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Sleep-anticipating effects of melatonin in the human brain

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Melatonin, the hormone produced nocturnally by the pineal gland, is an endogenous regulator of the sleep–wake cycle. The effects of melatonin on brain activities and their relation to induction of sleepiness were studied in a randomized, double-blind, placebo controlled functional magnetic resonance imaging (fMRI) study. Melatonin, but not placebo, reduced task-related activity in the rostro-medial aspect of the occipital cortex during a visual-search task and in the auditory cortex during a music task. These effects correlated with subjective measurements of fatigue. In addition, melatonin enhanced the activation in the left parahippocampus in an autobiographic memory task. Results demonstrate that melatonin modulates brain activity in a manner resembling actual sleep although subjects are fully awake. Furthermore, the fatigue inducing effect of melatonin on brain activity is essentially different from that of sleep deprivation thus revealing differences between fatigues related to the circadian sleep regulation as opposed to increased homeostatic sleep need. Our findings highlight the role of melatonin in priming sleep-associated brain activation patterns in anticipation of sleep.

Introduction

Sleep is an orchestrated neurochemical process involving sleep-promoting and arousal centers in the brain (Espana and Scammell, 2004; Pace-Schott and Hobson, 2002; Saper et al., 2005). Sleep is predominantly regulated by the sleep need (sleep homeostat) and the time of day (circadian pacemaker) (Dijk and Lockley, 2002; Borb'ely and Achermann, 1999). The interaction between these processes forms the basis of remarkably standardized episodes of sleep and wakefulness while maintaining stable levels of neurobehavioral function during the day.

The circadian pacemaker resides in the suprachiasmatic nucleus (SCN) within the brain. The individual period of the SCN is usually longer than 24 h (average 24.2 h; Czeisler et al., 1999) and is normally entrained (synchronized) by the ambient light to match the environmental 24-h light–dark cycle (Moore, 1997). One of the most important time cues generated by the SCN is the nocturnal production of melatonin (*N*-acetyl-5-methoxytryptamine) in the pineal gland at night which occurs during the quiescent (nocturnal) periods of SCN electrical activity and is inhibited by the ambient light (Macchi and Bruce, 2004; Antle and Silver, 2005). Because the elimination half-life of melatonin in serum is only 40–50 min (Mallo et al., 1990; Arendt, 1994), the circulating melatonin is diminished during the light hours. Consequently, the dim light melatonin onset (DLMO), which is the initial surge in melatonin release in the early part of the night under low light conditions, is a consistent and reliable measure of the intrinsic circadian phase (Lewy, 1999). Furthermore, melatonin serves as a time cue (signal of darkness) to various organs including the SCN itself, and is therefore able to phase shift the endogenous circadian clock and, in the absence of light, to entrain the sleep–wake and neuroendocrine rhythms (Arendt and Skene, 2005; Zisapel, 2001a,b).

In addition, melatonin is an important physiological sleep regulator. Thus, the sharp increase in sleep propensity at night usually occurs 2 h after the onset of endogenous melatonin production in humans (Lavie, 1997; Cajochen et al., 2003). Administration of melatonin during daytime (when it is not present endogenously) results in induction of fatigue (feeling of weariness, tiredness, or lack of energy) and sleepiness (feeling drowsy with a tendency to actually fall asleep) in humans (Arendt et al., 1984; Dollins et al., 1994). Beneficial effects of melatonin in improving night sleep have been reported, particularly in elderly patients with insomnia who do not produce sufficient amounts of this hormone (Leger et al., 2004). Because melatonin does not increase and even somewhat suppresses the amount of slow wave sleep (Dijk et al., 1995), which is considered a marker of the homeostatic sleep need (Knoblauch et al., 2002), the sleep promoting effects of melatonin may be mostly ascribed to the circadian component of sleep regulation rather than to an increase in the homeostatic sleep pressure.

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The brain networks affected by melatonin have not been identified. From animal studies, it is known that wakefulness is promoted by brainstem and hypothalamic neurons; each of these arousal networks is capable of increasing wakefulness, but coordinated activity in all these pathways is required for complete alertness and cortical activation (Espana and Scammell, 2004). It has been suggested that melatonin participates in the regulation of the sleep–wake cycle by inhibiting the wakefulness generating system in the brain (Lavie, 2001; Liu et al., 1997). Consistent with a synchronizing effect on the biological clock, melatonin receptors [MT1, MT2] which are members of the G protein-linked receptor family, have a narrow distribution in the brain, and there is no proof for their functional activities in brain regions other than SCN (Liu et al., 1997; Hunt et al., 2001).

The logical assumption would therefore be that melatonin acts within the brain regions connected to sleep–wake regulation, particularly the SCN and areas controlling wakefulness. Unfortunately, activity in most brain regions related to sleep and arousal cannot be reliably investigated using current EEG or fMRI paradigms. The activity of melatonin in these brain regions awaits further developments in the neuroimaging field.

