Melatonin and S-20098 increase REM sleep and wake-up propensity without modifying NREM sleep homeostasis

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Cajochen, Christian, Kurt Kräuchi, David Möri, Peter Graw, and Anna Wirz-Justice. Melatonin and S-20098 increase REM sleep and wake-up propensity without modifying NREM sleep homeostasis. *Am. J. Physiol.* 272 (*Regulatory Integrative Comp. Physiol.* 41): R1189–R1196, 1997.—The pineal hormone melatonin has been implicated in the circadian regulation of sleep. In a crossover design, we investigated the effect of acute administration of 5 mg melatonin and a melatonin agonist (S-20098, 5 and 100 mg) in healthy young men when given 5 h before bedtime on sleep structure and electroencephalogram (EEG) power density. Each trial comprised a baseline, a treatment, and a posttreatment sleep episode. Relative to the placebo condition, all treatments phase advanced the core body temperature rhythm [Kräuchi, K., C. Cajochen, D. Möri, C. Hetsch, and A. Wirz-Justice. *Sleep Res.* 24: 526, 1995; and Kräuchi, K., C. Cajochen, D. Möri, and A. Wirz-Justice. *Am. J. Physiol.* 272 (*Regulatory Integrative Comp. Physiol.* 41): R1178-1188, 1997]. Rapid eye movement (REM) sleep was increased after both melatonin and S-20098. This increase in REM sleep was most pronounced in the first REM sleep episode. On the posttreatment night after melatonin and S-20098 administration, more wakefulness was present in the latter one-half of the sleep episode. EEG power density between 0.25 and 20 Hz during either non-REM (NREM) or REM sleep did not differ from placebo. Thus a single early evening dose of melatonin or the agonist S-20098 increases REM sleep propensity and advances sleep termination while, at the same time, the EEG in NREM sleep remains unaffected.

human sleep; spectral analysis; circadian phase

MELATONIN, AN INDOLEAMINE secreted by the pineal gland at night, has long been suspected to be involved in human sleep regulation. Early studies reported sleep-inducing effects (1) and increased sedation (8) after exogenous melatonin administration in pharmacological doses. There have even been suggestions that the pineal is a "tranquilizing" organ (32). On the basis of these and more recent studies (17, 18, 35, 39), the "hypnotic" effects have been hypothesized to be an integral part of the natural physiological role of melatonin, although whether melatonin should be considered in these terms at all is questionable (36). In contrast to the hyperthermic effect of bright light (3, 5, 6, 33), orally administered melatonin in doses ranging from 0.3 to 80 mg decreases body temperature (12, 17, 18). This suggests that the sleep-inducing effect of melatonin may be mediated through lowering of core body temperature (11).

Both animal (31) and human studies (26, 38) document that melatonin is capable of inducing phase shifts of the circadian system, the direction of which is dependent on circadian phase of administration. In humans, only one preliminary study so far has investigated melatonin's entrainment capacity in free-running conditions (28). Nevertheless, melatonin's entrainment power is not yet validated. More is known of its usefulness in ameliorating sleep disturbances associated with circadian dysfunction [in blind people, delayed sleep phase syndrome, and jet lag (for an overview, see Ref. 2)]. Therefore, taken together, there is strong evidence that acute administration of melatonin augments sleep propensity and phase shifts the circadian pacemaker.

Because sleep consolidation and sleep structure are dependent on an appropriate phase relationship between the sleep-wake cycle and the endogenous circadian pacemaker (14), the interpretation of studies investigating the effects of melatonin on sleep parameters is difficult (for a review, see Ref. 11). In addition, the effects of melatonin on the sleep electroencephalogram (EEG) are not yet well documented. The observed changes in REM sleep (22) are more consistent with the phase-shifting effects of melatonin, whereas changes in the sleep EEG spectra seen after daytime administration are likely to be related to an acute hypnotic and possibly noncircadian action of melatonin (15, 35). Dawson and Encel (11) suggest that the improvement in sleep after evening melatonin administration (later than 2100) may reflect the hypnotic effects, whereas afternoon administration is more likely to be related to circadian effects. Therefore, the relationship between the timing of exogenous melatonin application and sleep, and whether or not endogenous melatonin is being secreted, is of crucial importance when designing melatonin studies.

The aim of the present study was to use an experimental strategy that is able to distinguish between circadian and noncircadian effects of exogenous melatonin and its repercussions on sleep architecture and EEG power density. Second, we wanted to compare the effects of melatonin with the novel melatonin agonist S-20098 (37). S-20098 has been fully characterized to be assessed in human studies. S-20098 binds to the sheep pars tuberalis melatonin receptor with a dissociation constant of 8.10^{-11} M. Its effect on sleep and circadian rhythms has been assessed in in vivo animal models (27, 30, 34).

METHODS

Subjects

Eight male students (age 23–32 yr) 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. Medical or psychiatric disorders were screened

by history, physical examination, clinical EEG, clinical electrocardiogram (ECG), biochemical blood and urine screening, and a psychological screening questionnaire (Freiburger-Persoenlichkeits-Inventar-Liste). None of the subjects reported sleep disorders (Pittsburgh Sleep Quality Index <5) or were extreme morning or evening types (defined by scores ≤14 and ≥21 in the Torsvall-Åkerstedt morning-eveningness questionnaire). Further exclusion criteria included no shift work or transmeridian travel within 1 mo before study and no smoking, medication, or drug use. Before the study the subjects were asked to maintain a regular sleep-wake cycle for at least 2 wk. This was verified by ambulatory monitoring of the subjects' motor activity and a daily sleep diary. Subjects were asked to restrict their sleep times to between 2300 and 0700 for 1 wk before and during the entire study period. Continuously worn actometers (Gaehwiler Actigraph, Zurich) verified compliance to the latter instruction (data not presented). During the experiment the amount of 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 disorders such as sleep apnea. All subjects completed the study without any complaint.

