Report

Evidence that the Lunar Cycle Influences Human Sleep

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Summary

Endogenous rhythms of circalunar periodicity (~29.5 days) and their underlying molecular and genetic basis have been demonstrated in a number of marine species [1, 2]. In contrast, there is a great deal of folklore but no consistent association of moon cycles with human physiology and behavior [3]. Here we show that subjective and objective measures of sleep vary according to lunar phase and thus may reflect circalunar rhythmicity in humans. To exclude confounders such as increased light at night or the potential bias in perception regarding a lunar influence on sleep, we retrospectively analyzed sleep structure, electroencephalographic activity during non-rapid-eye-movement (NREM) sleep, and secretion of the hormones melatonin and cortisol found under stringently controlled laboratory conditions in a cross-sectional setting. At no point during and after the study were volunteers or investigators aware of the a posteriori analysis relative to lunar phase. We found that around full moon, electroencephalogram (EEG) delta activity during NREM sleep, an indicator of deep sleep, decreased by 30%, time to fall asleep increased by 5 min, and EEG-assessed total sleep duration was reduced by 20 min. These changes were associated with a decrease in subjective sleep quality and diminished endogenous melatonin levels. This is the first reliable evidence that a lunar rhythm can modulate sleep structure in humans when measured under the highly controlled conditions of a circadian laboratory study protocol without time cues.

Results and Discussion

We attempted to exclude confounders such as increased light at night, potential bias in perception regarding a lunar influence on sleep, and temporal information about the 24 hr day by retrospectively analyzing sleep structure, non-rapid-eyemovement (NREM) sleep electroencephalogram (EEG) activity, melatonin, and cortisol secretion measured in prior studies carried out under stringently controlled laboratory conditions, which allowed "unmasking" of circadian and potential lunar influences.

The distribution of sleep latencies (i.e., the time span between lights off and the first occurrence of stage 2 sleep in the EEG) yielded a distinct modulation by lunar phase that

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could be fitted by a sinusoidal function with peak sleep latencies around full moon (Figure 1). For further analysis, the data were binned into three lunar classes (see the Experimental Procedures). As expected, we found significant effects for the factors age and/or gender in the following sleep variables: subjective sleep quality, total sleep time (TST), REM sleep latency (RL), deep slow-wave sleep (i.e., stage 4), and sleep EEG delta activity (0.5-1.25 Hz, Figure 2; see Tables S1 and S2 available online for descriptive and PROC MIXED statistics). Surprisingly, the factor lunar class also yielded significance for all the above mentioned sleep variables, as well as for sleep latency (SL) and evening melatonin levels (Table 1 and Table S1), while cortisol levels did not attain significance. We found lower sleep quality and TST, less deep slow-wave sleep and sleep EEG delta activity, longer SL and RL, and lower evening melatonin levels 0-4 days around the full moon compared to the other lunar classes (Figure 3 and the related Figure S1). Thus, we have evidence that the distance to the nearest full-moon phase significantly influences human sleep and evening melatonin levels when measured under strictly controlled laboratory conditions, where factors such as light and personal moon perception can be excluded. While lunar cycles have been much celebrated in different cultures, and despite the persistent belief that our sleep is affected by the phases of the moon, so far there has been no reliable quantitative evidence that the moon can influence cortical activity during sleep. Thus, to our knowledge, this is the first report of a lunar influence on objective sleep parameters such as EEG activity during NREM sleep and a hormonal marker of the circadian timing system (melatonin) in humans, when putative external masking factors have been diminished. In a prospective field study using daily sleep logs in 31 people over 6 weeks, Röösli et al. [4] found that people slept a mean of 19 min less on nights with a full moon compared with a new moon. These 19 min are a rather good match with the 20 min reduction in EEG-assessed total sleep time in our study.

A lunar influence on human brain electrophysiological properties has so far only been reported in patients suffering from epilepsy, such that the onset of the status epilepticus peaked 3 to 4 days after new moon [5] and the periodicity of partial seizures displayed a predominant circalunar rhythm in women [6]. While the latter findings can still be explained by a potential disturbing effect of nighttime light on sleep that may eventually result in overt seizure activity or by a "menstrual clock" and thus by hormonal changes in women with partial seizures, these factors cannot account for the lunar modulation in sleep and melatonin observed in our study carried out in the dark and screened from the environment. Although we carefully controlled that the study volunteers kept a very regular sleep-wake rhythm and light-dark pattern for at least 1 week prior to study begin (see the Experimental Procedures), we cannot completely rule out potential confounds of priming or entrainment to external synchronizers (i.e., photophases of the lunar rhythm) prior to entering the lab. Thus, the observed lower evening melatonin levels around full moon may (Figure S2) be indicative of more evening light prior to entering the lab. The significant three-way interaction between age, gender, and lunar class for the evening melatonin levels hints



