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ORIGINAL INVESTIGATION

Melatonin treatment for eastward and westward travel preparation

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Abstract
Introduction Melatonin is recommended for hastening adaptation to phase shift, but there is little information on appropriate formulations.
Materials and methods We evaluated the efficacy of three melatonin formulations for circadian phase advance and delay: (a) 3 mg regular release (RR), (b) 3 mg sustained release (SR), and (c) 3 mg surge-sustained release (SSR; consisting of 1 mg RR and 2 mg SR). Circadian phase was assessed by salivary melatonin dim light melatonin onset (DLMO) or offset (MelOff) using thresholds of (1) 1.0 pg/ml and (2) mean baseline+2 standard deviations (BL+2SD). Subjects spent from Tuesday evenings until Thursday in the laboratory. Melatonin (or placebo) was administered at 1600 hours (phase advance) Wednesday, with DLMO assessment on Tuesday and Thursday and at 0600 hours (phase delay) Wednesday, with DLMO assessment Tuesday, Wednesday, and MelOff Thursday morning. Phase advances using the 1.0 pg/ml DLMO were as follows: placebo, 0.73 h; RR, 1.23 h ($p<0.003$); SR, 1.44 h ($p<0.0002$); SSR, 1.16 h ($p<0.012$), with no difference between formulations.
Results and discussion Similar but smaller phase advances were found with BL+2SD. Using MelOff, posttreatment phase position for the RR formulation was delayed compared to placebo by 1.12 h ($p<0.012$), 1.0 pg/ml, and 0.75 h ($p<0.036$), BL+2SD. Phase shifts for the SR and SSR conditions could not be determined due to persistent high melatonin levels during sampling times. Similar phase advances were induced by all formulations, and slow clearance of slow release preparations impeded the determination of phase delays.
Conclusion Appropriately timed 0.5 mg melatonin doses may avoid these problems.

Keywords Jet lag · Melatonin treatment · Dim light melatonin onset · Circadian phase shift

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Introduction

Circadian misalignment, which can be induced by jet lag or shift work, is an operational concern in critical military and civilian occupations due to its adverse impact on sleep and the attendant fatigue, reduced alertness, and performance decrements (Arendt 2009; Waterhouse et al. 2007). Consequently, the Canadian Forces has initiated a multiple-study project to identify countermeasures effective at reducing circadian misalignment among its members engaging in transmeridian travel or shift work. Reduction of circadian misalignment by phase shifting should promote greater

alertness, facilitate better decision making, improve operational readiness, and reduce the possibility of fatigue-related accidents in military personnel.

The pineal hormone melatonin has a marked circadian rhythm driven by the suprachiasmatic nucleus, the central circadian pacemaker. It is extensively used as a "marker" rhythm for detecting circadian phase, being less "masked" than other peripheral rhythms such as core body temperature and cortisol.

In 1958, Lerner first identified melatonin and noted its ability to induce transient sleepiness during the day when endogenous melatonin levels are low. Early work on phase shifting with melatonin suggested that 2 mg taken in late afternoon for a month was sufficient to induce a phase advance in endogenous melatonin rhythm of 1–3 h (Arendt et al. 1985). Arendt et al. (1987) used a 5-mg dose of melatonin, taken at 1800 hours for 2 days, immediately prior to an eight time zone eastward transmeridian flight and the same dose at local bedtime at destination for 4 days to effect a phase advance in a successful attempt to counter jet lag. Subjects on melatonin were more alert and slept better than the subjects on placebo during the first five post-flight days. The endogenous melatonin and cortisol rhythms of the melatonin-treated subjects resynchronized at destination faster than those of the placebo subjects. Numerous other jet lag studies have since been carried out and melatonin is now recommended by the American Academy of Sleep Medicine (Morgenthaler et al. 2007).

Lewy and Sack (1996) stated that phase shifting with exogenous melatonin was more convenient than phototherapy and should be administered in late afternoon or early evening to cause a phase advance or on awakening to cause a phase delay. However, while the phase- advancing effects of appropriately timed exogenous melatonin have been confirmed across many studies, the phase-delaying efficiency of morning melatonin ingestion is not as certain (Arendt and Skene 2005). The precise timing of treatment can, in principle, be determined from a phase response curve (PRC; Burgess et al. 2008; Eastman et al. 2005; Lewy et al. 1992), but the best dose and formulation for suitable treatment have yet to be investigated.

There is very little information on the relative merits of different melatonin formulations for use in the alleviation of jet lag or for hastening adaptation to time zone change. Since it is not clear whether sustained release (SR) formulations might be more efficacious than regular release (RR) in the induction of phase changes, we wished to determine, in a group of subjects with a regular sleep wake cycle, the relative efficacy of three melatonin formulations [3 mg RR, 3 mg SR, and a surge-sustained release (SSR) (made up of 1 mg RR and 2 mg SR)]. Two studies were performed to compare these three melatonin formulations: one study for phase advance and another for phase delay. For study 1, the timing of melatonin treatment was based on the data of Lewy et al. (1998), which were fitted to a melatonin PRC by Revell and Eastman (2005). For study 2, the timing of melatonin treatment was a compromise between the PRC of Lewy et al. (1998), which is based on a 0.5 mg melatonin dose and was fitted to a curve by Revell and Eastman (2005) and the PRC reported by Burgess et al. (2008), which is based on a 3-mg melatonin dose.

Materials and methods

Various characteristics of the melatonin rhythm (acrophase, evening onset, synoff, morning offset, and 25% maximum rise/fall) can be used to determine phase (Arendt and Skene 2005). The dim light melatonin onset (DLMO) is found by sampling melatonin concentration in the blood or saliva at uniform intervals under dim light conditions (<10 lx; Arendt and Skene 2005; Benloucif et al. 2008; Lewy and Sack 1989, 1996; Voultsios et al. 1997). The first sample that exceeds a prescribed threshold is designated as DLMO. We chose DLMO to avoid overnight saliva collection, which disrupts sleep, as required when determining the acrophase of the melatonin rhythm and to compare our data with other related work using this phase marker. However, for study 2 (phase delay), the high residual levels of melatonin (after melatonin dosing) masked our ability to calculate posttreatment DLMO that evening. We therefore used salivary melatonin samples from the morning after melatonin dosing, for the calculation of melatonin offset (MelOff), to establish the phase delay attributable to the RR melatonin formulation. The high residual levels of melatonin also precluded the quantification of MelOff for the SR and SSR formulations.