Protocol

A double-blind placebo-controlled study was conducted according to a partial Latin square design. Each test period comprised 1 pretreatment day with placebo, 1 day of treatment [placebo, melatonin 5 mg (Mel), S-20098 5 mg (S5), or S-20098 100 mg (S100) in randomized order], and 1 posttreatment day with placebo. This schedule was repeated for 4 consecutive wk between April and July 1994. The subjects were not informed on which day they would receive the active substance. Subjects reported at 1500 to the chronobiology laboratory where the electrodes were attached. They remained supine and awake in bed in a sound-attenuated chronobiology room (temperature 22°C, humidity 60%, light <10 lx) from 1600 to 2300. During this 7-h period a "mini"-constant routine (mini-CR) was carried out, with protocol and procedure adapted from others (9) as used in our previous studies. Isocaloric meals (sandwiches) and water were given every hour to meet energy requirements. Saliva melatonin samples were collected in hourly intervals. Subjective sleepiness and mood ratings were assessed on visual analog scales every 30 min. One hour before and after the pill intake, a 5-min waking EEG was recorded. Results on subjective sleepiness ratings and waking EEG power density have been recently reported (7). The subjects were cared for by trained personnel and remained awake until 2300 without information about time of day. This mini-CR represents a standardized procedure that permits reduction of masking effects on circadian parameters measured, as well as no postural change at subsequent sleep onset. The sleep period was scheduled from 2300 to 0700 during which the lights were off. The pill was administered at 1800, 5 h before anticipated sleep onset.

Sleep EEG Recording and Analysis

During all sleep episodes two EEG signals (C3-A2, C4-A1), two electrooculogram (EOG) signals, and one electromyogram and electrocardiogram signal were recorded on polygraph paper (Nihon Kohden 4418 G; 10 mm/s paper speed). Self-adhesive silver-chloride electrodes (Sensor Medics, Skin electrode kit) were used for placements on the skin and silver-chloride disk electrodes (Nicolet, Biomedical Instruments) fixed with collodion for placements on the scalp. The EEG signals were high-pass filtered with a time constant of 1.0 s and low-pass filtered at 35 Hz (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 enable optimal synchronization of the sleep scoring with the spectral data. 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 omitted automatically by an artifact detection routine.

The sleep EEGs were scored manually according to established criteria (29) every 20 s and synchronized with the power spectra. NREM-REM sleep cycles were defined according to published criteria (20), with the following exception: for the last cycle analyzed (cycle 4) no minimum of REM sleep duration following on NREM sleep was required. For cycles 2–4, the first 20-s epoch after the last REM sleep epoch was defined as the onset of the cycle.

Statistics

The SAS statistical package (SAS Institute, Cary, NC) was used. Sleep stages were expressed as percent of total sleep time or percent of NREM sleep-REM sleep cycle duration. For some sleep parameters (e.g., sleep latency, REM sleep latency) and EEG power density, data were log transformed to meet the requirements of a normal distribution. Data were first averaged per subject so that all subjects would contribute with equal weight, and then the data were averaged over all subjects ($n = 8$).

Statistical significance between the four groups (placebo, Mel, S5, and S100) was assessed with two-way analyses of variance (ANOVA) for repeated measures, with the factors "group" and "night," or one-way ANOVAs for repeated measures with the factor "group." All P values derived from repeated-measure ANOVAs 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, Duncan's multiple-range post hoc tests and linear contrasts with H-F statistics were applied to locate significant differences between the means. Statistics on the time course of accumulated sleep stages were performed by the nonparametric Wilcoxon's signed-rank test.

RESULTS

Sleep Stages

Entire sleep episode. Table 1 summarizes the sleep parameters calculated over the entire sleep episode for the pretreatment night, treatment night (TN), and posttreatment night (PTN) of the placebo, Mel, S5, and S100 group. The six placebo nights (all pretreatment nights of each group and the TNs and PTNs of the placebo group) were tested for their homogeneity. First, to assess significant changes in the course of the three consecutive nights in the placebo condition, only the three nights (pretreatment night, TN, and PTN) of the placebo group were analyzed by a one-way ANOVA with the factor night. For none of the sleep parameters listed in Table 1 was a significant P value for the factor night

Table 1. *Sleep parameters calculated over entire sleep episode for pretreatment night, treatment night, and posttreatment night of placebo, melatonin, and S-20098 groups*