Figure 1. Time to Fall Asleep and Lunar Phase

Each data point (total of 64 nights double plotted) represents EEG-defined sleep-onset time (i.e., sleep latency: time between lights off and the first EEG occurrence of stage 2 sleep in minutes). The different color-coded symbols depict the different gender and age groups: pink for young women, blue for young men, white for older women, and gray for older men. Note: a lunar-phase (pictures upper abscissa)-dependent distribution could be fitted with a sinusoid function [f = y0 + a \cdot sin(2 \cdot pi \cdot x / b + c); goodness of fit, r = 0.46]. The colored boxes delineate the moon classes 1, 2, and 3, with moon class 1 comprising nights that occurred –4 and + 4 days around full moon, moon class 2 comprising nights that occurred 5 to 9 days before and after full moon.

to a complex interrelation between these factors, which is difficult to interpret. It is well known that with age, melatonin levels decrease, and more recently our and others' data indicate that premenopausal women have higher melatonin amplitudes than young men [7], older men, and postmenopausal women [8], which may have biased our findings related to lunar class.

Since the full moon does not have an explicit gravitational effect on terra firma, gravitational forces cannot explain lunar repercussions on human sleep. Although the moon clearly influences oceanic tides, it does not produce tides in smaller bodies of water such as lakes and even some seas, let alone in a human body [9]. Thus, we rather think that the observed rhythm represents an endogenous i.e., circalunar rhythm property, reminiscent of other endogenous rhythms such as the circadian (daily) and circannual (seasonal) rhythms. Indirect and preliminary evidence for this comes from the recent molecular and genetic dissection of a circalunar clock in a marine midge [2]. This circalunar clock is thought to tick inside many animals, running in synchrony with the tides and working in conjunction with the animal's circadian clock [1]. Wikelski and Hau [10] found that those Galapagos marine iguanas with the most accurate circalunar clock were most likely to survive tough times, presumably because they were the best at reaching feeding spots first-the predictive function of biological clocks is optimal timing [11]. Circalunar rhythms in nontidal-zone organisms may only be detected under laboratory conditions during which potential masking factors such as light, temperature, magnetic fields, hormonal status, etc. are stringently controlled for. Our findings of a 20 min shorter sleep duration, a 5 min longer time to fall asleep, and a 30% decrease in deep sleep are not small changes. Lunar rhythms are not as evident as circadian rhythms and are thus not easy to



Figure 2. Sleep EEG Power Density and Lunar Phase

EEG power density between 0.5 and 25 Hz during NREM sleep for moon class 1 (around full moon) and moon class 3 (10 to 14 days distant from the next full moon), expressed as percentage of the average of moon class 1, 2, and 3. Mean values \pm SEM in the occipital derivation (Oz) are shown. Symbols near the abscissa indicate frequency bins for which a significant effect for the factors "lunar class," "age," and "gender" was found (PROC MIXED; open triangles facing down illustrate the factor "gender," filled triangles facing up mark the factor "age," and the open circles with a central dot delineate the significant effect of "lunar class"). See also Figure S2.

document—but they exist [11]. Their role is mysterious, and there are probably large individual differences that underlie the contradictory evidence for their existence—some people may be exquisitely sensitive to moon phase. It remains challenging to unravel the neuronal underpinnings of such a putative lunar clock in humans. Combining functional imaging techniques and the EEG in controlled chronobiological study settings my help unravel such brain mechanisms.

