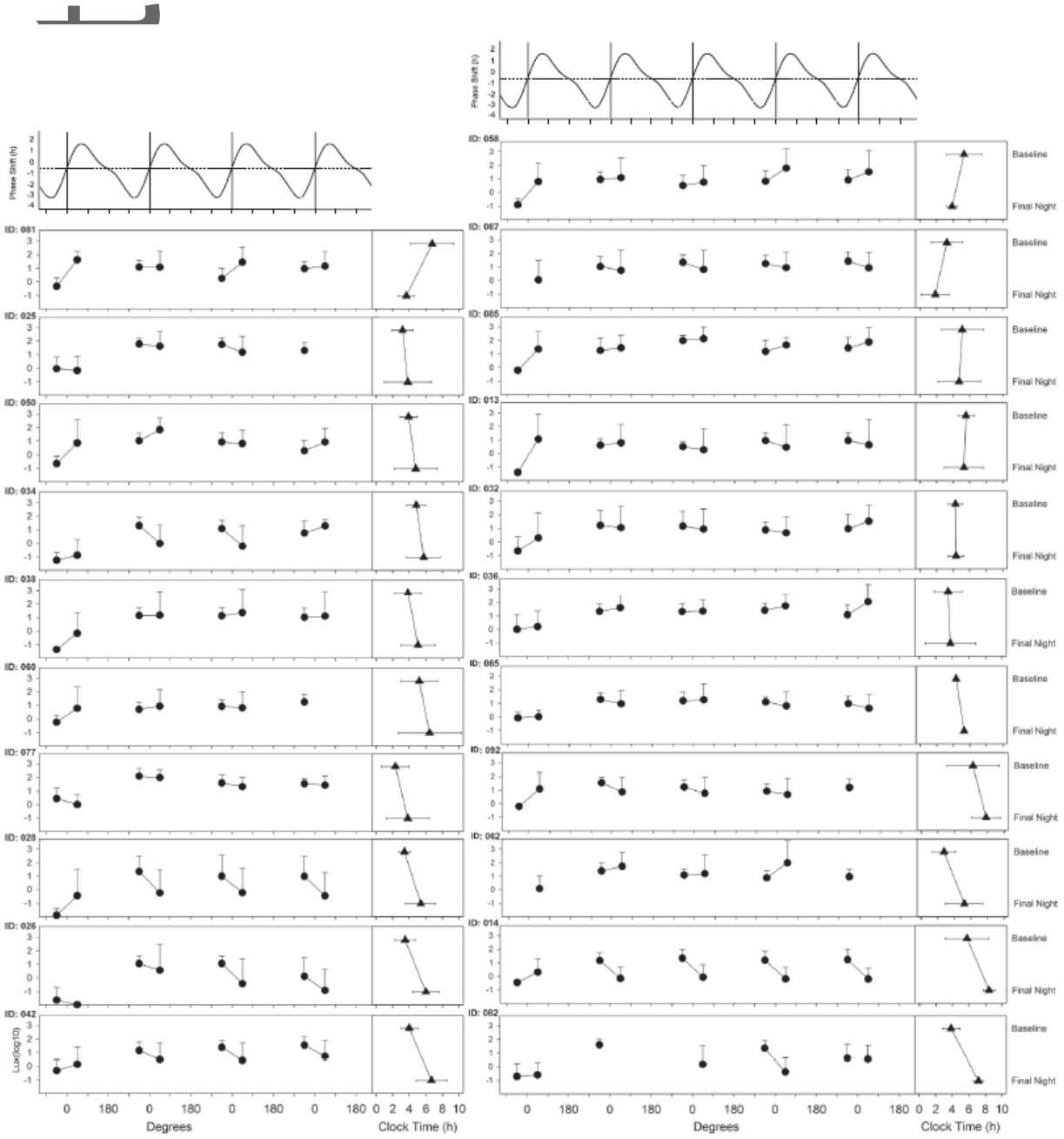

Figure 4. Relationship between phase shift and the difference between delay and advance light in the 90 degrees either side of acrophase time at baseline, first night shift (N1), second night shift (N2), third night shift (N3), fourth night shift (N4), and averaged over all night shifts (Mean Night).

