# Evening administration of melatonin and bright light: Interactions on the EEG during sleep and wakefulness

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Both the pineal hormone melatonin and light exposure are considered to play a major **SUMMARY** role in the circadian regulation of sleep. In a placebo- controlled balanced cross-over design, we investigated the acute effects of exogenous melatonin (5 mg p.o. at 20.40 hours) with or without a 3-h bright light exposure (5000 lux from 21.00 hours–24.00 hours) on subjective sleepiness, internal sleep structure and EEG power density during sleep and wakefulness in healthy young men. The acute effects of melatonin, bright light and their interaction were measured on the first day (treatment day), possible circadian phase shifts were assessed on the post-treatment day. On the treatment day, the evening rise in subjective sleepiness was accelerated after melatonin and protracted during bright light exposure. These effects were also reflected in specific changes of EEG power density in the theta/alpha range during wakefulness. Melatonin shortened and bright light increased sleep latency. REMS latency was reduced after melatonin administration but bright light had no effect. Slow-wave sleep and slow-wave activity during the first non-rapid eye movement (NREMS) episode were suppressed after melatonin administration and rebounded in the second NREMS episode, independent of whether light was co-administered or not. Self rated sleep quality was better after melatonin administration whereas the awakening process was rated as more difficult after bright light. On the post-treatment day after evening bright light, the rise in sleepiness and the onset of sleep were delayed, independent of whether melatonin was co-administered or not. Thus, although acute bright light and melatonin administration affected subjective sleepiness, internal sleep structure and EEG power density during sleep and wakefulness in a additive manner, the phase shifting effect of a single evening bright light exposure could not be blocked by exogenous melatonin.

KEYWORDS acute and phase shifting effects, circadian rhythms, EEG spectral analysis, sleep quality, sleepiness

# INTRODUCTION

In humans and other mammals, circadian rhythms are generated by the circadian pacemaker located in the

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Present addresses: (CC) Laboratory for Circadian and Sleep Disorders Medicine Brigham Women's Hospital, Harvard Medical School, Boston, USA. (VD) Institute of Physiology, Siberian Branch of the Russian Academy of Medical Sciences Novosibirsk, Russia. suprachiasmatic nuclei (SCN). It is well established that light is the major synchronizer for circadian rhythms for a number of physiologic and behavioral measures including alertness, plasma melatonin, body temperature and the internal sleep structure (Czeisler 1995; Dijk and Czeisler 1995a; Minors *et al.* 1991). Properly timed application of light even at low levels can induce phase shifts in circadian rhythms (Boivin *et al.* 1996; Danilenko *et al.* 1997). There is growing evidence that administration of the pineal hormone melatonin can also phase shift circadian rhythms in humans (Attenburrow *et al.* 1995; Deacon and Arendt 1995; Kräuchi *et al.* 1997b) and other mammals (for reviews see Redman 1997; Lewy *and* Sack 1997).

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Besides their chronobiotic properties, light and melatonin both evoke immediate short lasting effects. Bright light is thermogenic and alerting when applied in the early evening or at night (Badia et al. 1991; Cajochen et al. 1992 for a review see Campbell et al. 1995). In contrast, orally administered melatonin in doses ranging from 0.5 mg to 10 mg causes hypothermia and an increase in subjective sleepiness (Cajochen et al. 1996; Deacon and Arendt 1995; Dollins et al. 1994, Hughes and Badia 1997; Kräuchi et al. 1997a,b, Reid et al. 1996; Tzischinsky and Lavie 1994). Whether these acute effects are independent of circadian time of application, and whether they are a prerequisite for circadian phase shifts, is still unclear. Acute light and melatonin effects are dependent on the actual plasma melatonin and illuminance level. Bright light suppresses plasma melatonin levels in an intensity-related fashion (McIntyre et al. 1989) and light above 2000-2500 lux completely suppresses the nocturnal rise of endogenous melatonin (Lewy et al. 1980). Conversely, elevated rectal temperature produced by all-night bright light is reversed by melatonin infusion (Strassman et al. 1991). This is associated with reduced alertness (Badia et al. 1991, Sack et al. 1992). Bright light and melatonin are therefore considered to antagonize each other, at least in regard to their acute action. It may be therefore possible that the capability of light to induce circadian phase shifts is dependent upon its ability to suppress melatonin secretion. Sleep consolidation and sleep structure are dependent on an appropriate phase relationship between the sleep-wake cycle and the endogenous circadian pacemaker. Many studies have investigated the effects of bright light and melatonin on sleep. Sleep onset latency was longer after bright light application prior to bedtime (Cajochen et al. 1992; Carrier and Dumont 1995; Dijk et al. 1991, Drennan et al. 1989; Sack et al. 1986) whereas bedtime administration of melatonin reduced sleep onset latency (for a review see Zhdanova and Wurtman 1997). However, evening or morning bright light did not affect nonrapid eye movement sleep (NREMS) nor rapid-eye-movement sleep (REMS) in the subsequent nocturnal sleep episode (Bunnell et al. 1992, Cajochen et al. 1992, Dijk et al. 1989; Drennan et al. 1989) and the time course of slow-wave activity (SWA, EEG power density in the 0.75-4.5 Hz range) was only slightly affected after evening bright light exposure (Cajochen et al. 1992). Interestingly, both early evening administration of melatonin (Cajochen et al. 1997b) and morning bright light exposure (Dijk et al. 1989) increased wake-up propensity without modifying NREM sleep homeostasis. These findings are more consistent with the phase shifting effects of bright light and exogenous melatonin, while changes in the sleep (Dijk and Czeisler 1995a) and waking EEG spectra (Cajochen et al. 1996), seen after daytime melatonin administration, are likely to be related to its acute soporific action. The present experiment was aimed at documenting:

1 The immediate effects of a single evening application of melatonin or placebo, with or without bright light exposure, on subjective sleepiness and EEG activity during wakefulness and sleep.