Experimental unit All trials took place in a clinical investigation unit, separated from other buildings. Windows were covered and sealed to insure exclusion of all outdoor light. Temperature in the unit was kept at 21°C. Room illumination was less than 10 lx in the angle of subject gaze. A television monitor was kept at least 2.0 m distant from the subjects to limit extraneous light from it to less than 10 lx. Light intensity from the overhead room lighting and the television was measured at the beginning of each condition using an Advanced Broad Range Lux/FC Light Meter (Sper Scientific, model 840022, Scottsdale, Arizona, USA; www.sperscientific.com). For each of the four conditions of experiment 1, subjects reported to the laboratory on a Tuesday at 1700 hours (night 1), remained overnight, retired at 2345 hours, arose at 0700 hours, and were given breakfast and released from the lab at 0730 hours wearing dark glasses. They returned later the same day (Wednesday) at 1500 hours (night 2), again remaining

overnight, arising at 0600 hours Thursday morning. They had breakfast and were released at 0700 hours wearing dark glasses and returned at 1700 hours on Thursday (night 3) and were released from the laboratory at midnight. For each of the four conditions of experiment 2, subjects reported to the laboratory at 1700 hours on Tuesday (night 1) and remained until released at noon on Thursday. Experiment 1 was conducted over a 3-month period from August to October. Experiment 2 was conducted over a 3-month period from January to March.

Subject exclusion/inclusion criteria Subjects for each study passed a screening medical including physician review. Smokers were excluded, as there is a possible interaction of cigarette smoking with endogenous melatonin levels (Ozguner et al. 2005). All subjects completed the morningness–eveningness questionnaire to exclude extreme larks/owls (Horne and Ostberg 1976). The habitual caffeine intake of the participants varied between zero and three caffeinated beverages daily. Participants were instructed to avoid alcohol and caffeine-containing substances during all experimental days. In the rare instance where an analgesic was required by a participant during experimental days, they were given acetaminophen, since non-steroidal anti-inflammatory drugs such as aspirin and ibuprofen can suppress melatonin (Murphy et al. 1996).

To quantify sleep time (via activity recording) and ensure that all subjects maintained a regular sleep–wake cycle, a wrist activity monitor (Ambulatory Monitoring Inc, Ardsley, NY; www.ambulatory-monitoring.com) was worn the week preceding and throughout the studies. Activity recording was in 1-min epochs. The sleep–wake pattern to be maintained consisted of a 2300-hour bedtime and 0700-hour arise time. Actigraph data was downloaded and analyzed each week to ensure that subjects were compliant (Cole et al. 1992). Subjects also kept a sleep log to assist in the analysis of actigraphic data.

Saliva collection and melatonin assay Saliva samples for measurement of melatonin were collected using cotton swab salivettes (Sarstedt, Germany) using the collection procedures of Wright et al. (2001). Immediately following collection, the samples were spun in a centrifuge to extract all fluid and placed in a freezer for preservation pending radioimmunoassay (RIA) by the Gamma–Dynacare, London ON, Canada laboratory.

Subjects

Both protocols were approved by the DRDC-Toronto Human Research Ethics Committee and met the ethical standards of the Declaration of Helsinki. Subjects were compensated for their participation according to the DRDC-Toronto guidelines for subject stress allowance and were tested in groups of three to five volunteers. All were normal healthy non-smoking males who passed a screening medical designed to exclude subjects for whom supplementary melatonin might be contraindicated or who might be on medications, which could confound experimental results (i.e., beta blockers, which decrease endogenous melatonin levels). All subjects agreed to maintain normal bedtime hours between sessions [i.e., retiring to bed each day (between sessions) between 2300 and 2400 hours and remain in bed for 8 h of sleep] to insure they were well-rested and in a known circadian phase when they report to the laboratory. They also agreed to avoid alcoholic beverages for 24 h prior to each data collection session.

Experiment 1: The 13 normal healthy male volunteers who participated in experiment 1 ranged from 26 to 53 years in age (37.3±8.9 years; mean ± SD).

Experiment 2: The nine normal healthy male subjects who participated in experiment 2 were 26–54 years in age (40.7±10.5 yr; mean ± SD).

Procedures

Experiment 1 A double-blinded four-arm placebo-controlled repeated measures protocol with randomized treatment order was used to compare three melatonin formulations, which were identical in appearance: 3 mg RR, 3 mg SR, and a SSR formulation (made up of 1 mg RR and 2 mg SR) to a matching placebo for efficacy in circadian phase advance. These melatonin formulations including the matching placebo were manufactured by JAA PHARM Canada Inc., Woodbridge, Ontario. All melatonin formulations were taken at 1600 hours.

Subjects arrived in the laboratory on night 1, immediately after having a meal. For the duration of the evening, they remained semi-recumbent on lounge chairs in dim light watching television or videos. They were permitted to get up to use the toilet, but not in the 15 min prior to saliva collection, and were instructed to drink water responsibly to preclude frequent restroom use. Subject wakefulness was monitored by the data collectors to ensure no one fell asleep. Saliva samples for measurement of melatonin were collected every 30 min between 1800 hours and 2330 hours during night 1. During that time, the use of tooth paste and other oral hygiene products was prohibited. After the 2330-hour saliva sample, participants prepared for bed and were in their laboratory bedroom with the lights off, trying to sleep no later than 2345 hours.

On day 2 (Wednesday), subjects were awakened at 0700 hours, given breakfast and allowed to leave the

laboratory at 0730 hours wearing dark glasses. They returned later that day (Wednesday) at 1500 hours, ingested their assigned treatment at 1600 hours and retired to bed (night 2), remaining in bed until 0600 hours the following morning. The subjects resumed the semi-recumbent posture on lounge chairs in dim light at 0610 hours and provided a single saliva sample at 0625 hours, had breakfast, and were released at approximately 0700 hours (Thursday, day 3) wearing dark glasses.