Negative phase shift indicates phase delay; positive phase shift indicates phase advance. Negative lux difference indicates a greater amount of delaying light (90 degrees before acrophase), while positive lux difference indicates a greater amount of advancing light exposure (90 degrees after acrophase) compared to delaying. Men are represented as closed triangles, women as open circles.

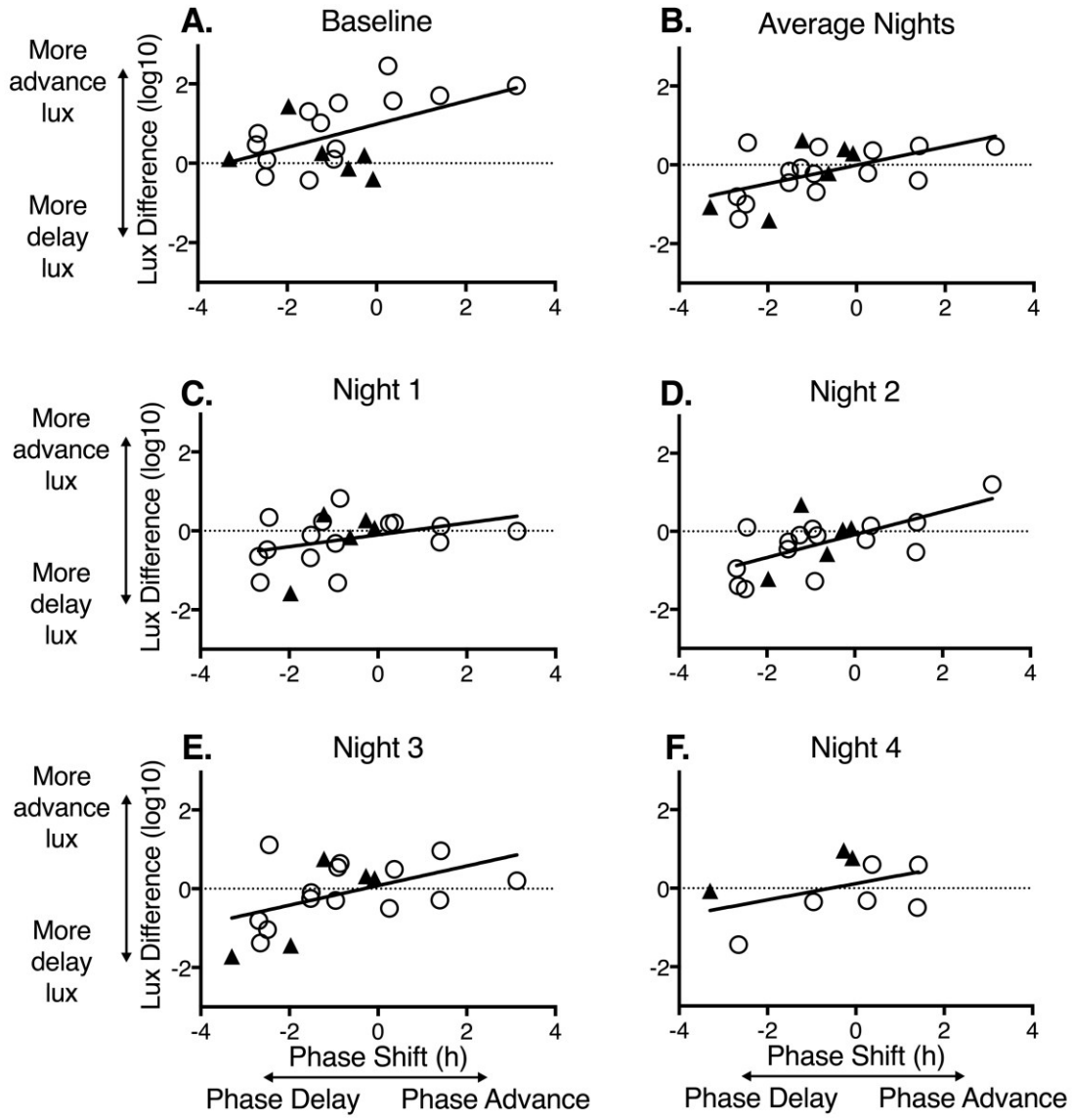

Figure 5. Predicted vs actual phase shift for each participant from two models: **A.** phase shift predictions calculated using regression model incorporating the difference between light in the delay and advance zones at baseline, and averaged across the night shifts; **B.** phase shift predictions

