J. Pineal Res. 2002; 32:1–5

Journal of Pineal Research
ISSN 0742-3098

No evidence for a phase delay in human circadian rhythms after a single morning melatonin administration

Wirz-Justice A, Werth E, Renz C, Müller S, Kräuchi K. No evidence for a phase delay in human circadian rhythms after a single morning melatonin administration. J. Pineal Res. 2002; 32: 1–5. © Munksgaard, 2002

Abstract: Although there is good consensus that a single administration of melatonin in the early evening can phase advance human circadian rhythms, the evidence for phase delay shifts to a single melatonin stimulus given in the early morning is sparse. We therefore carried out a double-blind randomized-order placebo-controlled study under modified constant routine (CR) conditions (58 hr bedrest under ~8 lux with sleep 23:00–07:00 hr) in nine healthy young men. A single (pharmacological) dose of melatonin (5 mg p.o.) or a placebo was administered at 07:00 hr on the first morning. Core body temperature (CBT) and heart rate (HR) were continuously recorded, and saliva was collected half-hourly for assay of melatonin. Neither the timing of the mid-range crossing times of temperature (MRCT) and HR rhythms, nor dim light melatonin onset (DLMOn) or offset (DLMOff) were phase shifted the day after melatonin administration compared with placebo. The only change was an altered wave form of the CBT rhythm: longer duration of higher-than-average temperature after melatonin administration. Under the same modified CR conditions we have previously demonstrated a clear phase advance of the above circadian rhythms following a single administration of 5 mg melatonin in the evening. This study's failure to find significant delays to a single administration does not negate other positive findings with multiple doses, which may be necessary for a 'weak zeitgeber'.

Anna Wirz-Justice, Esther Werth, Claudia Renz, Simon Müller and Kurt Kräuchi

Centre for Chronobiology, Psychiatric University Clinic, Basel, Switzerland

Key words: human circadian rhythms, morning melatonin, phase delay

Address reprint requests to Anna Wirz-Justice, Centre for Chronobiology, Psychiatric University Clinic, Wilhelm Kleinstr. 27, CH-4025 Basel, Switzerland.
E-mail: anna.wirz-justice@unibas.ch

Received March 7, 2001;
accepted May 2, 2001.

Introduction

In many species, melatonin is a chronobiotic (Redman, 1997). In humans, melatonin administration phase shifts and/or stabilizes circadian rhythms (reviews: Lewy and Sack, 1997; Arendt et al., 1999). A phase-response-curve (PRC) to melatonin based on administration for four consecutive days showed approximately opposite timing to the PRC to light (Lewy et al., 1992, 1998; Lewy and Sack, 1997). A somewhat different timing was found after a single infusion of melatonin, both advances and delays occurring later (Zaidan et al., 1994).

A 'classic' PRC measures phase shifts following a single exposure to a zeitgeber under free-running conditions. In humans, this has been performed for the PRC to light (Honma et al., 1987; Minors et al., 1991) but is obviously a difficult undertaking. An acceptable variant is to measure entrained phase position under the controlled unmasking conditions of a constant routine (CR) protocol before and after zeitgeber administration (e.g. a single light pulse, Dawson et al., 1993; Jewett et al., 1994; Kräuchi et al., 1997c). In particular, the measurement of multiple circadian rhythms that shift in parallel (such as core body temperature and melatonin) provides convincing evidence that indeed a phase shift in the circadian pacemaker has been achieved.

There is good consensus that a single application of melatonin in the early to late evening can induce a phase advance of about 1 hr, with some dose dependency (e.g. Deacon and Arendt, 1995; Kräuchi et al., 1997a; Wirz-Justice et al., 1999). However, few studies have investigated the putative phase delay after a single application of melatonin in the early morning, and the most controlled study has been equivocal (Deacon et al., 1997). The present design completed a series of identical protocols investigating the phase shifting potential of a single administration of 5 mg melatonin on multiple circadian markers, and focused on the most probable timing for a delay at 07:00 hr (Lewy and Sack, 1997; Lewy et al., 1998).