Recent functional imaging studies of sensory processing during sleep have indicated that brain activities related to both auditory and visual stimuli are modulated by sleep (Portas et al., 2000; Czisch et al., 2002, 2004; Tanaka et al., 2003; Born et al., 2002; Altman and Bernal, 2001; Martin et al., 2000). We hypothesized that administration of melatonin during the day will affect brain circuits that are involved in induction of sleep, and may thus impinge on areas involved with auditory and visual information processing. In addition, evidence for the involvement of the hippocampus as well as the parahippocampus in memory processes and the role of sleep in memory consolidation led to an additional hypothesis that melatonin's effects will also be evident in these areas (Walker and Stickgold, 2004; Gais and Born, 2004; Peigneux et al., 2004; Staba et al., 2002; Burgess et al., 2002; Piefke et al., 2005; Schon et al., 2004).

In a double-blind, placebo controlled study, we examined the effects of melatonin on brain activities during visual, auditory and memory tasks using functional magnetic resonance imaging (fMRI). All our studies were conducted during the afternoon hours (1600–1800 h). These hours were chosen as they represent considerably high sleep need (homeostatic); low sleep propensity (circadian component) and low endogenous melatonin levels (Cajochen et al., 2003; Arendt et al., 1984). Accordingly, it is expected that the effects of melatonin in combination with relatively high homeostatic sleep need during the afternoon hours will substantially resemble the conditions which prevail at night, when melatonin is produced internally and sleep normally occurs.

Following oral administration, melatonin is rapidly absorbed with peak plasma levels occurring between 20 min and 2 h (Mallo et al., 1990; Arendt, 1994). We therefore performed our studies within this time window. The peak concentration of melatonin in the blood following ingestion of a 2 mg dose would be 4–20-folds higher than the endogenous peak levels at night (Dollins et al., 1994). Because brain melatonin concentrations are estimated to be 6–20-folds higher than serum levels, the ingestion of the 2 mg dose would yield brain melatonin levels comparable to those present normally in the brain at night (Nicholson, 1999).

Importantly, melatonin is not sedating; its sleep-promoting effects become significant about 2 h after intake (Wesensten et al., 2005) consistent with the physiological situation at night, when the rise in endogenous melatonin increases sleep propensity but does not force undesired sleep. Therefore, it was not expected that melatonin would enforce sleep under the experimental conditions used here, especially since subjects were not allowed to sleep during the fMRI sessions and were tested during the first 2 h after drug administration.

Methods

Subjects

12 subjects (2 men, 10 women, body mass 19.2–25.7 kg/m^2; age 25 ± 4.8 year) were studied. Subjects had no history of psychiatric illness, drug or alcohol dependencies, sleep disorders or a recent history of a trans-meridian trip. The study protocol was approved by the Tel Aviv Medical Center Human Sciences Ethics Committee. All subjects received a detailed explanation of the study and gave written informed consents.

Experimental design

The study had a double-blind, crossover design, balanced according to the order of treatment. Each subject attended two trials (2–3 h each, starting at 1600–1700 h), separated by at least 10 days. During each trial, melatonin (2 mg in 100 ml of 1% ethanol in water) or placebo (100 ml of 1% ethanol in water) was administered orally.

Experimental protocol

Subjects were instructed to abstain from alcohol for 24 h, maintain a regular sleep–wake schedule (sleep 2300 h–0700 h) on the night prior to the trial and abstain from caffeine during the trial. Each trial comprised two fMRI sessions: a baseline session and a randomized treatment session, commencing 1 h later. The drugs (melatonin or placebo) were given orally after the baseline session and subjects were instructed to remain awake in ambient room light for 1 h to allow the ingested melatonin to reach maximal levels in the blood.

Psycho-behavioral assessment

Mood and sleepiness were assessed using the Bond–Lader questionnaire (Bond and Lader, 1974). This is a self-report form that includes 16 10-cm visual analogue scales and provides the subjective ratings of mood and sleepiness. The Bond–Lader has previously been used to demonstrate fatigue inducing effects of melatonin in humans (Terlo et al., 1997). Questionnaires were filled twice in each trial, once before the baseline session and once after the treatment (melatonin or placebo) session.

Experimental tasks

Three tasks were designed in order to assess the effects of melatonin on activation of areas involved with auditory and visual information processing and of the hippocampal region. The visual task designed was attention-demanding (Visual search) whereas the auditory task was designed to be a passive task (Listening to music). The autobiographic memory task was selected with the intention of probing hippocampal activity.

Visual search task

An orientation "pop out", target detection task was used. Subjects were presented with a matrix of 20 half circular forms with one form pointing in the opposite direction. They were asked to identify the exceptional form in each matrix. The protocol was block designed with 6 task blocks of 9 s interleaved with fixation baseline blocks of 6/9 s. Each task block included 5 sets of matrices appearing for 1 s followed by 0.8 s fixation.

Listening to music task

This task included 5 min of passive listening to classical music (F. Schubert, Impromptus, Opus 142, A flat major). Task block was interleaved between two baseline blocks of magnet noise only (1.5 min each). During the experiment, subjects lay relaxed with eyes closed. We designed this protocol (comprising a single, relatively long, task block) with the intention of creating a setting with minimal demands of attention and minimal stress. The design allowed for linear trend removal and in pilot experiments demonstrated highly significant activation in the auditory cortex during the music block compared to the background noise blocks.