The subjects returned to the laboratory at 1700 hours on Thursday (night 3) and provided saliva samples every 30 min between 1755 and 2325 hours and were released from the laboratory at approximately midnight. They were dismissed from the laboratory following the 2330-hour saliva sample on night 3. Each condition produced a total of 26 saliva samples per subject.

Experiment 2 A double-blinded four-arm placebo-controlled repeated measures protocol with randomized treatment order was used to compare the same three melatonin formulations used in experiment 1. Since the objective of experiment 2 was to achieve a phase delay, all doses were ingested at 0600 hours. For all experimental conditions, subjects reported to the laboratory on Tuesday at 1700 hours (night 1), provided saliva samples from 1755 to 2325 hours and retired to bed at 2345 hours. They were awakened at 0600 hours (in darkness) to take their scheduled treatment, returned to sleep, and were awakened at 1030 hours. They returned to their lounge chairs in semi-recumbent posture in dim light to provide a salivary melatonin sample at 1055 hours, consumed breakfast between 1055 and 1110 hours to insure that 15 min elapsed without food or water prior to the 1125-hour saliva sample. Saliva sampling continued every 30 min until 1625 hours. Subjects were served a standardized meal between 1630 and 1730 hours. The saliva sampling routine continued from 1755 to 0025 hour. Subjects retired to bed at 0030 hour, arose at 0700 hours, and saliva sampling began at 0710 hours with samples taken every 30 min from 0725 to 1255 h. They were released from the laboratory just after 1300 hours. They consumed a sandwich immediately after the 1025 sample. For both experiments, each new test session commenced the week following the conclusion of the previous test session, i.e., one session per week for each of 4 weeks. The experimental designs for both studies are illustrated in Fig. 1.

Analysis

Actigraphy Sleep start and sleep end time were derived from the activity measures using the manufacturer's software. Mean values for each subject were calculated for the week preceding the intervention study for each leg of the study.

Melatonin All saliva samples were assayed for melatonin content via RIA (Voultsios et al. 1997). The assay characteristics were as follows: intra-assay precision was 5.7% at 4.3 pg/ml; inter-assay reproducibility was 15.3% at 1.9 pg/ml and 10.6% at 19 pg/ml; and the functional least detectable dose was 0.9 pg/ml. All samples from the same subject in a condition (e.g., subject 1, condition A) were assayed together.

Dim light melatonin onset In experiment 1, DLMO was designated in two ways: initially as the first sample that exceeded the predetermined threshold of 1.0 pg/ml. Linear interpolation was used in this case. Secondly, for comparison with other published data regarding the PRC for melatonin, another definition of DLMO was used: the time point at which values exceeded the mean baseline (BL) concentration by 2 SD (by interpolation). The mean BL was defined as the average of the first rise point sample and the previous three BL samples after initiation of evening sampling (for DLMO) and the last decline point sample and subsequent three BL samples prior to the termination of morning sampling (for MelOff). The difference between the DLMO of nights 1 and 3 was the measure of overall phase advance. The differences between the DLMO of placebo night 3, and the melatonin formulations on night 3 was the measure of phase advance due to melatonin only.

Melatonin offset Since in experiment 2, melatonin was ingested at 0600 hours, the ability to determine posttreatment DLMO (commencing 12 h after ingestion at 1800 hours) was masked by the high residual melatonin levels from the morning ingestion. Therefore, we compared post-ingestion MelOff times for placebo and melatonin formulations, where possible.

Statistics The total decimal hours of circadian phase shift were analyzed using a single-factor, repeated measures ANOVA. Paired Student's t tests were used to assess the change in DLMO from nights 1 to 3 in each condition of experiment 1. To compare DLMO times across night 1 and across night 3 for experiment 1, a two-factor, repeated measures ANOVA [four conditions×two trials (nights 1 and 2)] was used. To compare the posttreatment MelOff times for the RR melatonin and placebo conditions in experiment 2, a paired Student's t test was used. Statistical significance was defined as the 95% level of confidence. The Geisser–Greenhouse correction for repeated measures was applied to all ANOVAs. Significant main effects and interactions were assessed with the Tukey honestly significant difference (HSD) test.

Results

Experiments 1 and 2

Actigraphy

Subjects complied with the imposed prestudy sleep schedule. Average sleep start time for the week prior to each study iteration ranged from 23.65±0.17 to 23.88±0.15 h for experiment 1 and from 24.00±0.33 to 24.58±0.24 h for experiment 2 (mean±SEM).

Salivary melatonin data

To determine if there were any seasonal differences in the timing of DLMO, ANOVA was used to compare all night 1 DLMOs across the two experiments. No differences across conditions were observed [$F(5,35)=1.53$, $p<0.21$]. This was expected as the subjects were instructed to sleep from 2300 to 0700 hours, and compliance with the prescribed sleep schedule was verified via wrist actigraphy.

Raw salivary melatonin profiles (for DLMO assessment) for each condition of experiment 1 are illustrated for subject 2 in Fig. 2a, while the profiles for posttreatment MelOff assessment comparing RR against placebo for subject 25 are shown in Fig. 2b.

Pretreatment (night 1) and posttreatment (night 3) DLMO and phase advance data for all subjects were collated and averaged by condition for experiment 1. These data are presented in Table 1. Posttreatment MelOff data for all subjects were collated and averaged by condition for experiment 2. These data are presented in Table 2. The average pretreatment (night 1) DLMO over all conditions of experiment 1 was 20.15 h±0.10 for the 1.0 pg/ml threshold and 20.94 h±0.12 for the mean BL+2SD threshold. The corresponding N1 DLMO values for experiment 2 were 20.82 h±0.31 for the 1.0 pg/ml threshold and 21.72 h±0.33 for the mean BL+2SD threshold.

Experiment 1 Using the 1.0 pg/ml threshold, the analysis of DLMO times revealed a significant main effect of trials (i.e., nights) [$F(1,12)=188.77$, $p<0.001$] and significant conditions×trials interaction [$F(1.92, 23.02)=9.70$, $p<0.001$]. Post hoc analysis demonstrated that night 3 DLMO values for each of the RR, SR, and SSR formulations were different from the night 3 DLMO value of the placebo condition ($p<0.0001$ for all three comparisons) but not different from each other. Mean pretreatment (night 1) DLMOs were not significantly different from each other.