Methods

Study design

The experiment lasted 22 days. Subjects remained under normal ambulatory conditions and were required to keep their habitual bedtime constant for the entire duration of the study except for two blocks of three nights and 2 days in the laboratory. Here they remained supine under a modified CR protocol for 58 hr (Fig. 1). Day 1 was used to measure acute effects (Kräuchi et al., 2000; Werth et al.,

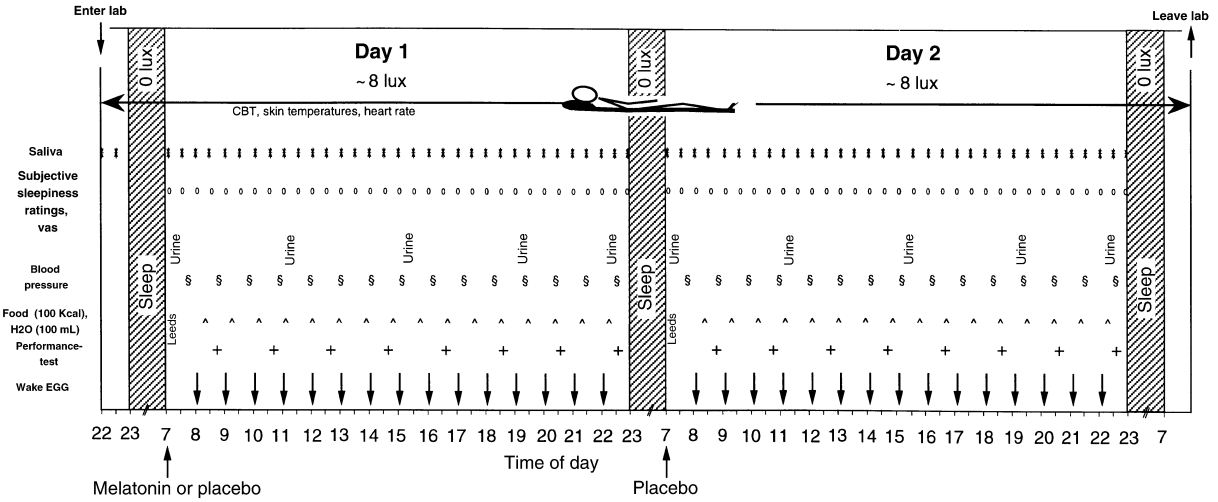

Fig. 1. Protocol of the modified constant routine (CR) protocol lasting 58 hr. Subjects entered the lab in the evening and were allowed three consecutive nights of sleep, with 2 days under supine constant routine (CR) conditions (no change in posture throughout). Melatonin (5 mg p.o.) or placebo was given at 07:00 hr on day 1, and a placebo given at 07:00 hr on day 2. Saliva was collected half-hourly for assay of melatonin; core body temperature (CBT) and skin temperatures and heart rate (HR) were measured continuously throughout. The data from day 1 were used to estimate acute effects (not shown here), data from day 2 for the effects on circadian phase.

2000; Graw et al., 2001), day 2 (presented here) to measure effects on circadian phase position. The design was a placebo controlled, double-blind crossover administration of melatonin (5 mg p.o.) or placebo at 07:00 hr, with placebo also given at 07:00 hr on the second day.

Subjects

Nine male volunteers [aged 23.6 ± 2.8 yr (S.D.); body mass index (BMI) 22.5 ± 2.2] completed both blocks of the study between November 1998 and August 1999 after an adaptation night in the laboratory. All subjects were non-smokers, free of medication and underwent a medical examination prior to study. Neither shift work nor transmeridian travel (>2 time zones) in the month before the study was allowed. Subjects were requested to refrain from alcohol and excessive exercise, and to consume no caffeine products after noon for 3 days before the experiment until 3 days after it was finished. The protocol was approved by the ethical committee of the University of Basel Medical School and subjects gave written informed consent. No deleterious side-effects were registered.