calculated using a regression model using the difference between in the delay and advance zones at baseline, averaged across the night shifts, and MEQ score. Predictions using this model (B) show less prediction error compared to predictions obtained without including MEQ score (A). Black symbols indicate actual phase shift; open symbols indicate predicted phase shift. Men are represented with triangles, women with circles. Negative phase shift = phase delay, positive = phase advance. Participants are ranked according to their phase shift.

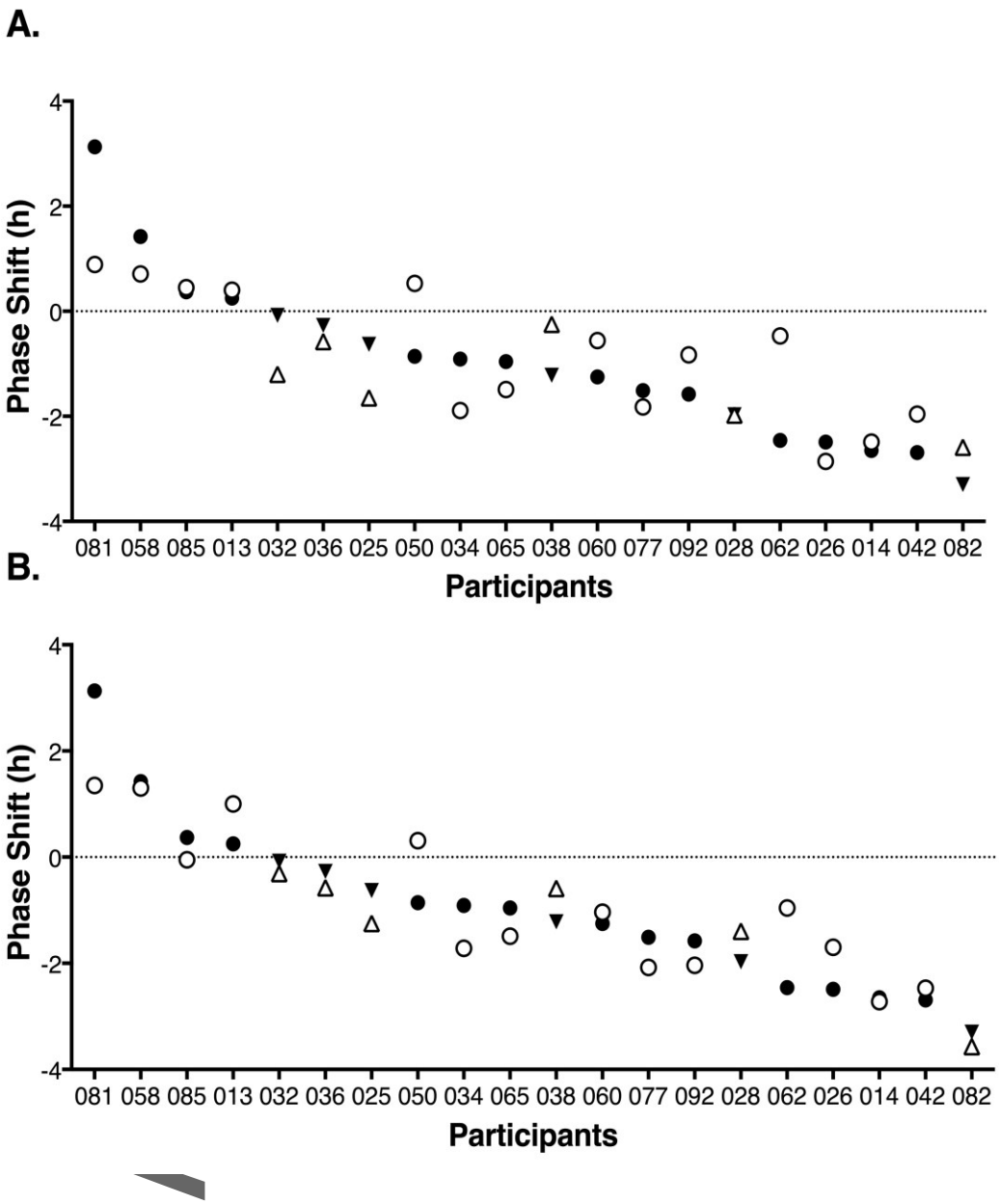

Table 1. Characteristics for participants included in acrophase dataset (n=25).