The mean phase shifts for each condition in experiment 1, calculated as the difference in DLMO from nights 1 to 3, were 1.23±0.11 h advance for the RR formulation, 1.41±0.14 h advance for the SR formulation, 1.16±0.13 h advance for the SSR formulation, and 0.73±0.09 h advance for the placebo condition (Fig. 3a). Assessment of phase changes demonstrated a significant main effect between conditions [$F(1.92, 23.11)=9.57$, $p<0.001$]. Post hoc analysis with the Tukey HSD test confirmed that each of the three melatonin formulations resulted in a significantly larger phase advance compared to placebo ($p<0.003$, $p<0.0002$, and $p<0.012$ for RR, SR, and SSR, respectively), but there were no significant differences between melatonin formulations (Fig. 3a).

Using the mean BL+2SD definition of DLMO, the analysis of DLMO times revealed a significant main effect of trials (i.e., nights) [$F(1,12)=46.82$, $p<0.00002$] and significant conditions×trials interaction [$F(2.69, 32.32)=4.18$, $p<0.016$]. Post hoc analysis demonstrated that night 2 DLMO values for each of the RR, SR, and SSR formulations were significantly different from the night 3 DLMO value of the placebo condition ($p<0.0003$, $p<0.0001$, and $p<0.002$ respectively) but not different from each other. Mean pretreatment (night 1) DLMOs were not significantly different from each other.

The mean phase shifts for each condition in experiment 1, calculated as the difference between DLMO from nights 1 to 3, were a 1.08±0.16 h (SEM) advance for RR formulation, a 1.01±0.20 h advance for the SR formulation;, a 0.95±0.20 h advance for the SSR formulation, and a 0.45±.13 h advance for the placebo condition (Fig. 3b). Assessment of the different phase changes yielded a significant main effect between conditions [$F(2.69, 32.33)=4.18$, $p<0.012$]. Post hoc analysis with the Tukey HSD test confirmed that the phase advances from the RR ($p<0.015$) and the SR ($p<0.036$) formulations were significantly larger than for the placebo conditions but not the SSR ($p<0.08$) formulation. There were no significant differences in phase advances between the melatonin formulations (Fig. 3b).

Using the mean BL+2SD definition of DLMO, the phase advances for each of the RR, SR, and SSR formulations relative to placebo were 0.74±0.24, 0.83±0.31, and 0.62±0.32 h (mean ± SEM), respectively. Using the 1.0 pg/ml threshold definition of DLMO, the phase advances for each of the RR, SR, and SSR formulations relative to placebo were 0.63±0.18, 0.87±0.26, and 0.61±0.18 h, respectively.

Experiment 2 In experiment 2, whether using the 1.0 pg/ml definition or the mean BL+2SD definition of DLMO, high residual levels of melatonin post-ingestion, masked our ability to determine DLMO on night 2 (i.e., the night after treatment) for all formulations. Therefore, we employed MelOff on day 4 as the phase marker for the RR formulation. MelOff was still masked by exogenous melatonin from the other two formulations (SR and SSR) on day 3, 31 h after ingestion. Since we had not taken pretreatment morning melatonin levels to establish BL

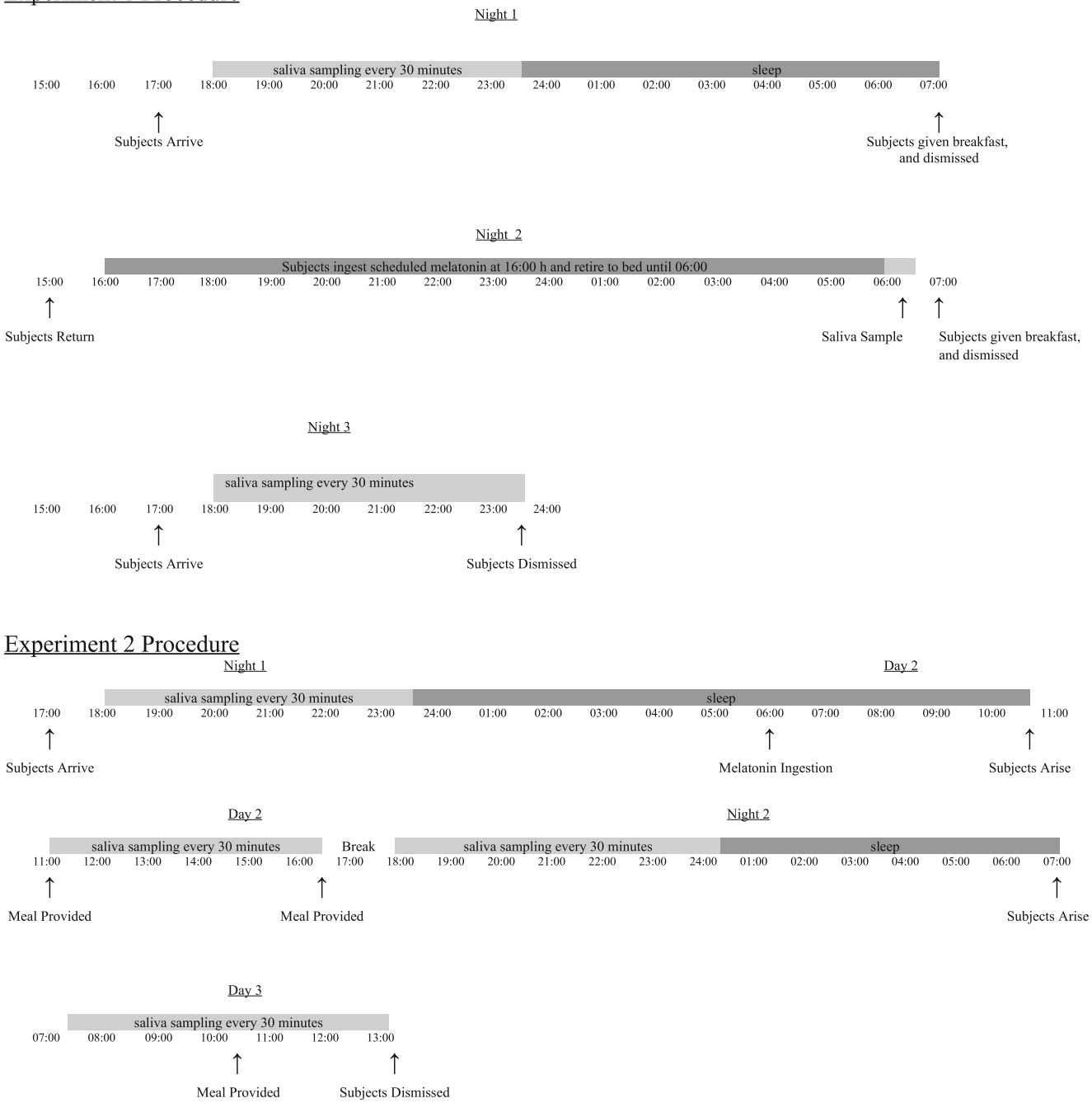

Fig. 1 Schematic illustrations of experimental design for each of the two experiments