Experimental Procedures

Subjects

Seventeen healthy young volunteers (nine women and eight men; age range, 20-31 years; mean, 25.0 ± 3.6 years [SD]) and 16 healthy older volunteers (eight women and eight men; age range, 57-74 years; mean, 65.0 ± 5.5 years) participated in a 3.5-day laboratory study on circadian and homeostatic aspects of human sleep/wake regulation [16]. Thus, the aim of exploring the influence of different lunar phases on sleep regulation was never a priori hypothesized, nor was it mentioned to the participants, technicians, and other people involved in the study. We just thought of it after a drink in a local bar one evening at full moon, years after the study was completed. Thus, this study reflects a post hoc assessment of the potential influence of lunar phase on sleep in a cross-sectional setting. All subjects were nonsmokers, took no drugs (drug screening before study begin) or medication, and were free from medical, psychiatric, and sleep disorders. Five young female volunteers used oral contraceptives. Young women were studied during the follicular phase of their menstrual cycle (follicular days 1-5). The clinical health of all subjects was assessed by questionnaires, physical examination, blood samples (only with older subjects). interviews, and a polysomnographically recorded adaptation night. This night served as screening night for potential restless legs syndrome and sleep apnea, as well as for good sleep quality (sleep efficiency >80%) for both young and older study participants. During the baseline week preceding the study, the subjects were instructed to keep their individual bed- and wake-time within a self-selected range of ± 30 min and to attempt to sleep for 8 hr. as assessed by a wrist activity monitor (Cambridge Neurotechnologies) and sleep logs. Adherence to this was accurately checked for each volunteer prior study begin by inspection of the volunteer's sleep logs and checking of sleep times derived from the actimetry recordings. Study participants were asked to abstain from excessive caffeine and alcohol

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Table T. Sleep and Endocrine variables and Lunar Phase							
	Lunar Class 1		Lunar Class 2		Lunar Class 3		Significance
Variable	Mean	SE	Mean	SE	Mean	SE	for Lunar Class
Bedtime	23.2	0.2	23.5	0.2	23.5	0.2	
Rise time	7.2	0.2	7.5	0.2	7.5	0.2	
Sleep quality	51.2	3.7	58.6	3.5	56.4	3.5	*
TST	409.0	7.9	433.2	6.8	424.8	11.0	*
WASO	13.9	2.1	8.3	1.7	10.6	2.9	
SL2	16.3	1.9	10.9	1.6	12.1	1.3	*
RL	89.3	8.0	58.9	5.2	76.5	7.4	*
MT	2.9	0.4	2.0	0.2	2.7	0.3	
Stage 1	14.0	1.2	14.7	1.5	12.7	1.2	
Stage 2	57.6	2.3	54.8	2.2	54.2	2.1	
Stage 3	8.7	1.1	8.0	0.9	9.2	1.2	
Stage 4	2.4	0.6	4.2	1.3	6.1	1.5	*
SWS	11.1	1.6	12.1	2.0	15.2	2.0	
NREM	78.1	2.2	75.6	2.5	74.5	2.5	
REM	17.2	1.2	18.3	1.2	17.5	1.3	
Melatonin	3.9	0.6	7.5	2.2	8.2	1.9	*
Cortisol	2.1	0.7	1.0	0.1	1.2	0.2	

Subjective sleep quality, sleep parameters of the second baseline night (always on a Tuesday to Wednesday) based on visual scoring for all lunar classes 1–3, and melatonin levels 2 hr prior bedtime. Except for two, each volunteer contributed with two baseline nights. If both nights of a volunteer occurred in the same lunar class, the nights were averaged per subject, this resulted in n = 21 for lunar class 1, n = 17 for lunar class 2, and n = 15 for lunar class 3. TST, total sleep time; WASO, wake after sleep onset (in percent of TST); SL2, sleep latency to stage 2; RL, REM latency; MT, movement time after sleep onset (in percent of TST); Stage 1–Stage 4, sleep stages 1–4 (in percent of TST); NREM, non-REM sleep (sum of sleep stages 2–4 in percent of TST); REM, REM sleep in percent of TST. Asterisks indicate variables that yielded significance for lunar class.

consumption. The study protocol, the screening questionnaires, and consent form were approved by the Ethical Committee of Basel, Switzerland and were in agreement of the Declaration of Helsinki. After a long personal discussion of all protocol details with an investigator, the study participants gave their written informed consent.

Protocol

The entire study was carried out between June 17, 2000 and December 2, 2003 across all seasons. In general, there were more study protocols in summer than in winter, independent of lunar class. The protocol consisted of two baseline nights in the sleep laboratory either followed by a 40 hr episode of sleep deprivation or a 40 hr sleep satiation (i.e., naps) and an 8 hr recovery sleep episode (for a detailed description of the sleep deprivation/satiation protocol, see [12-14]). There was a least 1 week in between the two protocols. The order of the protocols was balanced between age and gender. The entire protocol was carried out under constant routine (CR) conditions (<8 lux, temperature 21°C, semirecumbent posture in bed, regular small isocaloric snacks and water, and no time cues [15]). The timing of the 8 hr sleep episodes during the laboratory study was scheduled by centering of the midpoint of the subject's habitual sleep periods at home during the baseline week (as assessed by actigraphy). Continuous polysomnographic recording started before the second baseline night, which always occurred at the same day of the week (Tuesday-Wednesday) in order to avoid potential weekend variability. The older study participants received a daily low-dose heparin injection (Fragmin ± 0.2 ml, 2,500 IE/UI, Pharmacia) while recumbent in the CR (more details in [16]).