	Pretreatment Night	Treatment Night	Posttreatment Night
Total sleep time			
Placebo	443.6 ± 7.3	438.2 ± 10.6	452.6 ± 3.7
Melatonin (5 mg)	450.6 ± 4.0	445.7 ± 6.4	438.3 ± 6.4
S-20098 (5 mg)	444.4 ± 6.9	450.3 ± 4.7	438.8 ± 8.2
S-20098 (100 mg)	449.8 ± 6.5	454.5 ± 1.9	431.3 ± 15.1
Pooled placebo	446.5 ± 4.6		
Sleep efficiency			
Placebo	92.3 ± 1.5	91.2 ± 2.2	94.3 ± 0.8
Melatonin (5 mg)	93.8 ± 0.8	92.9 ± 1.3	91.2 ± 1.3
S-20098 (5 mg)	92.5 ± 2.0	93.7 ± 1.0	91.4 ± 1.7
S-20098 (100 mg)	93.6 ± 1.4	94.6 ± 0.4	89.9 ± 3.2
Pooled placebo	92.9 ± 1.0		
Sleep latency			
Placebo	7.9 ± 0.9	8.3 ± 0.8	11.0 ± 1.2
Melatonin (5 mg)	9.6 ± 1.8	7.5 ± 1.1	9.3 ± 1.4
S-20098 (5 mg)	8.8 ± 2.0	8.3 ± 1.1	12.0 ± 1.6
S-20098 (100 mg)	7.8 ± 1.3	5.5 ± 0.5	11.0 ± 1.6
Pooled placebo	8.9 ± 0.9		
REMS latency			
Placebo	61.8 ± 5.1	60.3 ± 5.5	62.5 ± 6.2
Melatonin (5 mg)	67.2 ± 4.2	64.2 ± 5.1	67.0 ± 6.5
S-20098 (5 mg)	65.2 ± 4.0	61.4 ± 4.7	62.4 ± 5.2
S-20098 (100 mg)	71.8 ± 9.1	62.1 ± 7.3	62.5 ± 2.8
Pooled placebo	64.8 ± 4.4		
Stage 2			
Placebo	54.5 ± 1.7	52.7 ± 2.2	53.2 ± 1.2
Melatonin (5 mg)	55.4 ± 2.3	51.1 ± 1.5	52.6 ± 1.8
S-20098 (5 mg)	59.6 ± 2.1	53.8 ± 1.3	54.4 ± 1.6
S-20098 (100 mg)	54.0 ± 2.0	51.6 ± 1.5	49.9 ± 2.2
Pooled placebo	54.9 ± 1.3		
Slow-wave sleep			
Placebo	11.9 ± 1.9	12.0 ± 2.0	11.8 ± 1.9
Melatonin (5 mg)	12.6 ± 1.9	11.9 ± 1.9	13.2 ± 2.5
S-20098 (5 mg)	12.5 ± 1.5	11.7 ± 1.9	13.2 ± 2.4
S-20098 (100 mg)	12.5 ± 2.1	12.6 ± 2.2	13.6 ± 2.6
Pooled placebo	12.2 ± 1.7		
REM sleep			
Placebo	22.3 ± 0.8	22.1 ± 1.5	22.9 ± 1.4
Melatonin (5 mg)	20.8 ± 1.6	25.4 ± 1.5*†‡	20.6 ± 1.2
S-20098 (5 mg)	18.2 ± 1.6	24.0 ± 1.1*‡	20.4 ± 1.2
S-20098 (100 mg)	20.9 ± 1.1	24.5 ± 1.2*†‡	23.0 ± 1.3‡
Pooled placebo	21.2 ± 0.9		
Arousal			
Placebo	6.0 ± 0.8	7.2 ± 1.0	5.3 ± 0.5
Melatonin (5 mg)	5.2 ± 0.6	5.9 ± 0.5	7.1 ± 0.8
S-20098 (5 mg)	5.3 ± 0.8	5.2 ± 0.5	6.3 ± 0.7
S-20098 (100 mg)	6.0 ± 0.8	5.3 ± 0.5	7.7 ± 1.8
Pooled placebo	5.8 ± 0.6		

Sleep parameters (mean values ± SE; $n = 8$) are expressed as a percentage of total sleep time except for sleep latency, rapid eye movement (REM) sleep latency, and total sleep time (min). Arousal = waking (after sleep onset) + stage 1 + movement time. *Melatonin, 5 mg S-20098, 100 mg S-20098 (Mel, S5, S100) vs. pretreatment night; †Mel, S5, S100 vs. corresponding placebo ($P < 0.05$, Duncan's multiple-range test; ‡Mel, S5, S100 vs. pooled placebo ($P < 0.05$, linear contrasts).

found ($P > 0.2$). Second, to assess significant changes in the course of the experimental protocol (week effect) all pretreatment nights were assorted according to their timing (*week 1* through *week 4*). A one-way ANOVA with the factor week revealed no significant changes for any sleep parameters, except for the variable sleep latency. Post hoc comparison between the first week and the last week revealed a trend to shorter sleep latencies ($P = 0.07$). Third, all six placebo nights were compared using a one-way ANOVA with the factor night. No significant changes were found for any of the sleep variables ($P > 0.2$). Because none of the sleep variables calculated for the entire sleep episode differed significantly between the six placebo nights, corresponding values of these six placebo nights were averaged per subject ("pooled placebo," Table 1).

For each sleep parameter a two-way ANOVA for repeated measures with the factors group and night was performed (Table 2). A significant interaction factor group × night was found for REM sleep, and a tendency was observed for arousals within sleep. The *P* values for the factor night yielded significance for REM sleep, stage 2, sleep latency, and REM sleep latency. In addition, the factor group was significant for stage 2. Post hoc comparisons were performed on the parameter REM sleep, for which a significant interaction group × night was found. REM sleep in the TN was significantly enhanced by all treatments (Mel, S5, S100) in comparison with the corresponding pretreatment nights and with the pooled placebo. Comparisons with the TN of the placebo revealed a significant increase in REM sleep for Mel and a tendency for S100. REM sleep in the PTN was still significantly enhanced in the S100 group when compared with the pooled placebo.