MelOff, we compared posttreatment MelOff between the RR formulation and placebo using two definitions of MelOff (i.e., mean BL+2 SD and the 1.0 pg/ml threshold). Using the mean BL+2 SD definition of MelOff from nine subjects, the phase delay for the RR formulation relative to placebo was 0.75±0.30 h (mean ± SEM; $p<0.036$). Using the 1.0 pg/ml threshold definition of MelOff from the seven subjects for whom we could determine MelOff, the RR formulation phase delay (relative to placebo) was 1.12± 0.34 h ($p<0.012$). The phases delay results are illustrated in Fig. 4.

Discussion

In both studies reported, melatonin administration induced significant phase changes. A phase shift is normally calculated by the pre- and posttreatment differences in the

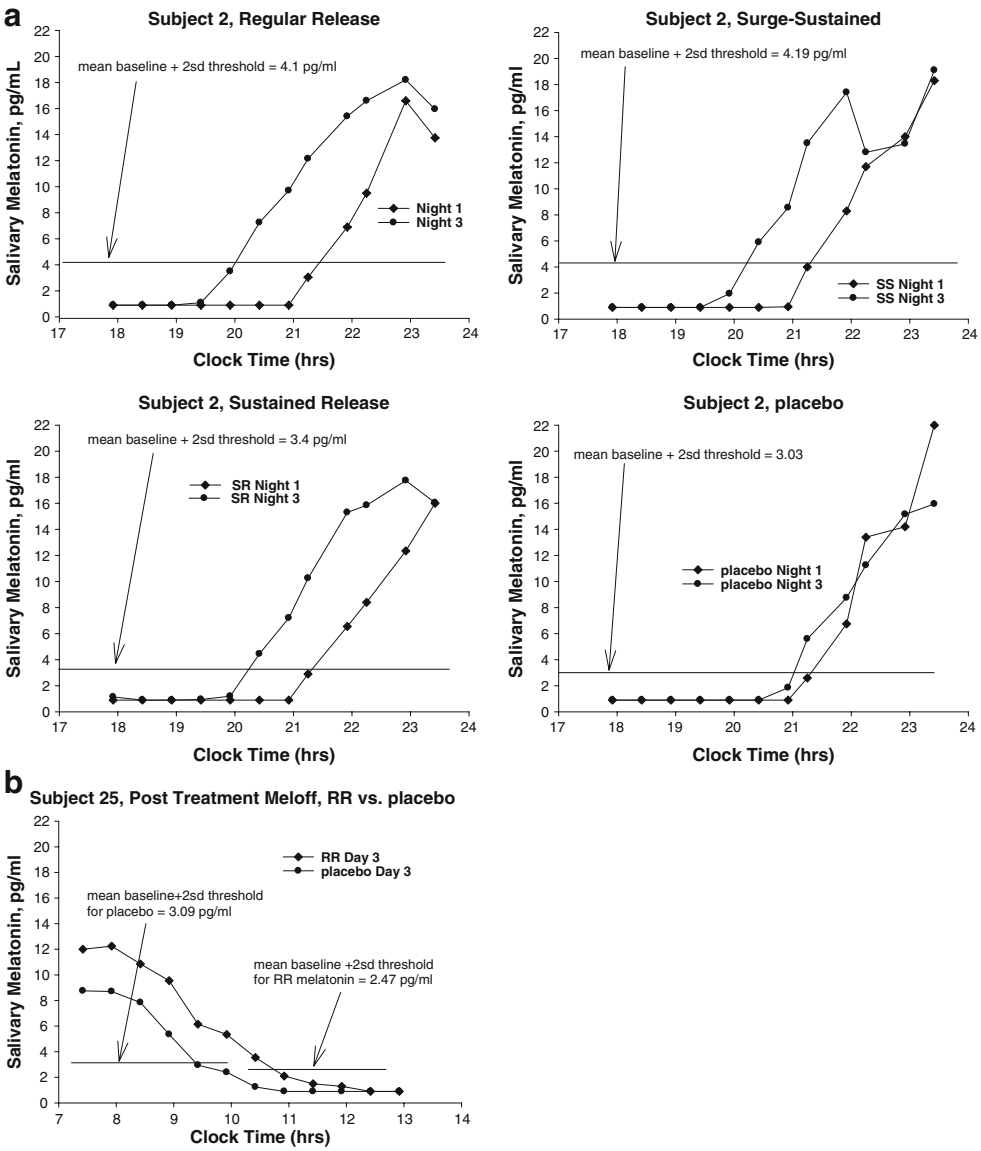

Fig. 2 **a** Raw salivary melatonin profiles pre- and post-treatment for subject 2 during each of the four phase advance conditions. **b** Raw salivary melatonin post-treatment profiles for subject 25, for RR and placebo

phase marker (e.g., DLMO or MelOff or core temperature) for each individual subject. These individual phase shifts are then averaged to yield an average phase shift for the treatment in question. In the present protocol, the subjects' sleep and wake up times were also manipulated. In order to control for any phase shifts due to factors other than melatonin administration, a placebo leg was introduced. The difference between placebo phase shift and melatonin phase shift provides the measure of the shift due to melatonin alone, rather than the combination of treatment

Table 1 DLMOs and phase advances in decimal hours by condition for each of 1.0 pg/ml and mean baseline plus 2 SD thresholds

