EEG Delta Activity During Undisturbed Sleep in the Squirrel Monkey

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The squirrel monkey (Saimiri sciureus) exhibits a robust daily rhythm of sleep-wakefulness that is under circadian control, but the nature of homeostatic sleep regulation in this diurnal primate is poorly understood. Since delta frequency (0.5-2.0 Hz) activity in the electroencephalogram (EEG) during non-Rapid Eye Movement (NREM) sleep is thought to reflect homeostatic factors contributing to sleep tendency, we measured EEG delta power density and slow wave incidence and amplitude during NREM sleep during spontaneous sleep, occurring when monkeys were housed undisturbed in a 24-hour light-dark (LD) cycle and in constant light (LL). In LD and LL conditions, monkeys exhibited circadian rhythms in delta power density, wave incidence and wave amplitude that peaked in the middle of the subjective night, several hours after consolidated sleep onset. These results differ from predictions of a purely homeostatic model of sleep that would include maximal levels of delta activity at sleep onset.

CURRENT CLAIM: Delta frequency (0.5-2.0 Hz) activity in the electroencephalogram during non-Rapid Eye Movement sleep during both undisturbed light-dark and constant light conditions in a diurnal primate, the squirrel monkey (Saimiri sciureus), does not fit predictions of a purely homeostatic model of sleep.

One of the proposed functions of sleep is to provide a physiological and psychological environment during which restorative processes can occur. A marker of such a process might be expected to have increasing levels during wake and decreasing levels during sleep. Experimental observations have suggested that slow wave sleep (SWS) and the delta frequency (0.5-4.0 Hz) components of the electroencephalogram (EEG) during NREM sleep, high levels of which define SWS, have such homeostatic characteristics in humans and nocturnal rodents. SWS and delta frequency activity levels are positively related to prior wake duration and are conserved in the face of experimentally decreased (Akerstedt and Gillberg, 1981; Webb and Agnew, Jr., 1971a, 1971b; Feinberg et al., 1980, 1982; Feinberg and Floyd, 1982b; Hume and Mills, 1977a, 1977b), increased (Feinberg et al., 1980, 1982, 1985) or fractionated sleep time (Karacan et al., 1970). In addition, delta activity is usually observed to be maximal at sleep onset, when sleep need and intensity in a homeostatic feedback system should, theoretically, be maximal (Borbely et al., 1981; Church et al., 1975; Feinberg and Floyd, 1982; Feinberg et al., 1982; Koga, 1965). Although a circadian influence on delta activity has been previously reported, the data are not conclusive (Akerstedt and Gillberg, 1981; Dijk et al., 1990; Agnew, Jr. et al., 1967; Campbell, 1984; Campbell and Zulley, 1987; Gagnon et al., 1985; Hume and Mills, 1977a, 1977b; Webb, 1986; Weitzman et al., 1980) since total prior wake time or amount of wake within a sleep episode was not controlled and therefore a circadian rhythm independent of homeostatic sleep-wake processes could not be determined.

To understand the relative strengths of the homeostatic and circadian effects on the amount of sleep and on delta activity in the EEG during sleep, these studies need to be extended beyond the species–nocturnal rodents and diurnal humans–in which they have previously been conducted. The circadian and homeostatic effects on EEG measures and sleep should be tested for comparison across all species: monophasic versus biphasic versus polyphasic sleepers, diurnal versus nocturnal animals, and primate versus rodent versus non-mammalian species. For example, when both non-human diurnal primates and humans are studied, cognitive factors and investigator-determined sleep timing can be analyzed as sources of species differences in results. In this report, spontaneous sleep and the delta activity in the EEG during NREM sleep were studied under undisturbed light-dark (LD) and constant light (LL) conditions in the squirrel monkey, a diurnal primate with consolidated sleep-wake rhythms similar to humans. If delta activity reflects a measure of sleep homeostasis independent of circadian processes, it should be maximal at sleep onset in both LD and LL conditions.