- 2 It was hypothesized that the phase delay induced by evening bright light would generate a phase angle difference between the imposed sleep/wake cycle and the circadian rhythm of core body temperature strong enough to affect internal sleep structure on the post-treatment night.
- **3** It was expected that contemporaneous melatonin application would reverse bright light's immediate and perhaps also the phase delaying effects. Results on melatonin and bright light's interaction on core body temperature, the phase marker in this study, were recently reported in Kräuchi *et al.* (1997a).

# METHODS

# Subjects

Ten healthy male students  $(27 \pm 5 \text{ years})$  were paid to participate in the study to which they gave their informed consent. The experimental protocol was accepted by the Human Research Committee of the Department of Medicine, University of Basel, Switzerland. Medical disorders were screened by history and physical examination. The subjects were free of self-reported sleep complaints, were neither extreme morning nor evening types (defined by scores = <14 and > = 21 in the Torsvall-Åkerstedt morning-evening questionnaire). Further exclusion criteria included: no shift work nor transmeridian travel within one month prior to the study, no smoking, medication or drug use. Prior to the study the subjects were instructed to maintain a regular sleep times between 23.00 to 07.00 hours for at least one week. This was verified by activity monitoring of the subjects' rest-activity cycle (Gaehwiler Actigraph, Zurich, Switzerland) and a daily sleep diary. During the experiment caffeine was limited to one morning cup of coffee per day, and no alcohol consumption was allowed. Each subject spent an adaptation night in the sleep laboratory to test his ability to sleep in a new environment and to exclude subjects with sleep apnoea. One subject was excluded from the analysis due to technical problems and one subject because of sleep onset REM. All subjects completed the study without any complaint.

# Design

A placebo-controlled 4-week cross-over design was conducted. Each test period comprised of one treatment day [melatonin (5 mg, p.o.) or placebo with or without a subsequent bright light exposure (5000 lux) in randomized order] and one post-treatment day with placebo. This schedule was repeated for 4 consecutive weeks so that the start of each test period was separated by 7 days. The subjects were not informed on which day they would receive melatonin, placebo or bright light. Subjects wore sunglasses in the morning before they reported to the laboratory at 10.00 hours. They remained in a room (<150 lux) where they ate lunch at 11.30 hours. After electrode attachment, they remained supine and awake in bed in a sound-attenuated chronobiology room (temperature 22°C, humidity 60%, light <10 lux) from 14.00 hours to 24.00 h, cared for by trained personnel. During this 10-h period, an abbreviated

constant routine protocol was carried out as used in our previous studies (Cajochen et al. 1996, 1997b, Kräuchi et al. 1997a,b). Isocaloric meals (sandwiches) and water were given every hour in order to meet energy requirements. Saliva melatonin samples were collected at half-hourly intervals, together with subjective mood and sleepiness ratings (VAS), the Karolinska Sleepiness Scale (KSS) and the Accumulated Time with Sleepiness Scale (ATASS; both described in Gillberg et al. 1994). A 6-min waking EEG was recorded at 20.00 hours (pre-intervention recording) and 22.00 hours (post-intervention recording). Melatonin (5 mg, p.o.) or placebo was administered at 20.40 hours followed either by a 3-h bright light exposure from 21.00 hours until 24.00 (5000 lux at eye level, 60 cm in front of the apparatus, J. Kriesi, Switzerland) or no bright light exposure (<10 lux). The sleep period was scheduled from 24.00 to 07.30 hours during which the lights were switched off. The Leed's Sleep Evaluation Questionnaire was completed 10 min after awakening. After waking up in the morning, subjects remained under <150 lux. Breakfast was at 08.00 h, lunch at 11.30 hours, and the protocol was repeated at 14.00 hours.

#### Sleep and waking EEG recording and analysis

During sleep and waking EEG recordings, two EEG signals (C3-A2, C4-A1), two EOG signals, and one EMG and ECG signal were recorded on polygraph paper (Nihon Kohden 4418 G, Japan; 10 mm/s paper speed). Self-adhesive silver-chloride electrodes (Sensor Medics, Skin Electrode Kit) were used for placements on the skin, and gold electrodes (Grass Instruments) fixed with collodion for placements on the scalp. The electrode impedance was measured on a regular basis to ensure that the levels were below 5K Ohm. The EEG signals were high-pass filtered with a time constant of 1.0s and low pass filtered at 35Hz (12 dB/octave), on-line digitized at a sampling rate of 128 Hz, and subjected to spectral analysis by a fast Fourier routine. Power spectra were computed for consecutive 4-s epochs and 0.25 Hz frequency bins by applying a Kaiser-Bessel window. Values of adjacent frequency bins were collapsed into 0.5-Hz bins in the range of 0.25-5.0 Hz, and into 1-Hz bins in the range of 5.25-25.0 Hz. In connection with the on-line calculation of the 4-s spectra, a time mark was written on the polygraph paper at 20-s intervals to synchronize the sleep scoring with the spectral data.

# Sleep EEG

Every five consecutive 4-s, EEG spectra were collapsed into 20-s spectra off-line. In this averaging procedure, 4-s epochs contaminated with artifacts were automatically eliminated by an artifact detection routine. The sleep EEGs were scored manually according to established criteria (Rechtschaffen and Kales 1968) per 20 s and synchronized with the power spectra. NREMS – REMS cycles were defined according to Feinberg and Floyd 1979. For cycles 2–4 the first 20-s epoch following the last REM sleep epoch was defined as the onset of the cycle.