Study	Condition	1.0 pg/ml threshold			Mean baseline plus 2 SD threshold		
		Night 1	Night 3	Phase advance	Night 1	Night 3	Phase advance
Phase advance study	RR	20.17±0.16	18.94±0.14	1.23±0.11 h**	20.96±0.19	19.88±0.19	1.08±0.16 h*
	SR	20.07±0.25	18.66±0.21	1.41±0.14 h**	20.80±0.25	19.78±0.23	1.01±0.21 h*
	SSR	20.08±0.22	18.92±0.16	1.16±0.13 h**	20.94±0.27	19.99±0.26	0.95±0.20 h
	Placebo	20.26±0.20	19.53±0.17	0.73±0.09 h	21.08±0.26	20.62±0.37	0.46±0.13 h

Melatonin treatment was given at 1600 hours. Values are mean ± SEM

*$p<0.05$ compared to placebo, **$p<0.01$ compared to placebo

Table 2 Post melatonin treatment MelOffs in decimal hours by condition for each of 1.0 pg/ml and mean baseline plus 2 SD thresholds

1.0 pg/ml threshold				Mean baseline plus 2 SD threshold			
Number of subjects	placebo	RR	Phase delay	Number of subjects	Placebo	RR	Phase delay
7	09.96±0.78	11.08±0.64	1.12±0.34 h**	9	09.23±0.54	09.98±0.49	0.75±0.30 h*

Melatonin treatment was given at 0600 h. Values are mean ± SEM
*$p<0.05$, **$p<0.01$

and changing sleep time/other factors. In the case of experiment 1, the overall shift in DLMO, as well as the contribution of melatonin alone, could be determined. In the PA study, the contribution of melatonin to the overall phase shift is given by subtracting the placebo values from each of the three active treatment conditions on night 3. The phase advances for the 1.0 pg/ml threshold were the following (RR = 0.59±0.18 h, SR = 0.88±0.26 h, and SSR = 0.61±0.18 h) and for the mean BL+2SD thresholds were [RR = 0.74±0.24 h, SR = 0.83±0.31 h, and SSR = 0.62±0.32 h (mean±SEM)].

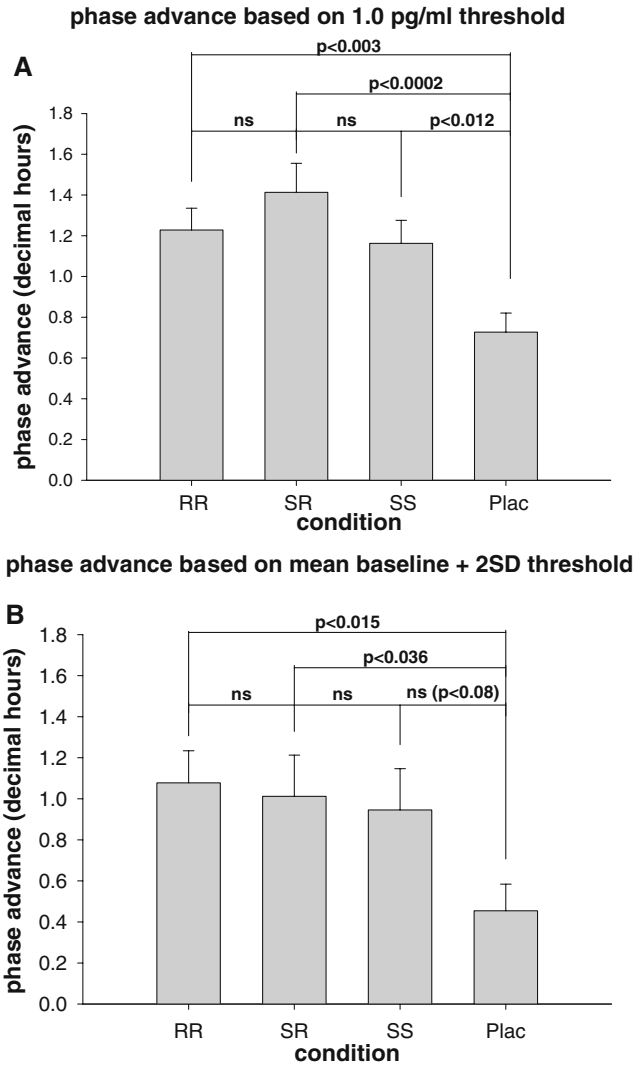

Fig. 3 Circadian phase advances marked by changes in DLMO across all three melatonin formulations of experiment 1 for the 1.0 pg/ml (**a**) and mn BL+2SD (**b**) thresholds. All values are mean±SEM and are plotted over the four treatment conditions

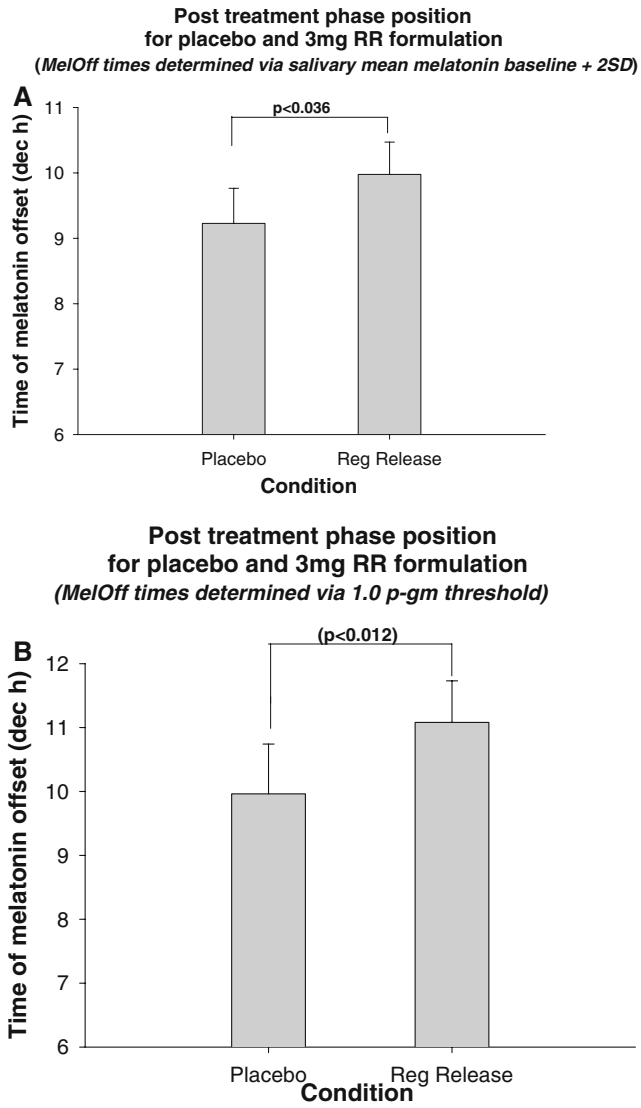

Fig. 4 Circadian phase delay attributable to the RR foumulation, marked by MelOff for mn BL + 2SD (**a**) and 1.0 pg/ml (**b**) thresholds. All values are mean ± SEM and are plotted over the RR and placebo post treatment conditions