Autobiographic memory task

Subjects were presented with written autobiographic questions and asked to answer these questions wordlessly. An odd/even task was used as a baseline. Subjects were presented with a 1 digit number appearing for 1.5 s and asked to acknowledge whether this is an odd or even digit. The protocol was block designed with 5 task blocks of 24 s interleaved with baseline blocks of 15–24 s.

Image acquisition

Imaging was performed on a 1.5 T GE MRI system. Functional imaging employs a gradient-echo, echo planar imaging (EPI) sequence. The TR during the visual search and memory task was 3000 ms. In the music task, where time resolution was less of an essence, we used a TR of 10000 ms in order to increase signal to noise ratio and reduce magnet noise frequency so as to interfere less with the music. The other parameters were TE = 50 ms, 27 4 mm thick axial slices with no gaps, acquisition matrix dimensions 80 × 80 (reconstructed to 128 × 128), field of view 240 × 240 mm^2 (acquired resolution of 3 × 3 × 4 mm^3). For each subject, a 3D T1 SPGR sequence was performed for anatomical overlay. Acquisition parameters for the SPGR were: TR/TE = 30/9 ms, Flip angle of 15° with resolution of 0.93 × 0.93 × 2 mm^3.

Image processing and statistical analysis

Data analysis was performed using BrainVoyager 4.9 software (Brain Innovation, Masstricht, The Netherlands). Preprocessing included 3D motion correction and linear trend removal. High pass filtering (filtering out the lowest three frequencies) was used in the visual search and memory tasks. In addition, in view of the long TR used in the music test, serial slice time correction was added to preprocessing of music trials. The first six functional volumes, before signal stabilization, were excluded from analysis. Functional 2D data were manually aligned and co-registered with 3D anatomical data and transformed into Talairach space. Spatial smoothing (Gaussian 4mm kernel) was applied.

Functional analysis was performed using a general linear model (GLM). The model included all trials: melatonin baseline and treatment; placebo baseline and treatment (12 subjects participating in 4 trials resulting in 48 runs).

Task-related activation was defined in correlation to predictors of the used protocol. To account for a hemodynamic response, predictors in the visual search and memory tasks were shifted one or two TRs. The shift was selected to result in maximized correlation between individual blood oxygen level dependent (BOLD) signal intensity time course and predictors. No shift was used with the long TR in the music task. A separate predictor for the mean of each study (confound predictor) was added to account for differences in the mean level of signal time course at a voxel between studies. Z normalization of the time course of each study was performed to address differences in the variance of voxel time courses between studies.

Drug effect was investigated by comparing the four different groups: before melatonin intake; after melatonin intake; before placebo intake; after placebo intake.

A hypothesis driven region of interest (ROI) analysis allows the use of a lenient p for appropriately addressing a possibility for a melatonin effect as well as lack of such effect. In addition, it allows a proper evaluation of decreased as opposed to abolished task-related activation. In agreement with our hypothesis and the designed tasks, we conducted an ROI based analysis. In the visual search task, our ROIs were the visual and parietal cortex as well as the thalamus. During the auditory task, we examined only activation in the auditory cortex. The memory task was a priority designed to evaluate the hippocampus and parahippocampus. Included within our ROI are only areas that demonstrated significant task-related activation in a group analysis when all trials are included.

Drug effect was defined by a conjunction analysis of two contrasts: activation after melatonin intake > activation before melatonin intake AND activation after melatonin intake > activation after placebo intake. This analysis identifies regions that are affected by melatonin while excluding changes resulting from placebo or second examination effects. Statistical threshold for this analysis was set at $P < 0.05$ with a cluster size of 50 continuous voxels. Voxels in ROIs demonstrating melatonin but not placebo effect were further analyzed using individual parameter estimates (correlation weights) from affected areas. Drug effect, placebo effect and the differences (delta) between baseline and treatment values in melatonin compared to placebo trials were examined using a two-tailed, paired, Student's t test. Significance was set at $P < 0.05$.

The effect of melatonin was also separately evaluated. Two separate GLM studies defining task-related activation were performed for trials before and after melatonin intake. Random effect analysis and correction for multiple comparisons were applied to each GLM. We used the False Discovery Rate (FDR) for the visual search and memory tasks and a Bonfferoni correction for the music task (P corrected < 0.01). The reason for the choice of a highly conservative correction during the music task is that with a less conservative correction the paradigm used (a single 5 min block of listening to music) results in task-related robust and widespread brain activation curtailing melatonin's effect in the auditory cortex. Drug effect was examined by comparing the two states (before and after melatonin intake).

Behavioral variables and correlation statistical analysis

The distance in millimeters from the left edge of each of the 10 cm Bond–Lader visual analogue scales was measured for each