#### Waking EEG

Each recording lasted for 6 min, during which the subjects were instructed to relax, to watch a small picture on the wall, to keep their eyes open and to avoid movement. These instructions were intended to maximize signal quality. At incipient behavioral signs of sleep during the task (lowering of eye lids, drowsiness, gazing or rolling eyes), the instructions were repeated or the subject was verbally entertained. In connection with the on-line calculation of the 4-s spectra, a time mark was written on the polygraph paper in 4-s intervals in order to allow synchronization with EEG spectra. All records of the waking EEG (C3/A2) were visually inspected on a 4-s basis. 4-s epochs with eye blinks or artifacts due to body movements, slow eye movements, and sweating were excluded from subsequent analyses.

#### Statistics

The statistical packages SAS® (SAS® Institute Inc., Cary, NC, USA) and Statistica® (StatSoft, Inc., Tulsa, OK, USA) were used. Sleep stages were expressed in minutes and as percentage of total sleep episode duration or percentage of NREMS-REMS cycle duration. For some sleep variables (e.g. sleep latency, REMS latency) and EEG power density, data were log transformed in order to meet the requirements of a normal distribution. Data were first averaged per subject, in order that all subjects would contribute with equal weight, and then the data were averaged over all subjects. The four conditions [placebo (PLAC), melatonin (MEL), bright light (LIGHT) and melatonin and bright light (ML)] represent in fact a composite of two different treatments namely melatonin and bright light. In other words, each lighting condition (i.e. with or without bright light) was studied with contemporaneous administration either of MEL or PLAC. The main effects of MEL and LIGHT, as well their interaction (MEL\*LIGHT) could be analyzed by two way ANOVAS for repeated measures (rANOVA). The main factors, MEL and LIGHT consist of two levels containing pooled values (MEL: placebo with light and placebo without light vs. MEL with light and MEL without light, and LIGHT: placebo without light and MEL without light vs. placebo with light and MEL with light). If there were several measurements of the same variable each day, a three-way rANOVA with an additional factor 'Time' was implemented. All p-values derived from rANOVAS were based on Huynh-Feldt's (H-F) corrected degrees of freedom, but the original degrees of freedom are reported. When the F-ratio proved significant, post hoc comparisons using Duncan's multiple range test were performed.

# RESULTS

#### Mid-range crossing time of core body temperature

For each subject, the mid-range crossing time of the evening core body temperature (CBT) decline was determined according to the method developed by Kräuchi *et al.* (1997b). Mean mid-range crossing times of CBT on the post-treatment day for the

#### Table 1

Mid-range crossing times of core body temperature (MRC-Time) on the post-treatment day for the placebo, light, melatonin and the combined light and melatonin condition

Condition	MRC-time	
Placeo	$23:53 \pm 28'$	
Light	24:39±39'*	
Melatonin	$23:38 \pm 32'$	
Melatonin & Light	$24:20 \pm 30$ *	

Values are expressed as a time of day (h), mean values  $\pm$  SE. (\*) indicates a significant LIGHT factor, for further statistics see Kräuchi *et al.*, 1997a.

four different conditions (placebo, melatonin, bright light and melatonin and bright light) are depicted in Table 1. As reported by Kräuchi *et al.* (1997a), a two-way ANOVA for repeated measures revealed significant MEL and LIGHT factors on the treatment day and a significant LIGHT factor on the posttreatment day (for statistics see Kräuchi *et al.* 1997a). No significant interaction MELxLIGHT was found on either day. *Post hoc* statistics showed that the CBT mid-range crossing time on the post-treatment day was significantly delayed in both conditions (LIGHT and ML) that received evening light on the treatment day.

#### Subjective sleepiness

The time course of subjective sleepiness ratings (KSS) across the treatment (TD) and post-treatment day (PTD) is illustrated in Fig. 1A. During TD, exogenous evening melatonin significantly enhanced sleepiness from 22.00 to 23.30 hours, whereas bright light significantly reduced sleepiness immediately after 21.00 hours (for statistics see Fig. 1 legend of Fig. 1A, *post-hoc* comparisons MEL, PLAC vs. LIGHT, ML and MEL, ML vs. LIGHT, PLAC; *P* at least <0.05). The time course in the ML group was similar as in the PLAC group. On the PTD, bright light delayed the evening rise in sleepiness indicated by a significant main factor LIGHT and TIME × LIGHT interaction (Fig. 1A legend). Sleepiness ratings for both light groups (LIGHT and ML) were significantly lower after 19.30 hours (*post hoc* comparisons MEL, PLAC vs. LIGHT, ML; *P*<0.05).

#### EEG power density during wakefulness

For each subject and frequency bin, waking EEG power density recorded at 22.00 hours was expressed relative to corresponding values recorded at 20.00 hours (Fig. 1B). This transformation reduces variability due to effects other than the intervention itself (i.e. melatonin, bright light) and permits standardization to a preintervention value. Pre-intervention EEG power density on the TD did not differ between conditions (MEL and LIGHT) in any frequency bin (data not shown).

Statistical analysis comparing waking EEG power densities after MEL, LIGHT, PLAC and ML to its pre-intervention values on TD revealed that MEL and LIGHT particularly affected frequencies in the theta/low alpha range (5.25–9.00 Hz) and higher alpha range (9.25–12.00 Hz; see Cajochen *et al.* 1997a and Fig. 1B). Therefore, relative EEG power values were averaged in the aforementioned frequency bands (Fig. 2). MEL significantly enhanced EEG power in the 5.25–9.00 Hz band ( $F_{1,7}$ =6.3, *P*<0.05) whereas the LIGHT induced decrease was not significant. However, EEG activity in the higher alpha band (9.25–12Hz) tended to be lower during bright light exposure (factor LIGHT:  $F_{1,7}$ =5.4, *P*=0.1; Fig. 2).