NREM-REM sleep cycles. To inspect the temporal evolution of different sleep parameters more closely, NREM-REM sleep cycles across each sleep episode were defined according to the above-mentioned criteria.

A two-way ANOVA for repeated measures with the factors group and night was performed for each cycle separately. The interaction factor group × night was significant for REM sleep in the first cycle ($F_{6,42} = 2.8$, $P < 0.03$), whereas no significant interaction factor was found in cycles 2 and 3. REM sleep in the first cycle of the TN was significantly longer in the Mel and S100 than in the placebo group. The comparisons of Mel, S100, and S5 vs. the corresponding pretreatment nights and the pooled placebo value were also significant (Table 3).

In the TN, a two-way ANOVA for repeated measures revealed significant changes in %REM sleep (percent of

Table 2. *Results of two-way ANOVA for repeated measures with the factor group, the factor night, and the interaction factor group × night for sleep parameters averaged over the entire night*

Sleep Parameter	Group ($F_{3,21}$)	Night ($F_{2,14}$)	Group × Night ($F_{6,42}$)
Total sleep time	0.1; NS	2.0; NS	1.4; NS
Sleep efficiency	0.1; NS	1.7; NS	1.4; NS
Sleep latency	1.0; NS	5.5; $P = 0.02$	1.0; NS
REM sleep latency	0.7; NS	4.5; $P = 0.03$	0.2; NS
Stage 2	4.4; $P = 0.02$	8.4; $P = 0.004$	1.0; NS
Slow wave sleep	0.6; NS	1.6; NS	0.2; NS
REM sleep	1.3; NS	12.4; $P = 0.0008$	3.1; $P = 0.01$
Arousal	1.3; NS	2.2; NS	2.0; $P = 0.08$

Group: placebo, Mel 5 mg, S5, S100; night: pretreatment, treatment, posttreatment; *F*, *F* value; *P*, Huyn-Feldt corrected *P* values.

Table 3. *REM sleep in the first REM sleep episode*

First REM Sleep	Pretreatment Night	Treatment Night	Posttreatment Night
Placebo	13.3 ± 1.5	10.6 ± 1.5	15.0 ± 3.1
Melatonin (5 mg)	7.7 ± 2.0	17.5 ± 2.6*†‡	12.0 ± 2.2
S-20098 (5 mg)	8.6 ± 1.8	14.8 ± 3.0*†	11.0 ± 1.2
S-20098 (100 mg)	11.0 ± 2.1	20.0 ± 1.6*†‡	13.4 ± 1.5
Pooled placebo	11.0 ± 1.4		

Values are means ± SE of percentage of non-REM-REM sleep cycle duration ($n = 8$). *Mel, S5, S100 vs. pretreatment night; †Mel, S5, S100 vs. pooled placebo ($P < 0.05$, linear contrasts); ‡Mel, S5, S100 vs. corresponding placebo ($P < 0.05$, Duncan's multiple-range test).

cycle duration) and REM sleep episode duration for the factors group and cycle (%REM sleep: group $F_{3,21} = 3.89$, $P < 0.03$; cycle $F_{2,14} = 7.22$, $P < 0.008$; REM sleep episode duration: group $F_{3,21} = 6.62$, $P < 0.003$; cycle $F_{2,14} = 25.2$, $P < 0.0001$); because the REM sleep episode in cycle 4 was not followed by a further NREM sleep episode in all subjects, the ANOVA was limited to three cycles (Fig. 1).

During the PTN, the above-mentioned effects disappeared. No significant differences were found for any of the sleep parameters.

Accumulation of REM sleep and the combined stages: wakefulness, stage 1, and movement time. For evaluation of REM sleep and wake-up tendency, the accumulation of REM sleep and the sum of sleep stages 0 and 1 and movement time (MT) after sleep onset were calculated and plotted in 10-min intervals. For statistical analysis, the nights were divided into four 2-h intervals. REM sleep and the sum of stages 0 and 1 and MT were accumulated within each single 2-h interval. A two-way ANOVA for repeated measures, with the factors group and night was performed for each interval separately. The interaction factor group × night was significant for the variable REM sleep ($F_{6,42} = 2.5$, $P < 0.04$) in the first 2-h interval, whereas for the *intervals 2, 3,* and *4* no significant interaction was found. Post hoc comparisons in the first 2-h interval revealed a significant REM sleep augmentation for the Mel and S100 group vs. the placebo group, the pooled placebo, and the pretreatment value. For the arousal measure (stages 0 and 1 and MT), a tendency ($F_{6,42} = 2.3$, $P = 0.07$) in the interaction factor was found when adding the values of 2-h *intervals 3* and *4*.