Table 1
Treatment effects on mood and sleepiness

Lader–Bond parameter	Difference from baseline mean + SD		*P* (*t* test)
	Placebo	Melatonin	
Sleepy–Alert	0.8 + 2.5	3.9 + 2.8	<0.001
Excited–Calm	1.2 + 2.6	−0.5 + 2	0.07
Weak–Vigor	0.4 + 1	1.4 + 2.5	0.2
Lucid–Confused	−0.5 + 1.3	−2.6 + 2.5	0.03
Clumsy–Well coordinated	0.4 + 1.3	2 + 2.8	0.1
Energetic–Fatigue	−0.4 + 1.7	−4.6 + 2.8	<0.001
Satisfied–Dissatisfied	−0.3 + 2.2	0.7 + 2.1	0.27
Calm–Worried	−0.7 + 1.8	0.2 + 2.5	0.45
Quick thinking–Slow thinking	−0.05 + 2.5	−2 + 3.9	0.13
Relaxed–Tense	−0.5 + 2.7	−0.6 + 2.7	0.93
Dreamy–Concentrated	0.9 + 3.3	3 + 3.5	<0.01
Very efficient–Inefficient	−1.6 + 2.2	−1.6 + 2.7	1
Sad–Happy	−0.08 + 1	0.45 + 1.7	0.41
Friendly–Hostile	0.2 + 1	−0.9 + 1.8	0.12
Bored–Interested	0.6 + 2	2.6 + 1.8	0.02
Sociable–Reclusive	0.6 + 1.6	−0.8 + 1.4	0.02

subject in each trial before drug intake (baseline) and after the treatment (melatonin or placebo). The mean differences (delta) between baseline and treatment values in the melatonin and placebo trials were compared using a two-tailed, paired, Student's *t* test. Significance was set at $P < 0.05$.

For each task, a correlation analysis was performed between behavioral and functional parameters. Each set was obtained independently. The behavioral variables were those found to be selectively affected by melatonin. The functional parameter estimates were derived from the brain areas that demonstrated melatonin's effect. The individual differences from baseline in both behavioral measurements and functional parameter estimates under melatonin and placebo treatments were used.

Results

Treatment effects on mood and sleepiness

Table 1 depicts the effects of melatonin 2 mg and placebo on the Bond–Lader questionnaire visual analogue scale items. Melatonin but not placebo caused significant increases from predosing scores in self-reported parameters of fatigue (item 6) and sleepiness (item 1). In addition, self-reported parameters of dreaminess (item11) and boredom (item 15) increased and lucidness decreased (item 4). The changes in these parameters as well as the reclusion parameter (item 16) were greater following melatonin than placebo treatment (Table 1).

Treatment effects on brain activities

Visual search task

Figs. 1 and 2 demonstrate the effect of melatonin on the activation of brain regions in the visual search task. While task-related activity was witnessed in an extensive bilateral network, drug effect analysis revealed a robust and statistically significant melatonin treatment effect within a specific area of the occipital cortex (Figs. 1a and 2). Specifically, an extensive region in the rostro-medial aspect of the occipital lobe (maximal effect centered around Talairach coordinates −3, −73, 5) shows reduced task-related activation after melatonin but not placebo ingestion. Fig. 1 represents the differential effects of melatonin over placebo in the affected area with fMRI parameter estimates indicating a significant drug effect. Positive activation is significantly reduced

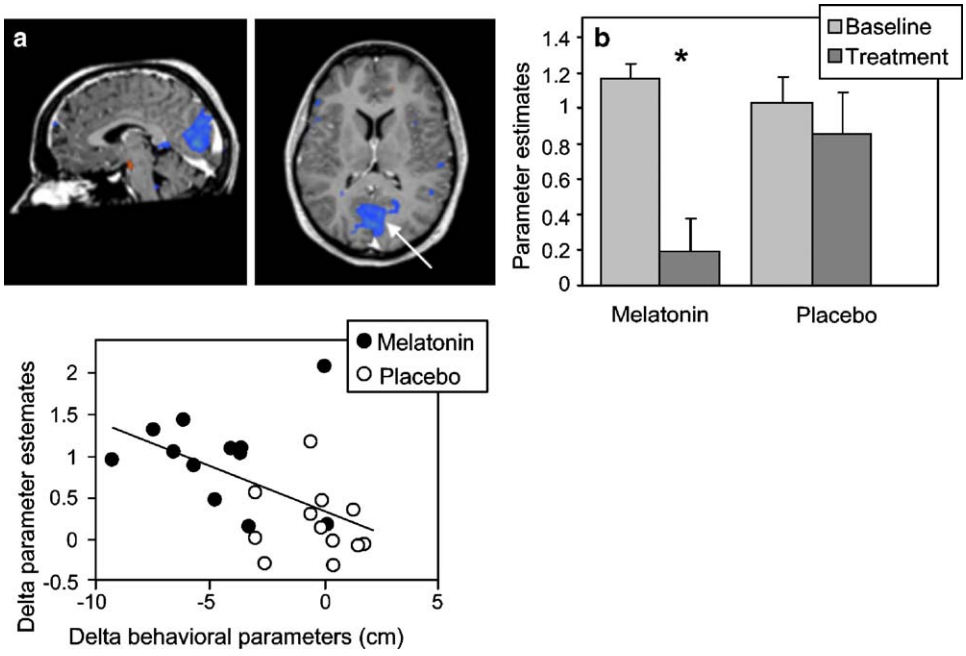

Fig. 1. The effect of melatonin in the visual search task. (a) Statistical parametric map of drug effect analysis (conjunction analysis, $P < 0.05$ uncorrected). The white arrow indicates reduced task-related activation (blue) in the rostro-medial occipital cortex following melatonin but not placebo intake. (b) Mean parameter estimates of activation in the rostro-medial occipital cortex. Error bars denote standard error. (c) Correlation between functional parameter estimates and corresponding behavioral parameters of fatigue.