Variables (timing intervals detailed in Fig. 1)

Actimetry documented the subjects' rest-activity cycle and thus compliance with the protocol. They wore a small, non-invasive activity monitor (Actiwatch® Cambridge Neurotechnology, Cambridge, UK) on the non-dominant wrist day and night throughout the entire experimental period (data not shown).

Body and skin temperatures were recorded on a Therm-5000-2 unit. Core body temperature (CBT, probe inserted 10 cm into the rectum) was continuously measured in 2-min-intervals (later collapsed in 30-min bins). The first 2 hr were excluded from the analysis because of postural effects after lying down (Kräuchi et al., 1997b). Thereafter, each curve was smoothed by 1-hr moving averages.

Standard electrocardiogram leads continuously recorded heart rate (HR; later collapsed in 30-min bins). After excluding the first 2 hr, each curve was smoothed by 2-hr moving averages.

Saliva was collected at 30-min intervals during waking. Samples were assayed for melatonin using a highly specific direct double-antibody RIA, validated by gas chromatography–mass spectrometry (GCMS), with an analytical least detectable dose of 0.15 pg/mL and a functional least detectable dose of 0.65 pg/mL (Bühlmann Laboratories, Switzerland; Weber et al., 1997).

The mid-range crossing times (MRCT) of the evening decline and morning rise in CBT and HR were used as circadian phase markers and calculated as detailed in Kräuchi et al. (1997a). For each subject, the minimum value (in °C and beats/min) and the maximum value (in °C and beats/min) of the 24-hr curve were averaged (= midrange value, in °C and beats/min).This value was taken to determine the MRCT for each subject, and averaged across subjects. The CBT and HR minima could not be used as a phase marker due to masking by sleep. Dim light melatonin onset (DLMOn), previously validated under identical conditions (Kräuchi et al., 1997a), was used as a sensitive marker for circadian phase. Morning melatonin offset (DLMOff) was also calculated, but it is a somewhat more problematic circadian phase marker (see Lewy et al., 1999). For each subject, the DLMOn and DLMOff was determined by linear interpolation as the time when melatonin levels crossed a 3-pg/mL threshold.

Statistics

Differences between the melatonin and placebo condition were analysed by two-way analyses of variance for repeated measures (rANOVA) with the repeated factor time and

the nominal factor group (placebo versus melatonin). All *P*-values derived from rANOVA were based on Huynh–Feldt's corrected degrees of freedom, but the original degrees of freedom are reported. Duncan's multiple range test was used for post hoc comparisons. Only significant results ($P < 0.05$) are presented.

Results

Exogenous administration of 5 mg melatonin showed classic pharmacokinetics, with a rapid peak attaining 4199 ± 1377 (S.D.) pg/mL 30 min after melatonin intake and a biological half-life of 49 ± 6 min.