Sleep EEG Recordings and Analysis

The sleep EEG was recorded from 12 derivations referenced against linked mastoids (A1, A2), together with two electrooculograms (EOGs), one electrocardiogram (ECG), and one submental electromyogram (EMG) using a digital ambulatory sleep recorder system (Vitaport-3 digital recorder, TEMEC Instruments). All signals were filtered at 30 Hz (fourth-order Bessel type antialiasing low-pass filter, total 24 dB/Oct). A time constant of 1.0 s was used prior to online digitization (range 610 \pm μ V, 12 bit AD converter, 0.15 μ V/bit, sampling rate at 128 Hz for the EEG). The raw signals were stored on a Flash RAM card (Viking) and downloaded offline to a local



Figure 3. The Influence of Lunar Phase on Sleep Variables and Melatonin From top to bottom: subjective sleep quality as assessed on the Leeds Sleep Evaluation Questionnaire (LSEQ) in the morning on waking, objective total sleep time and sleep latency in minutes from PSG recordings, stage 4 sleep and occipital EEG delta activity (0.5–1.25 Hz) as a percentage of the value at -9/+9 day around full moon, and salivary melatonin levels in the evening before lights off (average of 2 hr before lights off). Mean values \pm SEM (total n = 64) are shown. Data are plotted according to lunar classes: 0–4, 5–9, and 10–14 days distant from the nearest full moon phase. See also Figure S1 and Table S1.

computer hard drive. Sleep stages were visually scored per 20 s epoch according to standard criteria [17]. Artifact-free sleep EEGs (automated artifact detection algorithm: CASA, 2000 Phy Vision BV, Gemert) were subjected to spectral analysis with a fast Fourier transformation (10% cosine 4 s window), resulting in a 0.25 Hz bin resolution. For data reduction, artifact-free 4 s epochs were averaged over 20 s epochs. Sleep EEG power spectra were calculated during NREM sleep (stages two, three, and four) in the frequency range from 0.5 to 32 Hz. Here, we report EEG power density derived from the midline (Fz, Cz, Pz, Oz) during NREM sleep in the range from 0.5 to 25 Hz.

Classification of Lunar Phase

For each investigated study night date, we calculated the distance in days to the date of the closest full moon phase according to the "Münchner Astro Archiv" at http://www.maa.mhn.de/StarDate/moonphases.html. This difference was subdivided in three lunar classes: class 1, 0–4 days; class 2, 5– 9 days; and class 3, 10–14 days to closest full moon. This subdivision included both waxing and waning moon phases (see Table S2 for an example).

Salivary Melatonin and Cortisol Assays

Saliva collections for hormonal assays were scheduled every 30 min during scheduled wakefulness throughout the entire study protocol. A direct double-antibody radioimmunoassay (RIA) was used for the melatonin assay, which was validated by gas chromatography-mass spectroscopy with an analytical least detectable dose of 0.65 pg/ml (Bühlmann Laboratories) [18]. Cortisol was measured by RIA (Ciba Corning Diagnostics) with a detection limit of 0.2 nmol/liter. The intra-assay coefficient of variances was 4.0% above 0.4 nmol/liter and 10.0% for levels below.

Statistics

The statistical package SAS (SAS Institute; version 9.3) was used. The analysis of sleep stage, delta-EEG power density, and melatonin and cortisol differences was carried out with the mixed-model analyses of variance (PROC MIXED) with factors "age" (young, older), "gender" (women, men) and "lunar class" (1, 2, 3). Alpha adjustment for multiple comparisons was applied with the Tukey-Kramer test, which was also used for post hoc comparisons. For each EEG derivation (Fz, Cz, Pz, and Oz) and for each EEG power density bin in the range of 0.5–25 Hz, a PROC MIXED was applied with above mentioned factors. The number of nights, gender, and age distribution were equal among the three different lunar classes ($\chi^2 \leq 2.14$, p at least 0.15; see Table S3).

Supplemental Information

Supplemental Information includes two figures and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.06.029.

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