On the PTD, significant EEG changes between conditions were already detected at 20.00 hours (data not shown). Lower EEG power density after LIGHT was found in the following frequency bins: 4.25–4.5Hz ( $F_{1,7}$ =7.2, P<0.03) and 4.75–5Hz ( $F_{1,7}$ =5.8, P<0.05) and a tendency to lower values in the frequency bin 8.25–9Hz (P<0.1). In order to perform consistent analysis on the PTN as on TN, relative EEG power density at 22.00 hours were computed (as percentage of power density at 20 h) even though the 2000 *h*-values differed significantly between conditions. In this analysis, MEL and LIGHT had no significant effect on relative EEG power density at 22.00 hours.

# **Sleep variables**

#### Entire sleep episode

Mean values of EEG based sleep variables averaged over the entire sleep episode on the TN and PTN are summarized in Tables 2 A and 2B. In order to assess acute effects of melatonin and light on sleep, for each variable a two way rANOVA with the factors MEL and LIGHT was performed on the TN (see Table 3). On TN, sleep latency was significantly prolonged after LIGHT and reduced after MEL (Fig. 3, for statistics see Table 2 A and Table 3). The amount of stage 1 and arousal within sleep, a measure of superficial sleep, was significantly reduced after MEL. For none of the sleep variables was a significant interaction term MEL × LIGHT found. The same analysis performed on values corrected for sleep episode duration revealed similar results (data not shown).

To assess possible after-effects of MEL and LIGHT on sleep, a two-way rANOVA with the factors MEL and LIGHT was performed on the PTN for each sleep variable. The significant LIGHT effect for sleep latency persisted on PTN ( $F_{1,7}=5.4$ , P<0.05, Fig. 3). *Post-hoc* comparisons revealed significant longer sleep latencies for the conditions where light was administered (MEL, PLAC vs. LIGHT, ML P<0.05). Other sleep variables did not differ significantly.

#### Subjective sleep variables

Changes in subjectively rated sleep latency on the Leeds Sleep Evaluation Questionnaire in the morning after the TN and PTN, paralleled the changes seen in the objective measure of sleep latency (Fig. 3). In the morning after the TN, subjects reported significant longer sleep latencies after bright light (for rANOVAS see Table 3, *post hoc* comparisons MEL, PLAC vs.



**Figure 1.** Panel A, *left*: time course of subjective sleepiness as rated on the Karolinska Sleepiness Scale (KSS) after the intake of a single dose of 5 mg melatonin ( $\blacksquare$  MEL) and placebo ( $\bigcirc$  PLAC) at 20.40 hours and after single dose of 5 mg melatonin ( $\square$  MEl & LIGHT) and placebo ( $\bigcirc$  LIGHT) followed by a bright light exposure from 21.00 to 24.00 hours on the treatment day. Three-way rANOVA (factors: MEL, LIGHT and TIME): LIGHT:  $F_{(1,7)}=5.2$ ; *P*<0.05; TIME:  $F_{(11,77)}=11.2$ ; *P*<0.004; MEL × TIME:  $F_{(11,77)}=5.9$ ; *P*<0.002; LIGHT × TIME:  $F_{(1,7)}=2.4$  *P*<0.03, other factors were not significant. Panel A, *Right:* time course of subjective sleepiness (KSS) during the post-treatment day where in all groups a placebo pill was administrered at 20.40 hours. Three-way rANOVA (factors: MEL, LIGHT and TIME): LIGHT:  $F_{(1,7)}=9.5$ ; *P*<0.02; TIME:  $F_{(11,77)}=13.0$ ; *P*<0.002; LIGHT × TIME:  $F_{(11,77)}=2.9$  *P*<0.02, other factors were not significant. Panel B Waking EEG power density (%) in single frequency bins (0.75–20 Hz) at 22.00 hours on the treatment day and post-treatment day. For each frequency bin, subject and group, the values were expressed as percentage of the corresponding value at 20.00 hours. Log transformed values were averaged within groups and re-transformed for plotting [mean, + or - SEM].

LIGHT, ML *P*<0.01) and shorter sleep latencies after MEL (*post hoc* comparisons MEL, ML vs. LIGHT, PLAC *P*<0.02). As found for the objective measure, subjectively rated sleep latency remained significantly longer on the PTN after bright light (rANOVA factor LIGHT:  $F_{1,7}$ =5.6, *P*<0.05, *post hoc* 

comparison MEL, PLAC vs. LIGHT, ML P<0.05). The subjects rated their sleep quality significantly better after melatonin ingestion on the TN (Table 3, *post hoc* comparisons MEL, ML vs. LIGHT, PLAC P<0.01). Sleep quality in the PTN did not differ. The awakening process after the TN was



**Figure 2.** Acute effect of melatonin, bright light and the combined application of both treatments on EEG power in the 5.25–9 Hz and the 9.25–12 Hz frequency bands. For each frequency band, subject and group, the values were expressed as percentage of the corresponding value at 20.00 hours [mean values (min) + or - SEM]. n.s: no significant difference; P<0.05: a significant difference and P=0.1: trend.

rated as significantly more difficult after bright light exposure and tended to be easier after melatonin ingestion (Table 3, *post hoc* comparison MEL, PLAC vs. LIGHT, ML P<0.05). The 'feeling on awakening' did not differ significantly between conditions on either the TN or PTN.

#### NREMS-REMS cycles

In order to inspect the temporal evolution of different sleep variables more closely, NREMS-REMS cycles across each sleep episode were defined according to the above mentioned criteria (Tables 2 A and B).