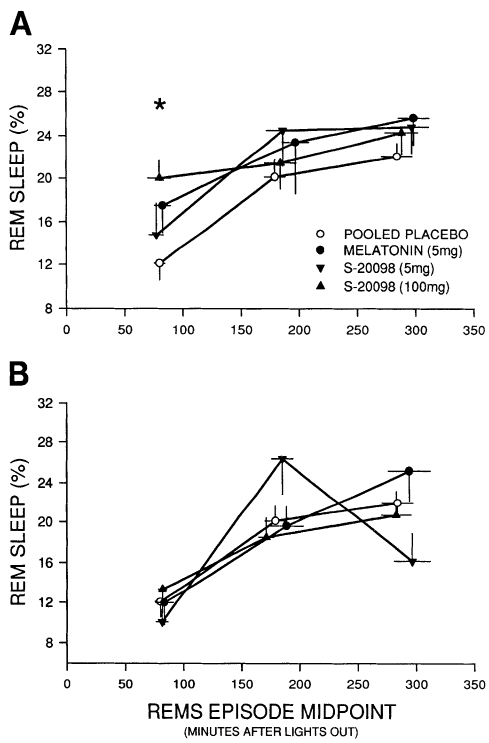

Fig. 1. Percentage of rapid eye movement (REM) sleep during first 3 non-REM (NREM)-REM sleep cycles in the treatment (*A*) and posttreatment (*B*) nights. Values are plotted against REM sleep episode midpoint [min after lights out; $n = 8$; vertical and horizontal lines represent SE; *$P < 0.02$ (linear contrasts) for melatonin and S-20098 (100 mg; S100) vs. pooled placebo].

Figure 2, *A* and *B*, shows that the REM sleep-curves for the Mel and S100 group separated 2 h after lights off from the pooled placebo curve on the TN but not on the PTN. The REM sleep accumulation curve in TN of the S5 group also dissociated from placebo 2 h after lights off (data not shown). REM sleep accumulated faster after Mel, S5, and S100. In contrast, the stages 0 + 1 + MT-curves were identical in the TN of placebo, melatonin, and S100, but differed in the PTN, where they significantly dissociated from placebo after 7 h in the Mel group and after 5 h in the S100 group. The combined stages 0 and 1 and MT accumulated faster in the second night after Mel or S100. The accumulation in TN and PTN of the S5 group was not significantly different from placebo (data not shown).

Correlation between the first REM sleep episode duration and core body temperature. To test whether there was a relationship between the prolongation of the first REM sleep episode and circadian timing, the mean first REM sleep duration was correlated with the midrange crossing time of core body temperature decline (24, 25) (Fig. 3). Data from all experimental days were included in the analysis. The negative correlation between the two variables was significant ($r = 0.79$; $P < 0.0025$; $n = 12$; product-moment correlation), i.e., the earlier the temperature phase, the longer the duration of the first REM sleep episode.

EEG Power Density

Entire sleep episode. EEG power density during NREM sleep is illustrated for both TN and PTN (Fig. 4). A one-way ANOVA for repeated measures revealed no significant changes for the factor group in any of the frequency bins (0.25–20 Hz), neither for NREM sleep nor REM sleep on either night (REM sleep data not shown). In addition, analyses limited to shorter time intervals (e.g., first one-third of the night or first hour of the night, etc.) revealed no consistent trends or significant changes in comparison to placebo (data not shown).

Dynamics of slow-wave activity in NREM sleep-REM sleep cycles. For a more detailed visualization of the time course of slow-wave activity (SWA), defined as EEG power density in the frequency range of 0.75–4.5