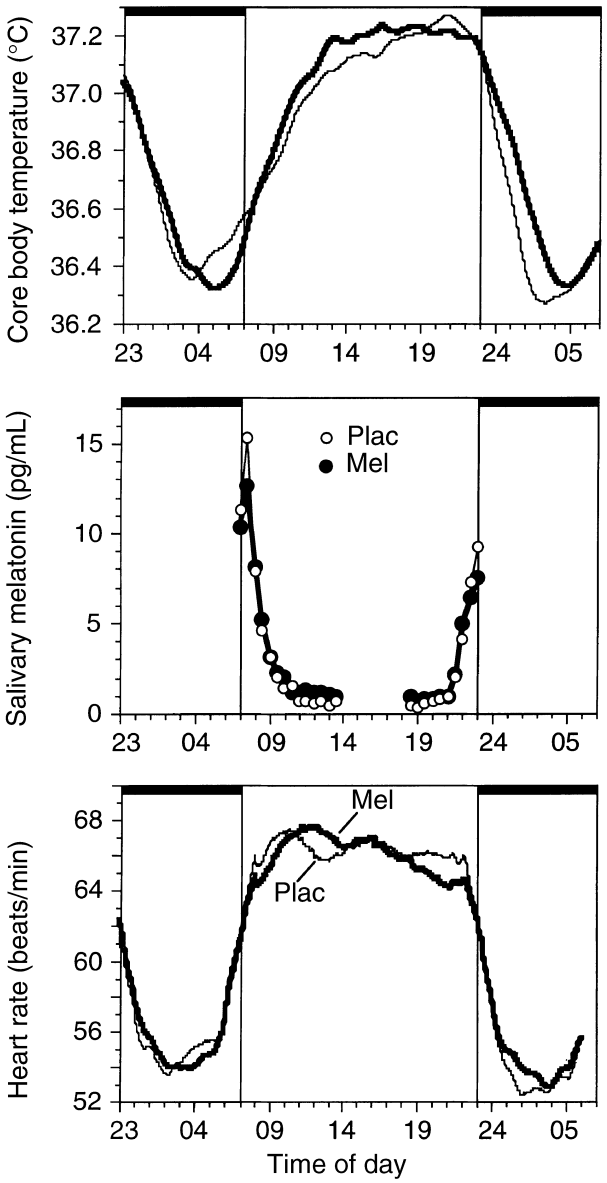

Fig. 2. Means (n = 9) of core body temperature (CBT) and heart rate (HR) (1-hr moving average), melatonin (30-min intervals beginning on night 2 (16 hr after melatonin or placebo administration) through night 3. As subjects remained supine throughout, there were no postural masking effects on CBT and HR after lights out; however, sleep between 23:00 and 07:00 hr did mask timing of the minima. The small peak after administration of placebo pill at 07:00 hr (to both groups) has been previously observed in our studies.

The mean raw data for each variable are presented across the last 32 hr of the 58-hr study, i.e. beginning 16 hr after morning melatonin intake (Fig. 2). In none of the measured circadian rhythms was a statistical difference between placebo and melatonin found. The apparent delay in CBT and HR minima during sleep was non-significant.

None of the circadian phase markers extracted from the CBT, HR, and salivary melatonin rhythms were shifted after morning melatonin administration (Fig. 3). The only significant difference was a longer duration of the circadian peak of CBT.

Discussion

A single administration of 5 mg melatonin at 07:00 hr did not induce the predicted phase delay in any circadian rhythm measured under controlled CR conditions. The caveat required of a negative finding is that only one time point and one dose was tested.

Entrainment to a zeitgeber cycle is theoretically not possible if the phase shift to a single pulse is not of sufficient magnitude. Our failure to demonstrate a phase delay may indicate that the delay region of a melatonin PRC is less important. As humans have a free-running period >24 hr, it is the phase advancing action of evening melatonin that is relevant for mediating entrainment. Although in some species only phase advances to melatonin within a narrow temporal window are found (Redman, 1997), this is not true for all species (e.g. Underwood, 1986; Rajaratnam and Redman, 1997; Sharma et al., 1999).

In contrast to the lack of effect on circadian phase, melatonin administration had immediate neurobiological effects lasting 4–6 hr: psychomotor vigilance was significantly impaired (Graw et al., 2001), daytime sleepiness augmented and theta power in the waking EEG increased (Werth et al., 2000). Our similarly designed CR studies of 5 mg melatonin given later in the day (at 13:00, 18:00 or 20:40 hr) have all increased sleepiness and theta power in the waking EEG (Cajochen et al., 1997).