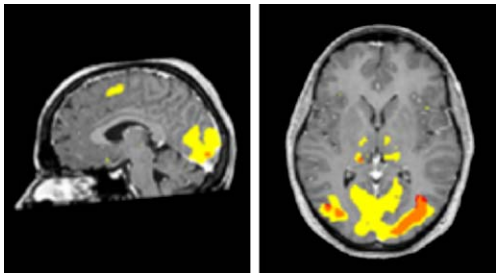

Fig. 2. The effect of melatonin in the visual search task. Statistical parametric map demonstrating task-related activation in the visual system before and after melatonin intake ($P < 0.01$ corrected). Yellow denotes activation before melatonin intake. Orange denotes overlapped activation (before and after). Note, significant activation in the caudal and lateral aspect of the visual system before and after melatonin intake as opposed to the rostro-medial aspect of the visual system showing melatonin's effect.

following melatonin but not placebo intake. The change from baseline (predosing) of this effect was significantly higher with melatonin than placebo ($P < 0.001$; Fig. 1b). All 12 subjects demonstrate reduced activations after melatonin intake with 2 subjects demonstrating negative activation. No placebo effect was demonstrated in this region. Fig. 2 shows task-related activation in the lateral occipital regions before and after melatonin intake while in the rostro-medial region activation is witnessed before but not after melatonin intake.

Furthermore, a significant correlation existed between an increase in self-assessed parameters related to sleepiness ($r = 0.49$, $P = 0.015$), fatigue ($r = 0.5$, $P = 0.013$); (Fig. 1c), dreaminess ($r = 0.5$, $P = 0.013$) and boredom ($r = 0.52$, $P = 0.01$) and the demonstrated reduction in functional activation in the rostro-medial aspect of the occipital cortex. For example, a larger increase in the score of self-reported fatigue is correlated with a larger reduction in the task-related parameter estimate from the affected area resulting in a negative correlation between fatigue and task dependent activation in this area (Fig. 1c).

Despite significant activation in the parietal cortex, thalamus and other visual regions during the visual task, these networks did not seem to be differentially affected by melatonin and placebo.

Passive listening to music task

Fig. 3 represents the differential effects of melatonin over placebo on the activation of brain regions in the passive listening to music task. Reduced bilateral task-related activation in the auditory cortex is demonstrated after melatonin but not placebo intake (Fig. 3a). In the right auditory cortex, a large cluster (maximal activation at coordinates 43, −23, 12) shows attenuated activation after melatonin but not placebo intake. In the left auditory cortex, a smaller cluster (maximal effect centered around coordinates −38, −25, 12) demonstrates a similar effect. Parameter estimates analysis confirms this drug effect. The difference between baseline and treatment values of this effect was significantly higher with melatonin compared to placebo (right $P < 0.01$; Fig. 3b, left $P < 0.05$). 11 subjects demonstrate reduced activity after melatonin intake in the right auditory cortex and 8 subjects demonstrate reduced activity after melatonin intake in the left auditory cortex. No placebo effect was demonstrated in this region. The effect of melatonin was also evident in activation maps employing random effect analysis and Bonfferoni correction for multiple tests (not shown).

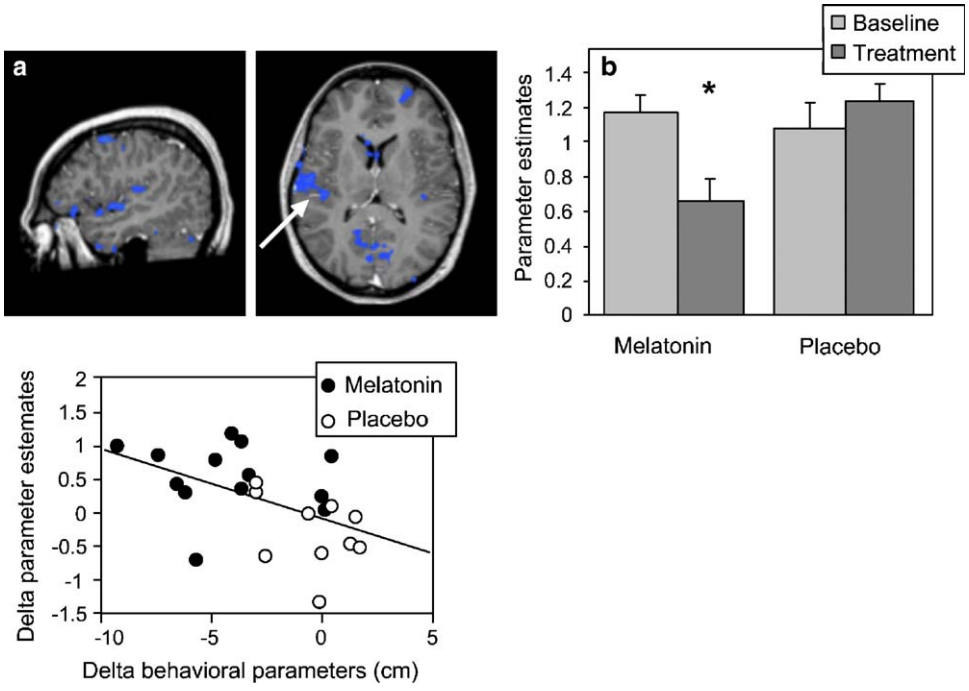

Fig. 3. The effect of melatonin in the music task. (a) Statistical parametric map of drug effect analysis (conjunction analysis, $P < 0.05$ uncorrected). The white arrow indicates bilateral reduced task-related activation (blue) in the auditory cortex following melatonin but not placebo intake. (b) Mean parameter estimates of activation in the right auditory cortex. Error bars denote standard error. (c) Correlation between functional parameter estimates and corresponding behavioral parameters of fatigue.

