Changes in the Concentration of Urinary 6-sulphatoxymelatonin during a Week of Simulated Night Work

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Abstract: The aim of the study was to examine the adaptation of participants to a common night work schedule using urinary 6-sulphatoxymelatonin (aMT6s) concentration as the circadian phase marker. Fifteen adults (7 male, 8 female, age = 21.9 yr) spent nine consecutive nights in the laboratory, including: (i) adaptation sleep, (ii) baseline sleep, and (iii) seven simulated night shifts (23:00–07:00 h) followed by daytime sleep. During the baseline and daytime sleeps, participants collected urine samples which were subsequently assayed for aMT6s. The concentration of aMT6s in urine for the first three day sleeps was significantly lower than for the baseline sleep, but there was no difference in aMT6s concentrations between any of the last three day sleeps and the baseline sleep. The data indicate that people may adapt to a pattern of work that includes seven consecutive night shifts if they adhere to a fixed sleep schedule, if their exposure to morning sunlight is minimised, and if they are provided with an ideal sleep environment.

Key words: Shiftwork, Adaptation, Melatonin

Shiftwork, particularly that involving night work, presents a challenge to the human circadian system. Specifically, shiftworkers are often required to sleep at a phase in their circadian cycle when they would usually be active (i.e. daytime) and work at a phase in their circadian cycle when they would usually be asleep (i.e. night-time). Typically, this results in sleep of diminished quality and quantity during the daytime and difficulty maintaining alertness and even wakefulness during the night-time¹–⁴).

In order to design reasonable schedules and/or formulate effective coping strategies for shiftworkers required to work at night, it is important to understand the magnitude and rate of adaptation to various night work schedules. As part of this process, our group undertook a laboratory-based study to consider the adaptation of participants to a simulated shiftwork schedule that included seven consecutive 8-h night shifts. Data regarding the neurobehavioural performance, salivary melatonin onset, and quantity/quality of sleep for participants in this protocol have been published elsewhere⁵, ⁶).

The aim of the current paper was to examine the adaptation of participants to this night work schedule using the principal metabolite of melatonin in urine—6-sulphatoxymelatonin (aMT6s)—as the circadian phase marker. For people on a normal sleep/wake schedule, pineal production of melatonin is low during the daytime and high during the night-time⁷). However, laboratory- and field-based studies indicate that daytime production of melatonin may increase as participants adapt to an inverted sleep/wake schedule⁸, ⁹). For the current
paper, it was hypothesized that participants would gradually adapt to being awake at night and asleep during the day, manifest in the concentration of aMT6s in urine during daytime sleep periods progressively increasing throughout the week.

Fifteen healthy adults (7 male, 8 female) gave written, informed consent to participate in the study as volunteers. Participants had a mean (± SD) age of 21.9 (± 2.7) years and a mean body mass index of 22.1 (± 2.3) obesipascals (kg/m²). Participants had not undertaken shiftwork or transmeridian travel in the month prior to the study. The study was approved by the University of South Australia Human Research Ethics Committee using guidelines established by the National Health and Medical Research Council of Australia.

The study was conducted at the Centre for Sleep Research’s sleep laboratory at the Queen Elizabeth Hospital, Woodville, South Australia. Participants spent nine consecutive nights in the sleep laboratory (Fig. 1). The first and second nights were adaptation and baseline sleep periods respectively. The next seven nights were simulated night shifts followed by daytime sleep. Curtains were drawn during the night shifts such that they were conducted in conditions of dim light (< 300 lux). During sleep periods, the bedrooms were completely dark (0 lux).

On the adaptation and baseline nights, participants arrived at the sleep laboratory at 17:00 h. For each participant, mean sleep onset time for the week prior to the adaptation night was determined using pre-study sleep diaries. This was assigned as their personal bedtime for the adaptation and baseline nights. On both nights, participants were allowed to sleep until they naturally woke.

Following the baseline night, participants began seven consecutive nights of simulated shiftwork. Participants were not permitted to sleep between the end of the baseline night sleep and the beginning of the first night shift. Participants arrived at the sleep laboratory no later than 19:30 h each evening. At 20:00 h, participants were confined to the living quarters until after they awoke from sleep the following day. During each night shift (23:00–07:00 h), participants completed approximately twenty minutes of performance testing each hour. Participants had free time in the other forty minutes of each hour, but they were not allowed to sleep, shower, exercise, or leave the living quarters. Participants went to bed between 08:00 and 09:00 h and slept until they naturally woke. Participants were then permitted to leave the laboratory, but had to return by 19:30 h. The only restriction placed on participants during this free time was that they were not permitted to sleep.