	3 Night Shifts				4 Night Shifts				Total			
	n	Mean (SD)	Min	Max	n	Mean (SD)	Min	Max	n	Mean (SD)	Min	Max
Demographics												
Sex (male, female)	3, 9				3, 10				6, 19			
Occupation (nurse, doctor)	12,0				9, 4				21,4			
Age (years)		33 (7)	24	45		33 (10)	25	58		33 (8)	24	58
BMI (kg/m^2)		24 (4)	16	30		23 (3)	17	30		24 (4)	16	30
PSQI		7 (3)	2	12		5 (2)	3	8		6 (2)	2	12
ESS		6 (4)	1	11		6 (3)	2	11		6 (3)	1	11
MEQ		40 (4)	35	49		37 (5)	27	45		38 (5)	27	49
Circadian Timing												
Baseline acrophase (hh:mm)		4:11 (1:10)	2:18	6:45		4:31 (1:02)	2:45	6:19		4:21 (1:05)	2:18	6:45
1st Night acrophase (hh:mm)		4:34 (1:01)	3:20	5:53		4:42 (1:04)	2:49	6:19		4:39 (1:02)	2:49	6:19
3rd or 4th Night acrophase (hh:mm)		5:20 (1:11)	3:37	6:46		5:16 (2:10)	1:51	8:24		5:18 (1:37)	1:51	8:24
Phase shift (hh:mm)		-1:09 (1:31)	-2:41	3:07		-00:45 (1:47)	-3:32	1:28		-1:08 (1:39)	-3:43	3:07
Shifts Prior to Baseline Acrophase (days)												
No. day shifts		5 (2)	2	8		6* (4)	0	14		6 (3)	0	14
No. evening shifts		5 (2)	1	9		3* (3)	0	10		4 (3)	0	10

No. days off	7 (2)	3	10	6 (6)	2	11	7 (2)	2	11
No. other	1 (1)	0	3	2* (3)	0	9	1 (3)	0	9
No. night shifts	0 (1)	0	3	1* (3)	0	7	1 (2)	0	7
Total days prior to baseline	18 (4)	6	21	17 (6)	3	29	18 (5)	3	29
Shift work History (years)									
Day shifts	10.50 (6.16)	3	23	7.08 (8.03)	0	30	8.72 (7.25)	0	30
Evening shifts	10.50 (6.16)	3	23	7.00 (8.12)	0	30	8.68 (7.32)	0	30
Night shifts	11.00 (5.86)	3	23	7.54 (7.59)	0	30	9.20 (6.90)	0	30

Note: BMI = Body Mass Index, PSQI = Pittsburgh Sleep Quality Index, ESS = Epworth Sleepiness Scale, MEQ = Morningness Eveningness Questionnaire. * $p<0.05$, 3 night shifts versus 4 night shifts. Negative phase shift indicates phase delay; positive phase shift indicates phase advance.

Author/s:
Stone, JE; Sletten, TL; Magee, M; Ganesan, S; Mulhall, MD; Collins, A; Howard, M; Lockley, SW; Rajaratnam, SMW

Title:
Temporal dynamics of circadian phase shifting response to consecutive night shifts in healthcare workers: role of light-dark exposure

Date:
2018-06-15

Citation:
Stone, J. E., Sletten, T. L., Magee, M., Ganesan, S., Mulhall, M. D., Collins, A., Howard, M., Lockley, S. W. & Rajaratnam, S. M. W. (2018). Temporal dynamics of circadian phase shifting response to consecutive night shifts in healthcare workers: role of light-dark exposure. JOURNAL OF PHYSIOLOGY-LONDON, 596 (12), pp.2381-2395. https://doi.org/10.1113/JP275589.