A two-way rANOVA with the factors MEL and LIGHT was performed on percentage-values (% of sleep cycle duration) for each cycle separately. Melatonin reduced the duration of the first NREMS episode significantly (MEL effect:  $F_{1,7}=8.7$ , P < 0.03) and tended to increase the first REMS episode duration (MEL effect:  $F_{1,7} = 4.5$ , P = 0.07). Furthermore, the percentage of REMS in the first REMS episode was significantly enhanced (MEL effect:  $F_{1,7}=9.8$ , P<0.02). In NREMS episode 2, slow wave sleep was significantly enhanced after MEL (MEL effect:  $F_{1,7}=9.8$ , P<0.02) at the expense of stage 2 (MEL effect:  $F_{1,7}$ =5.6, P=0.05), stage 1 ( $F_{1,7}$ =6.9, P<0.04) and the combined arousal measure (wake + stage 1 + movement time,  $F_{1,7}=9.2$ , P < 0.02). There were no significant LIGHT effects in the TN except for the arousal measure within cycle 3, where significant higher values were observed (LIGHT effect:  $F_{1,7} = 13.6$ , P < 0.007). For none of the sleep variables in none of the cycles, was a significant interaction term MEL × LIGHT found.

During the PTN the above-mentioned effects disappeared. No significant changes between conditions were found for any of the sleep variables (P>0.07, rANOVA).

#### EEG power density

Since we expected short-term effects of melatonin, pooled EEG power spectra for the factor MEL and LIGHT were averaged over each NREMS episode separately (Fig. 4). Since one subject

did not complete a fourth NREMS episode, the analysis was confined to the first three NREMS episodes.

Melatonin significantly suppressed EEG power density in the first NREMS episode in the following frequency bins: 0.25-0.5, 1.25-2 and 3.25-3.5 Hz was (rANOVA factor MEL, P<0.05 for each bin separately). In addition, LIGHT significantly affected EEG power density in the frequency bin from 13.25 to 14 and 15.25-16 Hz (P<0.05 for each bin). In contrast to the first NREMS episode, EEG power density in the lower frequency bins (0.25-3 Hz) of NREMS episode 2 was significantly enhanced after MEL and suppressed in the bins from 14.25 to 17 and 18.25-19 Hz (raNOVA P<0.05 for each bin separately). No significant LIGHT effects were found in the second NREMS episode. Except for a significant MEL effect in the frequency bins between 16.25 and 18 Hz (factor: MEL raNOVA P<0.05 for each bin separately) no MEL or LIGHT effects were found for the third NREMS episode.

The same analysis performed for the REMS episodes of the TN or either NREMS and REMS episodes in the PTN, revealed no consistent significant changes neither for the factor MEL nor LIGHT nor the interaction term MEL × LIGHT (data not shown).

# Dynamics of slow-wave activity

To investigate time dependent spectral EEG changes, slowwave activity (SWA), defined as EEG power density in the frequency range of 0.75–4.5 Hz, was percentilized into 10 parts for each NREMS episode and into 2 parts for each REMS episode. Relative SWA values (percentage of mean SWA during NREMS of the entire sleep episode) were calculated and log transformed for each subject (Figs 5 and 6). A three-way rANOVA with the factors MEL, LIGHT and part (10 levels) was carried out for each NREMS episode (1–3) separately.

In the first NREMS episode of the TN (Fig. 5), a significant main factor MEL ( $F_{1,7}$ =19.9, P<0.003) and part was found ( $F_{9,63}$ =34.0, P<0.0001). Other factors or interaction terms did not reach significance. *Post hoc* comparisons on pooled SWA

Sleep variable	Condition	Sleep episode	Cycle		
			1	2	3
Total sleep time	Placebo	423.5 (5.0)			
	Light	418.8 (6.7)			
	Melatonin	426.7 (6.1)			
	Mel & light	427.5 (4.2)			
Sleep efficiency	Placebo	93.9 (1.1)			
	Light	92.8 (1.5)			
	Melatonin	94.6 (1.3)			
	Mel & light	94.9 (0.9)			
Sleep latency	Placebo	12.2 (5.3)*#			
	Light	18.5 (6.2)*#			
	Melatonin	4.9 (0.7)*#			
	Mel & light	6.6 (0.9)*#			
REM sleep latency	Placebo	59.2 (3.9)#			
	Light	61.6 (3.9)#			
	Melatonin	53.1 (2.7)#			
	Mel & light	58.5 (4.8)#			
Arousal	Placebo	47.7 (2.8)*	2.3 (0.6)	10.1 (1.6)	11.1 (2.4)*
	Light	54.1 (2.7)*	3.5 (0.4)	10.8 (2.2)	16.8 (1.9)*
	Melatonin	48.5 (7.2)*	2.7 (0.7)	6.3 (1.7)	8.4 (1.1)*
	Mel & light	57.4 (7.3)*	3.8 (0.7)	8.1 (2.0)	12.1 (1.7)*
Stage 1	Placebo	32.5 (3.8)*	1.6 (0.5)	7.0 (1.3)#	8.3 (2.3)
	Light	40.1 (1.9)*	2.3 (0.4)	8.0 (1.8)#	11.3 (1.4)
	Melatonin	29.3 (3.4)*	1.7 (0.4)	4.3 (1.3)#	5.4 (0.7)
	Mel & light	41.2 (4.1)*	3.0 (0.6)	5.8 (1.1)#	9.5 (1.4)
Stage 2	Placebo	225.1 (9.9)	25.3 (2.6)	47.0 (5.4)#	66.4 (8.1)
	Light	216.0 (9.1)	29.0 (4.2)	57.5 (6.1)#	63.5 (4.5)
	Melatonin	220.9 (11.3)	25.1 (3.6)	47.0 (5.0)#	62.3 (2.9)
	Mel & light	211.8 (9.7)	27.5 (2.6)	38.8 (3.8)#	64.0 (6.1)
Slow wave sleep	Placebo	69.8 (6.9)	33.9 (3.5)	23.0 (6.2)#	11.1 (2.9)
	Light	66.4 (8.5)	32.0 (3.8)	24.5 (6.2)#	6.3 (2.5)
	Melatonin	75.0 (6.8)	29.0 (4.8)	35.5 (4.3)#	6.1 (2.1)
	Mel & light	75.3 (7.7)	29.5 (4.0)	38.3 (3.8)#	6.1 (3.3)
REM sleep	Placebo	96.0 (8.1)	9.3 (1.5)#	21.5 (3.9)	24.6 (5.8)
-	Light	96.3 (6.8)	13.9 (2.8)#	27.0 (3.4)	28.9 (2.5)
	Melatonin	101.5 (9.7)	15.3 (4.6)#	20.2 (3.5)	22.8 (3.9)
	Mel & light	99.3 (9.6)	15.9 (2.7)#	24.1 (2.2)	25.0 (6.4)
NREM sleep episode	Placebo		59.2 (3.9)#	74.6 (3.4)	79.8 (5.3)
duration	Light		61.6 (3.9)#	85.4 (6.1)	78.8 (3.7)
	Melatonin		53.1 (2.7)#	84.3 (4.4)	71.0 (3.6)
	Mel & light		58.5 (4.7)#	80.1 (3.7)	76.0 (3.9)
REM sleep episode	Placebo		11.6 (1.6)	27.1 (5.2)	33.3 (8.2)
duration	Light		16.7 (2.9)	34.3 (2.8)	36.8 (3.0)
	Melatonin		19.0 (5.0)	24.7 (3.9)	28.6 (4.5)
	Mel & light		18.1 (2.9)	29.0 (2.1)	31.2 (7.2)