The combined effects of melatonin and the other factors imposed in our protocol were clearly more powerful than melatonin alone. In experiment 2, the MelOff difference between RR and placebo in experiment 2 is the direct measure of the effect of RR melatonin controlling for all the other factors.

In the phase advance study, we woke the subjects up an hour early on the morning after the melatonin dose. These subjects were released from the laboratory approximately an hour after awakening. However, they left the laboratory wearing dark glasses, which provided some protection against the notion of morning light adding to the phase shift induced by the dose of exogenous melatonin. Nevertheless, the earlier wake-up time no doubt contributed to the phase advance in the placebo condition.

In the case of the phase delay study, the subjects remained in the dark 3.5 h later than their normal wake-up time. This would remove morning light from the phase advance portion of the PRC, thus promoting a phase delay.

We did not anticipate the length of time that the posttreatment DLMO would be masked by elevated residual postdose exogenous melatonin. It is possible that an additional posttreatment night of saliva sampling would have provided a suitable DLMO; however, at present, the time for complete clearance of these slow release preparations remains to be determined. The use of MelOff as a phase marker for the phase delay study gave a measure of the shift due to melatonin alone but not the shift due to all pertinent factors in the protocol: This would have required the collection of pretreatment morning saliva samples.

We administered our melatonin dose at a time that was a compromise between two PRCs. The PRC from Burgess et al. (2008) indicates that when using a 3-mg RR melatonin dose, the optimum time of administration for phase delay is about 0900 hours clock time rather than the 0600-hour timing used in our study.

The typical jet lag plan includes multiple days of treatment (Arendt 2009; Arendt et al. 1986, 1987; Eastman and Burgess 2009; Eastman et al. 2005; Revell et al. 2006; Revell and Eastman 2005). The small phase changes reported here are consistent with a single low-dose treatment (e.g., Deacon and Arendt, 1995). With more days of treatment we would expect a correspondingly larger cumulative phase shift.

Analysis of pretreatment DLMO determined that night 1 DLMO was not significantly different between conditions, confirming that the time away from the laboratory between treatment conditions was sufficient to restore the BL circadian phase of the subjects. The three 3-mg melatonin formulations were ingested at times based on established melatonin PRCs for optimum phase advance (experiment 1) and phase delay (experiment 2; Burgess et al. 2008; Lewy et al. 1998; Revell and Eastman 2005). In experiment 2, where MelOff was used as the phase marker, it is possible that the two SR formulations (SR and SSR), where we could not determine posttreatment phase, may have produced an unrecognized phase delay. By defining DLMO using a sensitive melatonin assay, any treatment used must have cleared completely from the circulation to successfully measure posttreatment melatonin onset or offset. This was not the case using two of the melatonin preparations, the SR and SSR treatments. However, it is also possible that the extended period of elevated melatonin levels provoked by these SR formulations would theoretically have "spilled over" (Lewy et al. 2002) into the phase advance portion of the melatonin PRC resulting in minimal or no net phase change. In the future, it would be advisable to avoid SR formulations in the phase delay section of the melatonin PRC, unless a PRC for each of the SR formulations used in this study (i.e., 3 mg SR and the surge-sustained formulation made of up 1 mg RR and 2 mg SR) can establish an ingestion time that would produce phase delays.

It is possible that smaller physiologic doses of melatonin (approximately 0.5 mg) would avoid the hypothetical "spill-over effect." Lewy et al. and Hack et al. have demonstrated the efficacy of 0.5 mg doses of melatonin in entraining free-running circadian rhythms of blind people (Hack et al. 2003; Lewy et al. 2001). Deacon and Arendt (1995) and Sharkey and Eastman (2002) demonstrated the efficacy of 0.5 mg melatonin for phase advances. Recent recommendations for preflight treatment for jetlag have suggested 0.5 mg, which may well be the most appropriate dose.

Based on the work of Deacon and Arendt (1995) and Sharkey and Eastman (2002), Burgess et al. (2008) state that a specific clock time of melatonin administration may be optimal for one dose but not another. Deacon and Arendt (1995) compared the effects of 0.05, 0.5, and 5 mg of melatonin administered at 1700 hours, which produced phase advances of 0.4, 0.7, and 1.4 h, respectively. Sharkey and Eastman (2002) administered 0.5 and 3.0 mg melatonin doses at the same clock times over four pulses (days) resulting in 3.0- and 3.9-h phase advances, respectively. Both these studies suggest that higher melatonin doses are more efficacious than the lower doses. However, Burgess et al. go on to cite work by Revell et al., where a 3-mg dose was administered 4.8 h prior to the DLMO and a 0.5-mg dose was administered about 2.4 h before the DLMO (Revell et al. 2006). The phase advances for each of these two doses were similar (i.e., no dose–response relationship was observed), suggesting that the timing of the phase advance portion of the PRCs differs with dose. In a dose–response field study on the use of melatonin to treat jetlag *after* eastward travel, Suhner et al. found that 5 mg fast release was more efficient than 2 mg slow release and better than 0.5 mg fast release with regard to the beneficial effects of melatonin on sleep (Suhner et al. 1998). Thus, it appears that lower doses can be

as effective as higher doses for circadian phase shift if timed appropriately, whereas higher doses post-travel also exploit the mild hypnotic properties of melatonin. Preflight administration of 0.5 mg with post-flight use of higher doses has been recommended (Arendt 2009). Therefore, a single-dose PRC specific to a lower dose (i.e., 0.5 mg) should be constructed such that lower doses may be effectively used preflight. Ideally, timing of a person's circadian rhythm should be known prior to treatment.