For all sleep periods, participants were wired with a standard montage of electrodes to record sleep by polysomnography (PSG). Brain activity was recorded via electroencephalogram (EEG), with electrodes placed at C3–A2 (i.e. left hemisphere-right mastoid) and C4–A1 (i.e. right hemisphere-left mastoid). Eye movements were recorded via electro-oculogram (EOG), with electrodes placed below the right and left outer canthus. Facial muscle tone was recorded via electromyogram (EMG), with two electrodes placed on the chin approximately 2 cm apart. The PSG data was recorded and analysed with a Compumedics 10/20 sleep system using standard criteria. Sleep duration was defined as the period of time from sleep onset to final wake up.

Production of melatonin during all sleep periods was inferred from urinary 6-sulphatoxymelatonin (aMT6s) concentrations. Participants voided urine immediately prior to going to bed. From that point, all urine passed during the sleep period was collected in a 2-litre plastic bottle. At the end of the sleep period, participants voided urine into the bottle, then transferred a 4 ml aliquot of urine by pipette to a 5 ml-sample tube containing a rice grain of the preservative boric acid. Urine samples were subsequently analysed at the Circadian Physiology Laboratory in the Department of Obstetrics and Gynaecology, University of Adelaide. The concentration of aMT6s in the urine samples was determined by radioimmunoassay, using reagents obtained from Stockgrand Ltd. (Surrey, UK).
Data from the sleep period on day 7 were excluded from all analyses as this was a truncated sleep period prior to participants leaving the laboratory. For each participant, sleep duration and the concentration of aMT6s in urine were determined for the baseline night sleep and each of the six day sleeps. Separate repeated measures analysis of variance (ANOVA), each with one within-subjects factor, were used to determine the effects of sleep type (baseline night, days 1–6) on the two dependent variables: (i) sleep duration, and (ii) concentration of aMT6s in urine. If the repeated measures ANOVA indicated a significant effect of sleep type on a dependent variable, planned means comparisons were performed to compare the value of that dependent variable for each of the daytime sleep periods with its value for the baseline sleep period. The Bonferroni correction for multiple comparisons was applied such that the alpha level was adjusted from .05 to .008.

The first repeated measures ANOVA indicated that sleep type did not have a significant effect on sleep duration (F_{6,84}=1.37, p=.237). In contrast, the second repeated measures ANOVA indicated that sleep type had a significant effect on the concentration of aMT6s in urine (F_{6,84}=9.50, p<.0001). Figure 2 shows that (i) the concentration of aMT6s in urine for the first day sleep was substantially lower than for the baseline sleep, and (ii) the concentration of aMT6s in urine increased progressively for each day sleep such that it reached a similar level as for the baseline sleep after six days. The planned means comparisons indicated that the concentration of aMT6s in urine for the first three day sleeps was significantly lower than for the baseline sleep (all p<.008), but that there was no significant difference between the last three day sleeps and the baseline sleep.

Participants in the current study adhered to a simulated shiftwork protocol that required them to be awake during the night-time and asleep during the daytime for a period of one week. The results indicate that the concentration of aMT6s in urine during daytime sleep periods increased throughout the week, which suggests that participants gradually adapted to the day-sleep/night-work schedule. This suggestion is supported by previously published data for these participants showing that salivary melatonin onset occurred progressively later, and neurobehavioural performance during night shifts progressively improved, over the course of the week. While the current data indicate that people may adapt to a pattern of work that includes several consecutive night shifts, two particular aspects of the protocol suggest that some caution should be taken in generalising these results to shiftworkers in their natural sleep and work settings.

First, participants in the current study had a fixed bedtime each day (i.e. 08:00–09:00 h). In contrast, people working at night are free to choose when they sleep each day. Previously, laboratory-based shiftwork simulations have shown that people on a fixed sleep schedule adapt to night work more quickly than participants allowed to sleep ad libitum. Second, field-based studies of night workers indicate that exposure to sunlight, especially in the morning between ending work and beginning sleep, may interfere with adaptation. This is consistent with predictions of phase shift magnitude based on the phase response curve of human circadian rhythms to bright light. Specifically, bright light in the morning provides a phase advance signal that is opposed to the phase delay required to adapt to working at night and sleeping during the day. Participants in the current study were exposed to sunlight each morning prior to going to bed, but the duration and/or intensity of exposure (i.e. 20 min of sunlight starting 16 min after sunrise) may not have provided a sufficiently strong advance signal to inhibit adaptation.

In summary, the data indicate that people may be able to adapt to a pattern of work that includes several consecutive night shifts if they adhere to a fixed sleep schedule, if their exposure to morning sunlight is minimized, and if they are provided with an ideal sleep environment (i.e. a dark, cool, quiet bedroom). It may be possible to create such conditions for shiftworkers in isolated work settings (e.g. at fly-in fly-out mine sites), but it would be less practical for most other shiftworkers.
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References