**Table 2A**Sleep variables calculated over entire sleep episode for treatmet (table 2A) and post-treatment night (Table 2B) of placebo, light,melatonin and the combined light and melatonin

Sleep variables (mean values  $\pm$  SE;) are expressed as min, except for sleep efficiency (%). Arousal=waking (after sleep onset) × stage 1+movement time. (\*) indicates a significant LIGHT factor, (#) a significant MEL factor; for further statistics see Table 2.

values with MEL vs. without MEL revealed significant lower SWA values after MEL during the first NREMS episode (P<0.004). Analyses of the second NREMS episode showed also a significant main factor MEL ( $F_{1,7}$ =18.8, P<0.005) and a significant main factor part ( $F_{9,63}$ =35.7, P<0.0001). Post hoc comparisons on pooled SWA values with MEL vs. without MEL showed significant higher SWA values after MEL during the second NREMS episode (P<0.004). For NREMS episode 3, a significant main factor LIGHT ( $F_{1,7}=9.6$ , P<0.02) and part was found ( $F_{9,63}=20.4$ , P<0.0001). Post hoc comparisons on pooled SWA values with LIGHT vs. without LIGHT showed significant lower SWA values after LIGHT during the third NREMS episode (P<0.02). During the PTN, the above mentioned effects on SWA disappeared except for the time effect (Fig. 6). Neither the main factor MEL nor LIGHT nor their interaction term reached significance.

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# Table 2B Post-treatment night

Sleep variable	Condition	Sleep episode	Cycle		
			1	2	3
Total sleep time	Placebo	416.2 (3.6)			
	Light	405.2 (9.6)			
	Melatonin	417.0 (7.5)			
	Mel & light	410.0 (5.3)			
Sleep efficiency	Placebo	92.4 (0.8)			
	Light	89.9 (2.2)			
	Melatonin	92.5 (1.7)			
	Mel & light	91.0 (1.2)			
Sleep latency	Placebo	17.1 (4.7)*			
1 2	Light	29.0 (10.3)*			
	Melatonin	14.5 (4.7)*			
	Mel & light	23.4 (7.6)*			
REM sleep latency	Placebo	57.6 (2.4)			
	Light	53.6 (7.3)			
	Melatonin	52.9 (2.3)			
	Mel & light	56.8 (3.0)			
Arousal	Placebo	57.5 (2.9)	3.8 (1.1)	8.1 (2.0)	13.0 (2.1)
	Light	57.1 (6.1)	5.5 (2.1)	10.4 (2.0)	16.0 (2.7)
	Melatonin	54.8 (7.3)	2.6 (0.4)	6.5 (1.1)	13.3 (2.9)
	Mel & light	53.1 (6.0)	2.2 (0.6)	9.3 (1.0)	12.0 (3.1)
Stage 1	Placebo	40.5 (2.2)	2.8 (1.1)	5.5 (1.7)	9.2 (1.6)
	Light	40.3 (4.9)	3.6 (1.6)	7.8 (2.0)	12.0 (2.7)
	Melatonin	35.6 (4.2)	1.9 (0.4)	9.7 (2.8)	9.7 (2.8)
	Mel & light	35.9 (2.8)	1.3 (0.5)	6.5 (0.9)	8.3 (2.1)
Stage 2	Placebo	214.7 (11.0)	26.1 (2.0)	45.4 (5.4)	63.2 (6.3)
	Light	207.7 (10.9)	31.4 (9.5)	52.5 (6.5)	61.1 (4.0)
	Melatonin	206.3 (5.6)	25.8 (2.3)	40.3 (4.0)	57.0 (4.7)
	Mel & light	214.8 (11.2)	24.2 (2.8)	47.7 (5.0)	57.6 (6.9)
Slow wave sleep	Placebo	73.9 (6.9)	33.5 (1.6)	27.5 (5.3)	9.9 (3.0)
*	Light	73.4 (4.5)	38.9 (5.4)	24.3 (3.3)	8.8 (3.0)
	Melatonin	81.8 (7.4)	27.8 (2.4)	38.6 (5.7)	7.8 (3.8)
	Mel & light	73.8 (8.2)	32.8 (4.6)	26.6 (5.5)	7.5 (3.4)
REM sleep	Placebo	87.1 (8.0)	12.0 (2.7)	18.3 (2.4)	31.2 (3.8)
	Light	83.8 (8.3)	12.8 (1.7)	26.8 (3.7)	22.4 (3.8)
	Melatonin	93.4 (7.5)	15.1 (3.3)	21.2 (3.0)	25.7 (5.1)
	Mel & light	85.5 (8.0)	10.3 (2.1)	20.4 (2.0)	22.5 (5.4)
NREM sleep episode	Placebo		79.8 (5.3)	57.6 (2.4)	76.7 (5.3)
duration	Light		71.4 (13.7)	78.4 (6.7)	72.5 (2.7)
	Melatonin		52.9 (2.3)	80.6 (4.2)	69.1 (5.6)
	Mel & light		56.6 (3.0)	78.1 (3.5)	71.0 (8.0)
REM sleep episode	Placebo		17.8 (4.2)	22.5 (3.6)	40.9 (5.2)
duration	Light		17.3 (2.6)	35.7 (5.0)	35.8 (6.9)
	Melatonin		18.5 (3.6)	30.0 (3.7)	34.8 (7.4)
	Mel & light		12.8 (2.6)	25.8 (2.4)	28.7 (7.0)