In the protocol most similar to ours, of a single application (albeit infused), no delay occurred when melatonin was given between 04:00 and 07:00 hr, but a delay was induced when melatonin was given between 12:00 and 15:00 hr (Zaidan et al., 1994). This study was carried out under 150 lux conditions, which now is known to be too high an intensity to provide unmasked melatonin onset times. The authors do note 'we suggest that it is easier to advance than to delay phase with melatonin'. The possible weak zeitgeber characteristic of melatonin has been commented on by Lewy: 'The phase advance after 1 or 2 days of 0.3 mg melatonin administration was less than after 4 days' (personal communication 7.7. 2000). An early study had shown that a single 0.5 mg dose could not advance circadian rhythms, whereas 7 days administration did so (Attenburrow et al., 1995). The most convincing demonstration of entrainment both by delays and advances has been shown in free-running subjects after repeated melatonin administration (Middleton et al., 1997). If melatonin is a weak zeitgeber, then a paradigm of successive daily doses may be

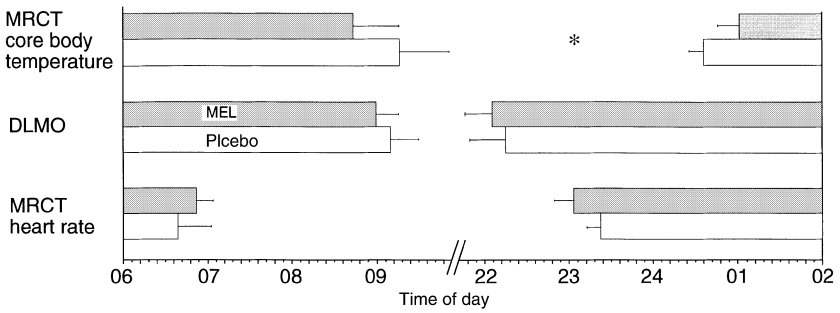

Fig. 3. Effect of melatonin on the three phase markers calculated on day 2 (n = 9). The mid-range crossing times (MRCT) of core body temperature (CBT) and heart rate (HR) increase and dim light melatonin offset (DLMOff) were measured in the morning; the MRCT of CBT and HR decline and dim light melatonin onset (DLMOn) were measured in the evening. As the threshold of 3 pg/mL for DLMOn was not reached by the last time-point at 23:00 hr in two subjects (one at baseline, one after melatonin administration), the average DLMOn is presented for n = 7. When a 3-pg/mL value was interpolated at 23:30 hr in order to calculate for n = 9, the results were similar. All subjects had clear DLMOff values in the morning. The only significant effect was an increased duration of higher-than-average CBT after melatonin (melatonin: 16:13 ± 0.44 hr versus placebo: 15:14 ± 0.53 hr; ANOVA, interaction: morning/evening versus plac/mel, $P < 0.025$).

better suited to reveal the characteristics of a melatonin PRC.

We chose this dose, because many studies, including our own previous CR investigations, have documented an unequivocal phase advance following a single 5 mg intake of melatonin. Also, a dose–response study found greater phase advances after 5 mg than after 0.5 mg (Deacon and Arendt, 1995). It has been argued that such high doses are not 'physiological' and thus invalid. Yet levels of melatonin in the CSF are much higher than in the blood (Shaw et al., 1989; Skinner and Malpaux, 1999), and it is not clear what CNS concentrations are necessary or sufficient for neurobiological response.

We chose this timing with reference to the PRC to melatonin (Lewy and Sack, 1997; Lewy et al., 1998). Here it is argued that exogenous melatonin should overlap the endogenous offset to optimize the magnitude of phase delays. It may also be argued that melatonin levels were still high around noon, which is approximately the time of the crossover from delays to advances (Lewy and Sack, 1997; Lewy et al., 1998). Thus, by overlapping into the phase-advance region in the afternoon, any delay initially induced would have been cancelled out. The same argument should also apply for evening administration of 5 mg of melatonin; here a phase advance is measurable in spite of overlapping into the putative phase-delay region at the end of the night. There is an alternative suggestion, that later timing, after endogenous melatonin had declined, might be more efficacious (as in Zaidan et al., 1994).