In summary, for phase advance, there appears to be no difference in phase shifting efficacy between our slow and fast release preparations. For phase delay, it is probably best to avoid slow release formulations. As a general principle, we consider that the smallest effective dose should be used. More research is required to establish a melatonin PRC for 0.5-mg doses for RR and for SR formulations especially in the phase delay region of the curve.

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References

Arendt J (2009) Managing jet lag: some of the problems and possible new solutions. Sleep Med Rev 13:249–256

Arendt J, Skene DJ (2005) Melatonin as a chronobiotic. Sleep Med Rev 9:25–39

Arendt J, Bojkowski C, Folkard S, Franey C, Minors DS, Waterhouse JM, Wever RA, Wildgruber C, Wright J (1985) Some effects of melatonin and the control of its secretion in man. In: Evered D, Clark S (eds) Ciba Foundation Symposium 117: photo periodism, melatonin and the pineal. Pitman, London, pp 266–283

Arendt J, Aldhous M, Marks V (1986) Alleviation of jet lag by melatonin: preliminary results of controlled double blind trial. Br Med J (Clin Res Ed) 292:1170

Arendt J, Aldhous M, English J, Marks V, Arendt JH (1987) Some effects of jet-lag and their alleviation by melatonin. Ergonomics 30:1379–1393

Benloucif S, Burgess HJ, Klerman EB, Lewy AJ, Middleton B, Murphy PJ, Parry BL, Revell VL (2008) Measuring melatonin in humans. J Clin Sleep Med 4:1542–1551

Burgess HJ, Revell VL, Eastman CI (2008) A three pulse phase response curve to three milligrams of melatonin in humans. J Physiol 586(2):639–647

Cole RJ, Kripke DF, Gruen W, Mullaney DJ, Gillin JC (1992) Automatic sleep/wake identification from wrist activity. Sleep 15:461–469

Deacon S, Arendt J (1995) Melatonin-induced temperature suppression and its acute phase-shifting effects correlate in a dose-dependent manner in humans. Brain Res 688:77–85

Eastman CI, Burgess HJ (2009) How to travel the world without jetlag. Sleep Medicine Clinics 4(2):241–255

Eastman CI, Gazda CJ, Burgess HJ, Crowley SJ, Fogg LF (2005) Advancing circadian rhythms before eastward flight: a strategy to prevent or reduce jet lag. Sleep 28:33–44

Hack LM, Lockley SW, Arendt J, Skene DJ (2003) The effects of low-dose 0.5 mg melatonin on the free-running circadian rhythms of blind subjects. J Biol Rhythms 18:420–429

Horne JA, Ostberg O (1976) A self-assessment questionnaire to determine morningness–eveningness in human circadian rhythms. Int J Chronobiol 4:97–110

Lewy AJ, Sack RL (1989) The dim light melatonin onset as a marker for circadian phase position. Chronobiol Int 6:93–102

Lewy AJ, Sack RL (1996) The role of melatonin and light in the human circadian system. In: Buijs RM, Kalsbeek A, Romijn HJ, Pennartz CMA, Mirmiran M (eds) Progress in brain research, vol. 111. Elsevier Science, Amsterdam

Lewy AJ, Ahmed S, Jackson JM, Sack RL (1992) Melatonin shifts human circadian rhythms according to a phase-response curve. Chronobiol Int 9:380–392

Lewy AJ, Bauer VK, Ahmed S, Thomas KH, Cutler NL, Singer CM, Moffit MT, Sack RL (1998) The human phase response curve (PRC) to melatonin is about 12 h out of phase with the PRC to light. Chronobiol Int 15:71–83

Lewy AJ, Bauer VK, Hasler BP, Kendall AR, Pires ML, Sack RL (2001) Capturing the circadian rhythms of free-running blind people with 0.5 mg melatonin. Brain Res 918:96–100

Lewy AJ, Emens JS, Sack RL, Hasler BP, Bernert RA (2002) Low, but not high, doses of melatonin entrained a free-running blind person with a long circadian period. Chronobiol Int 19:649–658

Morgenthaler TI, Lee-Chiong T, Alessi C, Friedman L, Aurora RN, Boehlecke B, Brown T, Chesson AL Jr, Kapur V, Maganti R, Owens J, Pancer J, Swick TJ, Zak R (2007) Practice parameters for the clinical evaluation and treatment of circadian rhythm sleep disorders. An American Academy of Sleep Medicine report. Sleep 30:1445–1459

Murphy PJ, Myers BL, Badia P (1996) Nonsteroidal anti-inflammatory drugs alter body temperature and suppress melatonin in humans. Physiol Behav 59:133–139

Ozguner F, Koyu A, Cesur G (2005) Active smoking causes oxidative stress and decreases blood melatonin levels. Toxicol Ind Health 21:21–26

Revell VL, Eastman CI (2005) How to trick mother nature into letting you fly around or stay up all night. J Biol Rhythms 20:353–365

Revell VL, Burgess HJ, Gazda CJ, Smith MR, Fogg LF, Eastman CI (2006) Advancing human circadian rhythms with afternoon melatonin and morning intermittent bright light. J Clin Endocrinol Metab 91:54–59

Sharkey KM, Eastman CI (2002) Melatonin phase shifts human circadian rhtyhms in a placebo-controlled simulated night-work study. Am J Physiol Regulatory Integrative Comp Physiol 282: R454–R463

Suhner A, Schlagenhauf P, Johnson R et al (1998) Comparative study to determine the optimal melatonin dosage form for the alleviation of jet lag. Chronobiol Int 15:655–666

Voultsios A, Kennaway DJ, Dawson D (1997) Salivary melatonin as a circadian phase marker: validation and comparison to plasma melatonin. J Biol Rhythms 12:457–466

Waterhouse J, Reilly T, Atkinson G, Edwards B (2007) Jet lag: trends and coping strategies. Lancet 369:1117–1120

Wright HR, Lack LC, Partridge KJ (2001) Light emitting diodes can be used to phase delay the melatonin rhythm. J Pineal Res 31:350–355