# DISCUSSION

The present study shows that bright light exposure or melatonin administration in the evening immediately affects the EEG during wakefulness and sleep, subjective sleepiness and perception of the previous night's sleep. Contemporaneous administration of bright light with melatonin results in mutual influence on their acute action. We suggest that light and melatonin given at these times may affect each other in an additive manner, since for none of the variables a significant interaction term was found. This is in accordance to the acute effects on core body temperature recently reported in Kräuchi *et al.* (1997a). Moreover, the single evening bright light pulse induced a phase delay in the mid-range crossing time of core body temperature on the post-treatment day (Kräuchi *et al.* 1997a). The light pulse changed the phase angle between the circadian system and the imposed sleep-wake rhythm which was reflected in a prolonged latency to sleep and a delay in the evening rise of subjective sleepiness. The phase delay occurred independently of whether melatonin or placebo was

Objective Sleep variable	LIGHT	MEL	$LIGHT \times MEL$
	$(F_{1,7})$	$(F_{I,7})$	$(F_{1,7})$
Total sleep time	n.s.	n.s.	n.s.
Sleep efficiency	n.s.	n.s.	n.s.
Sleep latency	8.4; <i>P</i> <0.03↑	7.6; <i>P</i> <0.03↓	n.s.
REM sleep latency	n.s.	8.7; <i>P</i> <0.03↓	n.s.
Stage 1	17.7; <i>P</i> <0.005↑	n.s.	n.s.
Stage 2	n.s.	n.s.	n.s.
Slow wave sleep	n.s.	n.s.	n.s.
REM sleep	n.s.	n.s.	n.s.
Arousal	17.7; <i>P</i> <0.005↑	n.s.	n.s.
Subjective sleep variable	LIGHT	MEL	$LIGHT \times MEL$
Sleep quality	n.s.	5.1; <i>P</i> <0.05↑	n.s.
Sleep latency	11.4; <i>P</i> <0.01↑	8.9; <i>P</i> <0.02↓	n.s.
Awakening process	7.1; <i>P</i> <0.03↓	$3.5; p = 0.08\uparrow$	n.s.

**Table 3** Results of two-way ANOVA forrepeated measures with the factor brightlight (LIGHT) and melatonin (MEL) andthe interaction factor LIGHT  $\times$  MEL forobjective and subjective sleep variablesaveraged over the entire sleep episode



Figure 3. Effects of melatonin, bright light and the combined application of both treatments on sleep latency (first occurrenc of stage 2) and subjective sleep latency as rated on the Leeds Sleep Evaluation Questionnaire, 10 min after lights on for the treatment and post-treatment night [mean values (min) + SEM]. Lower values in the subjective ratings indicate shorter sleep latencies. Statistics in the text.

administered, i.e. whether exogenous melatonin was present or not.

# Subjective sleepiness

On the treatment day, light decreased and melatonin increased subjective sleepiness. This finding does not support the concept

that the alerting action of nocturnal bright light exposure may be exclusively mediated via suppression of endogenous melatonin levels (Badia *et al.* 1991, Sack *et al.* 1992), since we did not find a significant interaction term between light and melatonin. On the post-treatment day, the time course of the evening rise in subjective sleepiness was delayed in both groups treated with light which together with the delay in the core





**Figure 4.** Effects of exogenous melatonin and bright light on EEG power density in NREM sleep during the first three NREMS episodes (main effects). For each frequency bin (0.75–20 Hz) and subject, the light values (LIGHT, ML) were expressed as percentage of the corresponding no-light values (PLAC, MEL), and the MEL values (MEL, ML) were expressed as percentage of the corresponding no-MEL values (PLAC, LIGHT) [mean + or - SEM]. Symbols ( $\bullet$ , $\bigcirc$ ) near the abscissa indicate significant effects of the factor MEL and LIGHT (two way-ranova).

(SWA) for the first four NREM-REMS cycles of the treatment night. NREM sleep episodes were subdivided into 10 equal parts (percentiles), REM sleep episodes into 2 percentiles. The interval between lights off and sleep onset was subdivided into 1 percentile. SWA is expressed as the percentage of the mean NREM sleep value (100%) for each group separately. The curves connect mean percentile values [n=8, except for the fourth NREM-REMS cycle] and are plotted relative to the mean onset and termination of each cycle. For a better comparison to placebo the melatonin ( $\bigcirc$ ), bright light ( $\bigcirc$ ) and melatonin & light ( $\blacksquare$ ) values are superimposed on placebo data (solid line).

body temperature rhythm indicate that evening bright light induced a phase delay in both conditions.