In blind persons, sleep onset that is delayed or free-running can be synchronized by advances after daily melatonin intake, yet rarely is the circadian system itself entrained (Arendt et al., 1997; Sack and Lewy, 1997). Recently, a number of free-running blind subjects have been able to entrain with melatonin given after prior determination of their circadian phase position (Lockley et al., 2000; Sack et al., 2000). It may be that high doses (10 mg) may even be initially necessary for such entrainment, although lower doses may subsequently maintain stable phase-position (Sack et al., 2000). In such circadian pathologies, precise timing of application of melatonin is probably crucial. Further controlled studies are required to establish unequivocally whether daily doses of melatonin (for one, three, or more days) can elicit phase delays.

Acknowledgements

This study was funded by the Swiss National Science Foundation (Grant # 31-53698.98). Data have been presented in abstract form at meetings of the SLTBR, SRBR, APSS. We thank Drs C. Cajochen and J. Elliott for constructive comments on an earlier version of the manuscript.

References

Arendt, J., D.J. Skene, B. Middleton, S.W. Lockley, S. Deacon (1997) Efficacy of melatonin treatment in jet lag, shift work, and blindness. J. Biol. Rhythms 12:604–617.

Arendt, J., B. Middleton, B. Stone, D. Skene (1999) Complex effects of melatonin: evidence for photoperiodic responses in humans? Sleep 22:625–635.

Attenburrow, M.E.J., B.A. Dowling, P.A. Sargent, A.L. Sharpley, P.J. Cowen (1995) Melatonin phase advances circadian rhythm. Psychopharmacol. 121:503–505.

Cajochen, C., K. Kräuchi, A. Wirz-Justice (1997) The acute soporific action of daytime melatonin administration: effects on waking EEG power density and alertness. J. Biol. Rhythms 12:636–643.

Dawson, D., L. Lack, M. Morris (1993) Phase resetting of the human circadian pacemaker with use of a single pulse of bright light. Chronobiol. Int. 10:94–102.

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

Deacon, S., J. English, J. Arendt (1997) Sensitivity of the human circadian pacemaker to melatonin timed to phase delay (Abstract). Chronobiol. Int. 14:41.

Graw, P., E. Werth, K. Kräuchi, F. Gutzwiller, C. Cajochen, A. Wirz-Justice (2001) Early morning melatonin administration impairs psychomotor vigilance performance. Behav. Brain Res. 121:167–172.

Honma, K., S. Honma, T. Wada (1987) Phase-dependent shift of free-running human circadian rhythms in response to a single bright light pulse. Experientia 43:1205–1207.

Jewett, M.E., R.E. Kronauer, C.A. Czeisler (1994) Phase-amplitude resetting of the human circadian pacemaker via bright light: a further analysis. J. Biol. Rhythms 9:295–314.

Kräuchi, K., C. Cajochen, D. Möri, P. Graw, A. Wirz-Justice (1997a) Early evening melatonin and S-20098 advance circadian phase and nocturnal regulation of core body temperature. Am. J. Physiol. 272:R1178–R1188.

Kräuchi, K., C. Cajochen, A. Wirz-Justice (1997b) A relationship between heat loss and sleepiness: effects of postural change and melatonin administration. J. Appl. Physiol. 83:134–139.

Kräuchi, K., C. Cajochen, K.V. Danilenko, A. Wirz-Justice (1997c) The hypothermic effect of late evening melatonin does not block the phase delay induced by concurrent bright light in human subjects. Neurosci. Lett. 232:57–61.

Kräuchi, K., M. von Arb, E. Werth, C. Renz, A. Wirz-Justice (2000) Morning melatonin administration and heart rate variability in healthy young men during 58 hours constant bedrest (Abstract). Sleep 23:A113–A114.

Lewy, A.J., R.L. Sack (1997) Exogenous melatonin's phase-shifting effects on the endogenous melatonin profile in sighted humans: a brief review and critique of the literature. J. Biol. Rhythms 12:588–594.