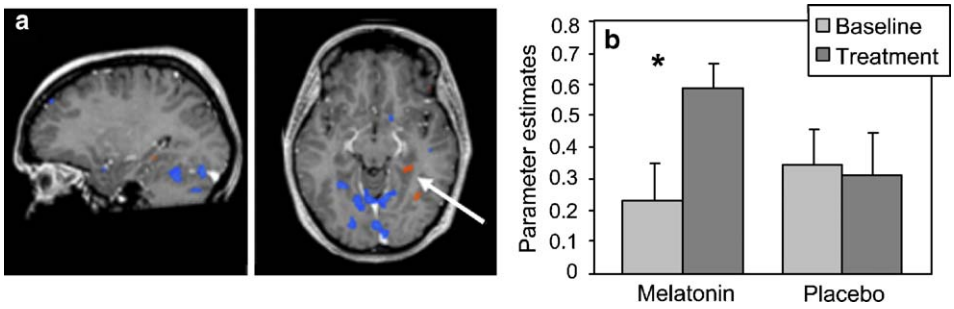

Fig. 4. The effect of melatonin during the autobiographic memory task. (a) Statistical parametric map of drug effect analysis (conjunction analysis $P < 0.05$ uncorrected). The white arrow indicates increased activation (red) in the left parahippocampus following melatonin but not placebo intake. (b) Mean parameter estimates of activation in the left parahippocampus. Error bars denote standard error.

While not within ROI analysis, attenuated activation within the medial occipital cortex was noted during the auditory task (Fig. 3b).

Significant correlations were found between the increase in perceived fatigue ($r = 0.50$, $P = 0.012$) (Fig. 3d), confusion ($r = 0.50$, $P = 0.013$), dreaminess ($r = 0.49$, $P = 0.016$) and boredom ($r = 0.63$, $P = 0.001$) and the reduction in the task-related activity of the right auditory cortex. For example, a larger increase in the score of fatigue is correlated to a larger reduction in parameter estimates from affected area resulting in a negative correlation between fatigue and brain activation in this area (Fig. 3c).

Significant correlation to the reduced activity in the left auditory cortex was found only with the subjective level of boredom ($r = 0.41$, $P = 0.045$).

Autobiographical memory task

Figs. 4 and 5 demonstrate the effect of melatonin on the activation of brain regions in the autobiographic memory task. Fig. 4 demonstrates a significant increase in the task-related activity in the left parahippocampus (maximal effect centered around coordinates −27, −33, −7) after melatonin but not placebo intake ($P < 0.01$; Figs. 4a and b). 10 subjects demonstrate increased activity in this region after melatonin intake. No placebo effect was demonstrated. As shown in Fig. 5, task-related activation in an extensive part of the left parahippocampus is witnessed only after melatonin intake.

No significant correlations were found between any of the subjective behavioral parameters assessed by the Bond–Lader questionnaire and the changes in parahippocampal activity ($r = 0.21–0.37$, $P > 0.05$ for all).

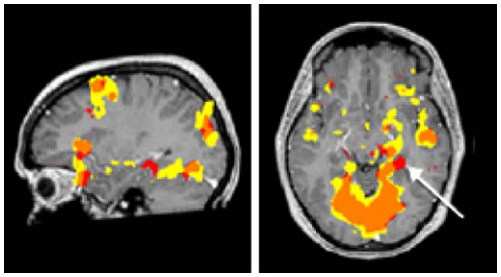

Fig. 5. The effect of melatonin during the autobiographic memory task. Statistical parametric map demonstrating task-related activation in the hippocampal region before and after melatonin intake ($P < 0.01$ corrected). Yellow denotes activation before melatonin intake. Red denotes activation after melatonin intake. Orange denotes overlapped activation (before and after). Note, there is a significant activation in an extensive part of the left parahippocampus only after melatonin intake.

Discussion

This study displays for the first time two different aspects of melatonin's action in the human brain: (1) attenuated activation within the visual system during a visual search task and within the auditory cortex during a music task. These effects are significantly correlated with increase in self-reported parameters of fatigue; (2) increased activation in the left parahippocampus during a declarative memory task. This effect does not correlate with any of the measures of fatigue or mood that are affected by melatonin.

The visual search task resulted in activation in the parietal and visual cortex as well as the thalamus. The effects of melatonin, however, were selectively evident in the rostro-medial aspect of the occipital cortex, a part of the visual system that covers the more eccentric part of the visual field (Grill-Spector and Malach, 2004). On the other hand, caudal and lateral visual areas, also showing task-related activity, did not demonstrate altered activity under melatonin. Surprisingly, neither the parietal cortex nor the thalamus, which are considered central for attention and arousal, exhibited drug-related effects. Nevertheless, it is possible that melatonin does have an effect on these brain areas, but these are overcome or compensated for during increased demands for attention.