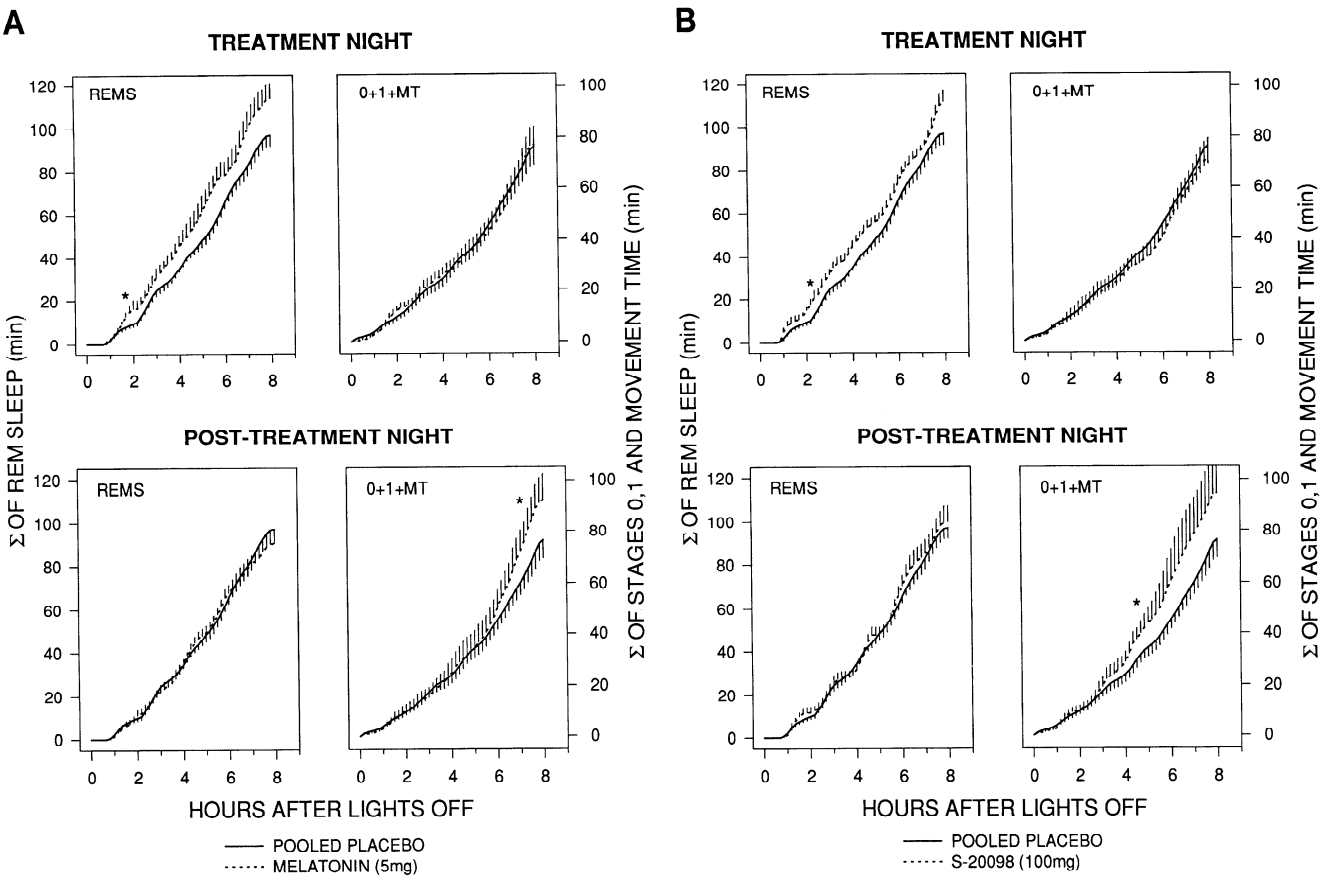

Fig. 2. Accumulation of REM sleep or combined stages: wakefulness (0), stage 1 (1), and movement time (MT) in 10-min intervals (n = 8; vertical lines represent SE). *$P < 0.05$, indicates first occurrence of a significant difference from placebo (Wilcoxon's signed-ranks test).

Hz, each NREM sleep episode was subdivided into 20 equal parts (percentiles) and each REM sleep episode into four equal parts (Fig. 5). SWA exhibited the well-known buildup in the first part of NREM sleep episodes and a rapid decline before REM sleep episodes. Also, the typical declining trend of SWA over successive NREM sleep episodes was present in all four groups.

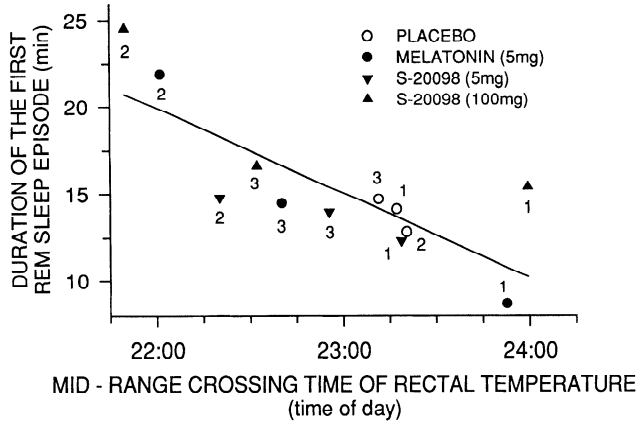

Fig. 3. Correlation between duration of first REM sleep episode and midrange crossing time of rectal temperature decline. Symbols represent mean values (n = 8) for each group [placebo, melatonin, S-20098 (5 mg), S100] on 3 experimental days (1, pretreatment day; 2, treatment day; 3, posttreatment day). Line was fitted to data points with linear regression ($r^2 = 0.63$; $P < 0.0025$).

On the TN (Fig. 5*A*), SWA did not differ significantly from placebo in any of the treatment groups in any of the percentiles (1-way ANOVA for repeated measures factor group, $P > 0.05$). A similar negative result was obtained in the PTN (Fig. 5*B*).

However in TN, due to the prolongation of the first REM sleep episode, the initiation of the following NREM sleep and REM sleep episodes was delayed after Mel and S100. To quantify the magnitude of this delay, the time course of SWA of the placebo ($SWA_{placebo}$) and treatment groups was analyzed by cross-correlation analysis. A mean cross-correlation coefficient was calculated separately for the eight subjects between $SWA_{placebo}$ and SWA of all treatment groups. For the determination of time lag with the highest absolute correlation coefficient, SWA data in 2-min intervals after the first NREM sleep episode were used. The maxima of cross-correlation curves of Mel, S5, and S100 were at positive lags, indicating a delay with respect to $SWA_{placebo}$. The mean maximal cross-correlations were at time lags of 14 ± 2.7 min for Mel, 14 ± 2.9 min for S100, and 5.5 ± 2.3 min for S5. The time lags of Mel and S100 were significantly different from *time lag 0* (Mel: $P = 0.03$; S100: $P = 0.04$). Although the mean onset times of the