Lewy, A.J., A. Saeeduddin, M.M. Latham-Jackson, R. Sack (1992) Melatonin shifts human circadian rhythms according to a phase response curve. Chronobiol. Int. 9:380–392.

Lewy, A.J., V.K. Bauer, S. Ahmed, K.H. Thomas, N.L. Cutler, C.M. Singer, M.T. Moffit, R.L. Sack (1998) The human phase response curve (PRC) to melatonin is about 12 hours out of phase with the PRC to light. Chronobiol. Int. 15:71–83.

Lewy, A.J., N.L. Cutler, R.L. Sack (1999) The endogenous melatonin profile as a marker for circadian phase position. J. Biol. Rhythms 14:227–236.

Lockley, S.W., D.J. Skene, K. Thapan, J. Wright, J. Arendt (2000) Melatonin administration can entrain the free-running circadian system of blind subjects. J. Endocrinol. 164:R1–R6.

Middleton, B., J. Arendt, B. Stone (1997) Complex effects of melatonin on human circadian rhythms in constant dim light. J. Biol. Rhythms 12:467–475.

Minors, D., J. Waterhouse, A. Wirz-Justice (1991) A human phase–response curve to light. Neurosci. Lett. 133:36–40.

Rajaratnam, S.M., J.R. Redman (1997) Effects of daily melatonin administration on circadian activity rhythms in the diurnal Indian squirrel (*Funambulus pennanti*). J. Biol. Rhythms 12: 339–347.

Redman, J.R. (1997) Circadian entrainment and phase shifting in mammals with melatonin. J. Biol. Rhythms 12:581–587.

Sack, R.L., A.J. Lewy (1997) Melatonin as a chronobiotic: treatment of circadian desynchrony in night workers and the blind. J. Biol. Rhythms 12:595–603.

Sack, R.L., R.W. Brandes, A.R. Kendall, A.J. Lewy (2000) Entrainment of free-running circadian rhythms by melatonin in blind people. N. Engl. J. Med. 343:1070–1077.

Sharma, V.K., M.K. Chandrashekaran, M. Singaravel, R.J. Subbaraj (1999) In the field mouse *Mus booduga* melatonin phase response curves (PRCs) have a different time course and wave form relative to light PRC. J. Pineal Res. 26:153–157.

Shaw, P.F., D.J. Kennaway, R.F. Seamark (1989) Evidence of high concentrations of melatonin in lateral ventricular cerebrospinal fluid of sheep. J. Pineal Res. 6:201–208.

Skinner, D.C., B. Malpaux (1999) High melatonin concentrations in third ventricular cerebrospinal fluid are not due to Galen vein blood recirculating through the choroid plexus. Endocrinology 140:4399–4405.

Underwood, H. (1986) Circadian rhythms in lizards: phase response curve for melatonin. J. Pineal Res. 3:187–196.

Weber, J.M., J.C. Schwander, I. Unger, D. Meier (1997) A direct ultrasensitive RIA for the determination of melatonin in human saliva: comparison with serum levels (Abstract). Sleep Res. 26:757.

Werth, E., K. Kräuchi, P. Graw, C. Renz, D. Mertz, F. Gutzwiller, A. Wirz-Justice (2000) Soporific effects of early morning melatonin administration (Abstract). Sleep 23:A112–A113.

Wirz-Justice, A., K. Kräuchi, C. Cajochen, K.V. Danilenko, C. Renz, J. Weber, M. Martin (1999) Additive and not interactive shifts in dim light melatonin onset after evening melatonin and bright light administration (Abstract). SLTBR Abstracts 11:5.

Zaidan, R., M. Geoffriau, J. Brun, J. Taillard, C. Bureau, G. Chazot, B. Claustrat (1994) Melatonin is able to influence its secretion in humans: description of a phase–response curve. Neuroendocrinology 60:105–112.