The music task resulted in attenuated activation in the auditory cortex following melatonin treatment. No such effect was witnessed following placebo intake; thus, design setting cannot be responsible for this apparent effect. Reduced task-related activation in the auditory cortex after melatonin intake is also compatible with the reported decrease in the number of correct responses on the Wilkinson auditory vigilance task in wake individuals following ingestion of melatonin (Dollins et al., 1994).

Interestingly, results of the music task point toward a difference in response to melatonin between right and left auditory cortex, namely, the effect in the right hemisphere are more extensive and significant. The current design is not sufficient to claim asymmetry in melatonin's effect. However, one possible explanation for the apparent asymmetry is that it is related to the particular task selected (Schubert's Impromptus); in the adult brain, melody and rhythm processing has been found to show different hemispheric dominance, with the right hemisphere apparently more sensitive to melody and the left hemisphere to rhythm (Zatorre, 2001; Peretz and Zatorre, 2005).

In essence, it is reasonable to compare the effects of melatonin on brain activation patterns with those of sleep deprivation (increase in homeostatic sleep drive) and actual sleep. Imaging studies addressing sleep deprivation did not report a similar effect in the visual cortex. More so, most studies reported on significant effects of sleep deprivation in the parietal cortex. Thus, in the studied networks, the effect of melatonin appears to be different from that of sleep deprivation (Drummond et al., 1999, 2000, 2001; Choo et al., 2005; Bell-McGinty et al., 2004). This may seem surprising at first considering that both melatonin and sleep deprivation increase sleep propensity. On the other hand, it should be kept in mind that our study used no manipulation of the sleep homeostat (only one hour in between sessions, the same for melatonin and placebo trials). Therefore, the distinct effects of melatonin on brain activities found here from those seen following sleep deprivation indicate differential involvement of distinct brain networks in homeostatic (sleep deprivation) and circadian (melatonin) sleep regulation. Notably, a recent preliminary study comparing fluorodeoxyglucose PET during wakefulness in morning and evening hours has demonstrated lower relative metabolism in the occipital lobe in the evening (Buysse et al., 2004). The authors hypothesize that this could reflect increased homeostatic drive. The results of our study point to the evening rise in endogenous melatonin as a possible mediator of such changes.

We find a greater degree of similarity between our observations on the effects of melatonin and changes that have been previously observed during sleep. In sleeping and sedated infants and in spontaneously sleeping adults, visual stimulation during sleep results in robust cortical BOLD signal decreases in the rostro-medial occipital cortex (Born et al., 2002; Altman and Bernal, 2001; Martin et al., 2000). Likewise, the sedative drug pentobarbital has been found to dose-dependently reduce BOLD signal in this brain area during visual processing (Martin et al., 2000). As pentobarbital took effect, the positive BOLD contrast became weaker and finally vanished. 3 subjects with the highest sedative dose in fact displayed a negative BOLD signal.

The current study demonstrates that the effect of melatonin during a visual task is evident in a similar location, the rosto-medial occipital cortex. More so, while a negative BOLD signal was observed only in 2 subjects after melatonin intake, all other subjects indeed demonstrate a significant reduction in the positive BOLD signal. Thus, the effect of melatonin resembles in locality and direction the pattern previously observed during sleep though it is not identical to it. Our findings indicate that attenuation of activation in this brain area does not require sedation or slow wave sleep, but may be related to the induction of fatigue.

Indeed, there is some evidence in animal studies for an association of fatigue with sleep-related local changes in brain activity. Pigarev et al. recorded cortical activity from area V4 in monkeys during a visual search task and observed reduced, or even blunted, responses when animals became drowsy; background activity in this area changed to a pattern typically observed in sleep while the animals continued to perform the visual task (Pigarev et al., 1997).

Decrease in activation in the auditory cortex is again comparable to findings regarding auditory processing during sleep. Czisch et al. (2002, 2004) reported, in a combined fMRI/electroencephalographic (EEG) study, that acoustic stimuli presentation during sleep leads to reduced auditory activation; they suggested that there is a sleep stage dependent cortical deactivation upon stimulation. Similarly, Tanaka et al. showed bilateral reduced signal intensity in the auditory cortex during stage 1 non-rapid eye movement (NREM) sleep (Tanaka et al., 2003). Furthermore, Czisch observed a pronounced negative signal in the visual cortex upon auditory stimulation during sleep (Czisch et al., 2002, 2004). Our findings that activation in the medial occipital cortex during the auditory task is attenuated after melatonin intake thus resemble changes observed during sleep in this brain area. Moreover, the location of this effect is very much similar to the observed in our study during the visual task.

The changes in both visual and auditory task-related activity were found to be correlated with self-reported measurements of fatigue. This firmly suggests that observed changes are manifestations of subjective sleepiness, or, alternatively, sensitivity to states of fatigue.