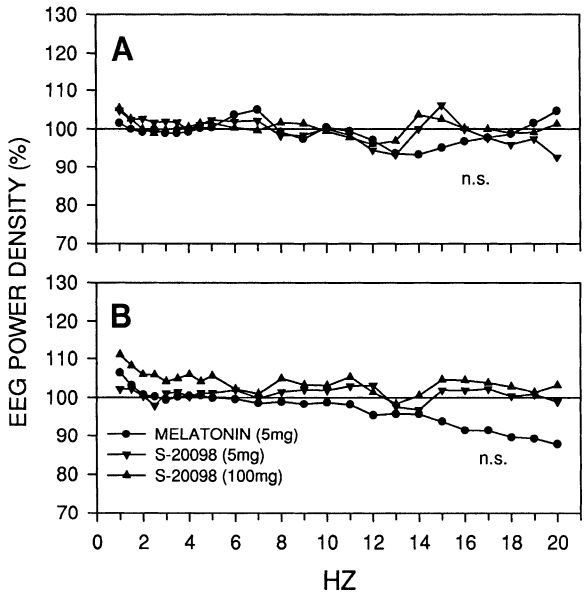

Fig. 4. Effect of melatonin and S-20098 on electroencephalogram (EEG) power density in NREM sleep in treatment night (*A*) and posttreatment night (*B*). For each frequency bin, subject and night values were expressed as a percentage of corresponding pooled placebo value. Log-transformed values were averaged within groups and retransformed for plotting (n = 8); n.s. indicates no significant differences between placebo and any of the treatment groups.

NREM-REM sleep cycles in the PTN (Fig. 5*B*) still appeared delayed, no significant differences were found.

DISCUSSION

Sleep Stages

The present study clearly shows that the acute phase advance of the circadian body temperature rhythm induced by melatonin or the melatonin agonist S-20098 (24, 25) is reflected in a selective modification of sleep stages. The shift to a long first REM sleep episode resembles the pattern seen when entrained subjects free run in temporal isolation (40). Under these circumstances the minimum of the body temperature rhythm advances to the beginning of the major sleep episode. The prolongation of the first REM sleep episode correlated with the midrange crossing time of body temperature decline. Because the timing of REM sleep is coupled to the circadian rhythm of core body temperature (10) and regulated to a large extent by the circadian pacemaker (14), it may be that the prolonged first REM sleep episode is a consequence of the phase advance in the core body temperature rhythm. A phase delay induced by evening bright light can produce the corollary effect of significantly shorter first REM sleep episodes (M. C. M. Gordijn, H. J. Korte, D. G. M. Beersma and R. H. van den Hofdahler, unpublished observations). However, in the PTN, where a phase advance in core body temperature was still observed (24, 25), this significant change in REM sleep was not maintained. The failure to find a persistent significant effect may have been due to the relatively small phase advance of ~1 h. This explanation would be in accordance with results from experiments where phase advances in core body temperature of ~1 h had no effect on REM sleep (13, 19). Because the amount of REM sleep is also likely to be dependent on the actual level of core body temperature, the REM sleep increase in the TN may also result from the acute hypothermic effect of melatonin and the melatonin agonist.

A clear indication for a persistent phase shift on the PTN after melatonin or agonist administration is in the early rise of the accumulated stages of wakefulness, stage 1, and MT, which together reflect the wake-up tendency during the course of a night. An earlier increase in the wake-up threshold is considered to be a reflection of a phase advance. For example, a phase advance induced by morning bright light not only reduced sleep duration (16) but the circadian phase of

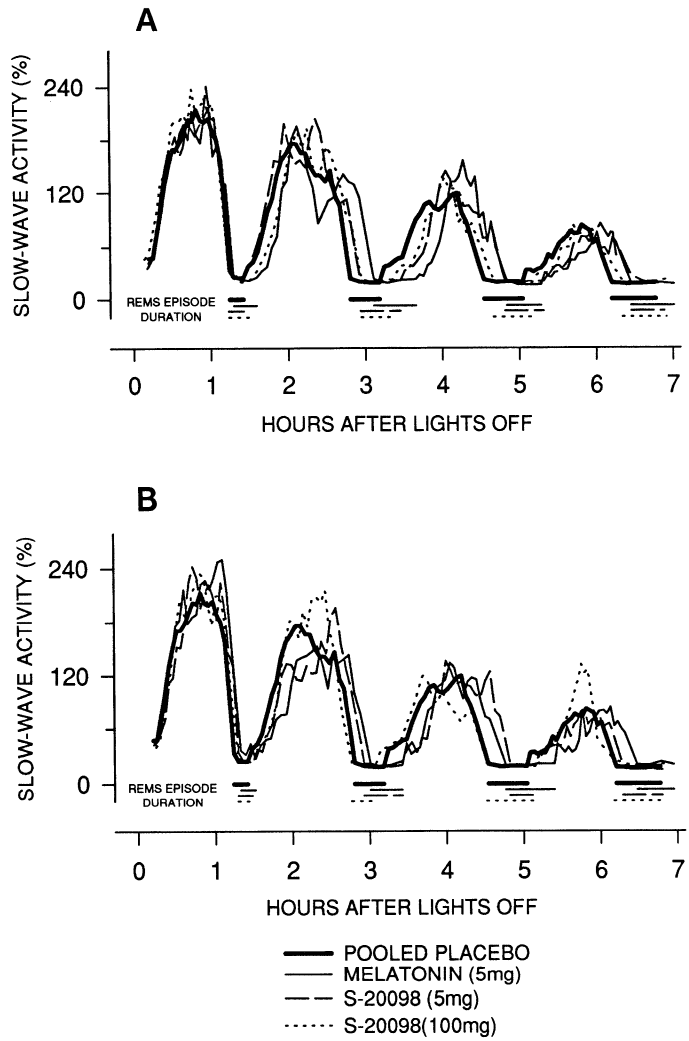

Fig. 5. Dynamics of EEG power density in the 0.75- to 4.5-Hz band (slow-wave activity; SWA) for the first 4 NREM-REM sleep cycles. NREM sleep episodes were subdivided into 20 equal parts (percentiles), REM sleep episodes into 4 percentiles. Interval between lights out and sleep onset was subdivided into 2 percentiles, but only the 2nd percentile is shown. SWA is expressed as the percentage of mean NREM sleep value (100%). Curves connect mean percentile values and are plotted relative to mean onset and termination of each cycle. Lines above abscissa indicate duration of first 4 REM sleep episodes (see RESULTS for statistics). *A*: treatment night; *B*: posttreatment night.