#### Sleep latency

On the treatment night, sleep latency, a measure of early night sleep propensity, was longer after bright light and reduced after melatonin administration. This is in agreement with other studies reporting difficulties initiating sleep after evening light exposure (Cajochen *et al.* 1992, Carrier and Dumont 1995, Dijk *et al.* 1991, Drennan *et al.* 1989) and shorter sleep latencies after bedtime administration of melatonin (for a review see Zhdanova and Wurtman 1997). Sleep latency was also shorter after the combined application of both melatonin and bright

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**Figure 6.** Dynamics of EEG power density in the 0.75–4.5 Hz band (SWA) for the first four NREM-REMS cycles of the post-treatment night. Methods as in legend to Figure 5.

light, due to the high circulating levels of melatonin. It is unlikely that these changes in sleep latency reflect a circadian effect, since changes in sleep latency on the treatment night are more consistent with an acute activating effect of light or an acute soporific effect of melatonin. However, alterations in sleep latency on the post-treatment night, are more likely to reflect the chronobiotic effect, that is the change in phase or the change in the phase angle between the sleep cycle and the circadian pacemaker. This is supported by the persistence of longer sleep latencies on the post-treatment night in both groups treated by light. Changes in estimated sleep latency on the morning after the treatment and post-treatment night clearly followed the changes in measured sleep latency and corroborated the separation of effects into acute and circadian.

# First REMS episode duration

Because the timing of REMS is coupled to the circadian rhythm of core body temperature (Czeisler et al. 1980, Zulley 1980), and regulated to a large extent by the circadian pacemaker (Dijk and Czeisler 1995), changes especially in the first REMS episode duration (Dijk et al. 1989; Zulley 1980) and REMS latency (Sack et al. 1986) are considered to reflect circadian alterations in REMS propensity. However, in order to induce an increase in REMS in the initial part of sleep episodes by phase advancing the circadian rhythm of REMS, a phase advance of at least 4-6 h is needed (Dijk and Cajochen 1997). We therefore suggest that the observed shorter REMS latencies and longer first REMS episodes in the treatment night after melatonin which have been also observed in a previous experiment giving melatonin earlier in the evening (Cajochen et al. 1997b) do not reflect phase advances of the circadian pacemaker. The longer REMS episode may be a consequence of the acute hypothermic effect of melatonin (see Fig. 2a in Kräuchi et al. 1997a), since REMS propensity increases when core temperature physiologically decreases (Wehr 1992).

#### Wake-up propensity

We could not observe the expected later rise in wake-up propensity during sleep after evening bright light. A delayed rise in wake-up propensity during the post-treatment night was expected in both light treatment groups, since the ascending limb of the circadian temperature rhythm occurred later in the sleep episode (Kräuchi *et al.* 1997b). It may have been that time in bed in our study was too short in unveiling delays in wake-up propensity, subjects must have the possibility to sleep as long they need, particularly when sleep latencies are longer after a phase delay. Since melatonin was not given at a time that would induce a large phase advance, if any, no phase advance in wake-up propensity was expected for the melatonin group, nor was it found for core body temperature (Kräuchi *et al.* 1997b).

#### EEG during wakefulness

Significant changes in EEG power density during waking were only observed during the treatment period. As reported earlier (Cajochen *et al.* 1996), melatonin induced a significant enhancement in waking theta activity which paralleled higher sleepiness ratings. Waking EEG activity in the theta and alpha range tended to be reduced during bright light exposure which is in accordance to a previous study (Cajochen *et al.* 1992). As for subjective sleepiness, melatonin and light acted in an additive manner. The phase delay in subjective sleepiness and the mid-range crossing time of CBT decline on the post-treatment day in both light treatment groups was reflected in a reduction of theta activity already at 2000 h, which did not further change. This also paralleled the lower sleepiness ratings.

# EEG during sleep

The same 5 mg dose of melatonin given 3 h earlier did not modify SWS or SWA (Cajochen et al. 1997b). In this experiment, when administered closer to sleep onset, both melatonin groups significantly suppressed SWA in the first NREMS-REMS episode, and this was followed by a rebound in the second NREMS-REMS episode, together with higher SWS values. Thus, a sufficiently high plasma level of melatonin prior to bedtime promotes REMS sleep at the expense of NREMS intensity. To what extent the observed changes in EEG power density are related to the hypothermia after melatonin administration are not yet clear. A recent report does indicate that drug induced changes on EEG activity may be due to nonspecific effects on body temperature (DeBoer and Tobler 1997). Reduced SWA and SWS have also been reported after melatonin ingestion during daytime sleep (Dijk et al. 1995b, Hughes and Badia 1997). In contrast to these studies, we could not observe a significant increase in sleep spindle activity. Although SWA was reduced in the first NREMS-REMS episode, the lack of spindle effects and the immediate intrasleep rebound of SWA do not support the idea of a benzodiazepinelike action of melatonin. Benzodiazepines exhibit strong SWA - and sleep spindle effects which persist, depending on their half-life time, for at least an entire night sleep episode (Trachsel et al. 1990). In addition, nocturnal REMS is, if at all, reduced after benzodiazepine ingestion, and the first REMS episode is often skipped (Achermann and Borbely et al. 1987).

EEG power density was not changed after the evening bright light exposure, but the timing of the NREM-REMS modulation of the EEG was delayed in the treatment and the post-treatment night mainly due to the longer sleep latencies.

#### CONCLUSION

Exogenous melatonin and bright light in the evening exhibited distinct acute effects on EEG power density during wakefulness and sleep, internal sleep structure and subjective measures (i.e. sleepiness ratings and sleep quality) — melatonin and light acted in an additive manner. In contrast, the circadian effects of evening light to induce a phase delay could not be blocked by contemporaneous exogenous melatonin.

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