It is most important to point out that subjects were not asleep during the study sessions. First of all, melatonin does not force sleep when undesired. Secondly, the visual experiment uses a very short paradigm (2.1 min) and subjects had verbal communications with the researchers at its beginning and ending and did not report on any sleep episodes. More so, it is quite clear that the effect of melatonin was not related to some decrease in perceiving the visual stimuli due to actual sleep. Had that been the case, the attenuated activation would be expected to encompass also the more caudal visual cortex, not just the rostro-medial aspect of the occipital cortex. Furthermore, if subjects were asleep, we would not expect the BOLD signal intensity in non-affected visual areas to accurately follow the given paradigm. Contrary, as shown in Fig. 2 that portrays task-related activity in the visual system before and after melatonin intake, melatonin had a selective effect in the rostro-medial aspect of the occipital cortex. The same arguments hold for the melatonin induced increase in activation in the parahippocampus during the memory test.

The passive music task may pose a greater confound regarding sleep during experiments because of its passive nature and the relatively long duration of the task. Although only one subject self-reported a questionable episode of sleep while listening to music under placebo treatment, the possibility of an occasional episode of stage 1 sleep during this task could not be ruled out. However, because sleep is associated with a decrease in the BOLD signal in the auditory cortex, we would expect a decline in the BOLD signal during episodes of actual sleep in the music period. It is important to note in this respect that the BOLD parameter estimates remained stable during the music period with respect to the signal before and after the music period. Thus, the reduced activation in the auditory system under melatonin is most probably not due to falling asleep during the trial. Nevertheless, future studies employing simultaneous EEG and fMRI may address this potential confound.

As the subjects were most probably awake during the sessions, we must conclude that attenuation in activation in the rostro-medial occipital cortex as well as in the auditory cortex do not require actual sleep. Thus, results converge to show that some of the brain responses to external stimuli previously ascribed to sleep may actually precede sleep. Possibly, melatonin suppresses the activation of specific neuronal networks in anticipation of sleep to protect the brain from arousing effects of environmental cues. This effect of melatonin may thus be a key element in the increase in sleep propensity at night (Lavie, 1997; Cajochen et al., 2003).

Contrary to decreases in sensory brain regions, the autobiographic memory retrieval task demonstrated increased activation in the left parahippocampus. As with the visual and auditory tasks, this effect also resembles changes in activation seen during sleep in this brain area. A relative increase in regional metabolism in the medial temporal lobe compared to wakefulness has been reported

in NREM as well as REM sleep (Nofzinger et al., 2002; Maquet, 2000). Furthermore, recordings from human hippocampal single neurons demonstrated greater burst firing during SWS compared with wakefulness (Staba et al., 2002). Information regarding declarative memory and sleep is fairly new, yet, Peigneux et al. (2004) demonstrated that both the hippocampus and the parahippocamus show increased rCBF during SWS and that this activity correlates with an overnight gain in performance.

In this respect, it is interesting to note that the change in activation of the parahippocampus was not correlated with any of the self-reported Bond–Lader parameters found to be affected by melatonin. This indicates that at least some of the melatonin-mediated changes in brain activation are not readouts of the subjectively perceived fatigue and mood.

We would like to point out that the rise in endogenous melatonin at night is a physiological and highly consistent phenomenon and that this rise does induce a very unique state of increased sleep propensity without sleep enforcement in humans. Our study contributes to the understanding of this phenomenon and demonstrates for the first time both an attenuated activation in the rostro-medial occipital cortex (an area known to show true deactivation during sleep) with parallel unaffected BOLD signal in other visual areas. In addition, it shows lack of effect in the thalamus and parietal cortex, reduced auditory activation and increased parahippocampal activity.

While effects of melatonin on the SCN could not be investigated with the currently available methods, the possibility that the results of our study are indirect effects mediated by hypothalamic or brainstem neurons should be kept in mind. Yet, the manifestation of melatonin's effect outside of these areas should not be ignored. Melatonin may also act locally within the visual and auditory networks and in the hippocampal region. Low levels of MT1 melatonin receptors mRNA were detected in various areas of the human brain including occipital and temporal cortices (Mazzucchelli et al., 1996) and striatum (Uz et al., 2005). In addition, in the human fetal brain, melatonin binding has been demonstrated in several cranial nerve nuclei including the cochlear nuclei (Thomas et al., 2002). Immunohistochemical evidence indicates the presence of melatonin receptors (both MT1 and MT2) in the human hippocampus (Savaskan et al., 2001, 2005). Low affinity melatonin binding sites and melatonin-mediated effects on neurochemical activities have been demonstrated in the preoptic area of the hypothalamus, ventral hippocampus, striatum, medulla-pons and cortex in rodents, particularly in rats (Zisapel, 2001a,b). The relevance of these activities to the mechanism by which melatonin induces fatigue and sleep anticipation in humans remains to be investigated. The results of our study show that the induction of fatigue by melatonin is associated with a wider brain network than apparent from the distribution of MT1 and MT2 receptors.

Conclusion

We show that melatonin ingested during afternoon hours induces specific changes in brain activation patterns. These resemble changes in brain activities that occur during sleep but not those observed after sleep deprivation. As subjects were awake, these findings indicate that melatonin induces sleep anticipation in the brain so that some sleep-related processes commence before actual sleep initiation. These data provide a physiological evidence for the pivotal role of melatonin in circadian sleep regulation, specifically in the opening of the sleep gate.

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