the wake-up threshold was earlier. The wake-up threshold on the TN was not affected, although the phase advance in core body temperature was larger than in the PTN. However, it is possible that any advance of the arousal threshold on the TN was antagonized by the hypnotic effect of melatonin or its agonist. Some evidence for this interpretation comes from the time course of the wake-up tendency after the higher dose of the agonist (S100), where the wake up tendency was somewhat, albeit not significantly, below that of placebo.

Although the time course of REM sleep in TN and the wake-up tendency in PTN changed after treatment, the percentage of slow-wave sleep (SWS) was not affected. The present data support the notion that SWS is regulated very accurately by a homeostatic control mechanism (4). In contrast, NREM sleep stage 2 was reduced at the expense of REM sleep. This indicates that stage 2, where most sleep spindles occur, may also be partially regulated via circadian mechanisms.

Hypnotic Effect of Melatonin and S-20098

Wake EEG data objectively document the acute soporific effect of melatonin and its agonist that is subjectively noted as increased fatigue (7). Although subjects felt more tired after melatonin and the melatonin agonist, sleep latency, a common measure of hypnotic activity, was shorter only after the higher dose of S-20098. A possible explanation for this is that, in contrast to a previous study that describes shorter sleep latencies after low melatonin doses administered in the late evening (39), the time gap between pill ingestion and lights out in our study was 5 h. Nevertheless, the average salivary melatonin levels at 2300 after exogenous melatonin administration were still seven times higher than the endogenous nocturnal rise (7) and still sleep latency was not significantly reduced. In addition, the usual problem of demonstrating a shortened sleep latency in subjects specifically screened for being good sleepers may also play a role. However, there was a clear soporific effect of melatonin and its agonist, both enhancing theta/alpha frequency activity in the waking EEG and increasing self-reported sleepiness (Ref. 7; unpublished data). Therefore, soporific effects may be only demonstrable when circulating levels of endogenous melatonin are low (11) or suppressed by bright light. This explanation favors the idea that the physiological increase of endogenous melatonin that occurs naturally late in the evening (typically 1 to 2 h before habitual bedtime) is involved in triggering sleep onset. Others have described this as a melatonin onset-related opening of the "sleep gate" (35). The sleep-inducing properties of exogenous melatonin when administered in the evening differ from those of the benzodiazepines. Benzodiazepines decrease the duration of REM sleep after a single administration (23) and also reduce SWS (21). Thus the use of the term hypnotic for melatonin is controversial, and the effect of such a chronobiotic drug depends on time of day of administration (36).

EEG Power Density

Analysis of EEG power densities during NREM sleep and REM sleep in the frequency range of 0.25–25 Hz revealed that melatonin and the agonist did not affect any of the EEG frequencies either during the TN or the PTN. In other words, when the endogenous levels of melatonin are high, application of exogenous melatonin or S-20098 did not affect EEG power density in the above frequency range. There are as yet no studies that have investigated the effects of late evening administration of melatonin on night sleep EEG power density. Daytime administration of melatonin has indeed some minor benzodiazepine-like effects on the sleep EEG power spectrum (15). Daytime administration, or administration of melatonin when endogenous melatonin is suppressed by bright light, is the only approach to evaluating what acute effects the exogenous compound may have on the EEG power spectrum.

Apart from the acute effect induced by melatonin and S-20098, the changed phase relationships between sleep and the circadian system had no effect on the mean EEG power density and the time course of EEG power density. It is known that changes in phase relationships of this extent (~1 h) have no (13) or minor effects (5) on EEG power density. A recent study has reported circadian modulation of sigma activity (12.75–15.0 Hz) with maximum activity at habitual bedtime (14). Although NREM sleep stage 2 was reduced by melatonin and S-20098 in our study, there was no decrease in sigma activity, which is associated with the highest density of sleep spindles within NREM sleep. Nevertheless, compared with placebo, sigma activity at the beginning of the sleep episode of the TN tended to be reduced after melatonin and S-20098 ($P < 0.1$; data not shown). A more detailed analysis of spindle activity and larger phase shifts are needed to elucidate whether melatonin is also able to shift the rhythm of sigma activity.

The changes in REM sleep and the earlier termination of sleep may be considered in association with the phase advance seen in several circadian markers in this study (24, 25). The time course of EEG power density within the sleep episode is, to a large extent, independent of circadian phase. The melatonin agonist S-20098 is very similar to the natural hormone in all its effects. Experiments are needed in which the direct hypothermic, hypnotic, and phase-shifting effects of melatonin are clearly distinguishable to further elucidate the impact of melatonin on polysomnographically recorded sleep and quantitative EEG analysis.

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