METHODS

General Conditions

Four male and four ovariectomized female adult squirrel monkeys (Saimiri sciureus) were studied. At the time of surgery, the females weighed 598-815 g and the males weighed 975-1217 g. The experimental protocols complied with the "Principals of Animal Care" (Publication 86-23, Revised 1985) and were approved by the Harvard Medical Area Standing Committee on Animals.

After surgery (described below), each animal lived in a stainless steel cage (46x46x55 cm) within a light-tight, sound attenuating, electrically shielded chamber in a temperature-
controlled experimental room. Temperature inside the chambers was maintained at 26-28°C. Lighting in the chamber was provided by a 7-watt incandescent lamp that generated a maximum of 23 lux. Continuous white noise was played in the room to mask extraneous sounds. The chambers were opened several times a week for replenishing food (Teklad diet TD76357, supplemented with fresh fruit) and water supplies, changing the litter, replacing broken cables and wires in the commutator swivel assembly, and checking on the condition of the animals. An effort was made to limit these openings to times when the animals were awake and active. The monkeys were kept first under daily light-dark cycles (LD 12:12, lights on at 08:00 h Eastern Standard Time), then under constant illumination (LL). LL recordings were made after the animals had been in LL for 2-3 weeks.

Surgery

Two surgical and EEG recording procedures were used. Group A animals (three males) were implanted with both cortical and subcortical electrodes. All electrodes were connected to preamplifiers enclosed in a small aluminum box (33x33x10 mm) that was fixed to the skull. Group B animals (one male and four females) had cortical EEG electrodes only and no preamplifiers.

The monkeys were anesthetized with pentobarbital (15 mg/kg) following premedication with atropine (0.02 mg/kg) and diazepam (1 mg/kg) and placed in a stereotaxic apparatus. In Group A animals, cortical EEG electrodes (gold-plated Amphenol pins) were inserted through the skull to rest on the dura in frontal (anterior/lateral coordinates relative to bregma: 14.5/-3.5 mm) and central (0/6 mm) positions. Each monkey also had two deep electrodes aimed at the lateral geniculate nucleus (LGN) and another brain region for recording multiple unit activity (Boulos et al.; unpublished observations). The guide tubes for the deep electrodes served as electrical ground. In Group B animals, gold-plated cortical EEG electrodes were placed in frontal (15/5 mm), central (0/5 mm) and occipital (20/5 mm) positions. An anchor screw served as ground.

In both groups, electrooculogram (EOG) electrodes were anchored to the outer canthi and electromyogram (EMG) electrodes were sutured to the lateral trapezius muscle. Glass microbead thermistors for recording body temperature were implanted under the temporalis muscle (Group A) or intracranially (Group B) (data not reported here). Mercury switches fixed to the skull were used for recording motor activity (data not reported here).

All animals were treated with antibiotics (Oxacillin) on the day of surgery and on the following three days. Group A animals also received dexamethasone to reduce the risk of edema. The animals were allowed a minimum of three weeks to recover from surgery before sleep recordings began in LD.

Data Collection and Analysis of Sleep State

Each animal was connected to a commutator swivel that allowed free movement within the cage. Wires carrying EEG (cortical and LGN), EOG, EMG, temperature and activity signals ran within shielded cable to data collection devices, including sleep recording equipment, located outside the chamber. Animals were continuously connected to the commutator swivel.

During a sleep recording, EEG, EOG or LGN, and EMG signals were passed from the swivel through the preamplifiers and filters of a Grass Model 77 12-channel polygraph. The outputs were recorded simultaneously on paper (usual paper speed of 2.5 mm/sec) and analog tape via a Hewlett-Packard FM tape-recorder. A slow paper speed was chosen because recordings lasted up to 144 hours. Polygraph filter settings were EEG 0.3/30, EOG 1/30, LGN 10/60 and EMG 10/90 Hz (low/high frequency cutoffs). The EEG derivation used was contralateral central/frontal for Group A animals and ipsilateral frontal/occipital or ipsilateral frontal/vertex for Group B animals.

Sleep records were scored in 1-minute epochs as one of five states by one person (EBK): awake, REM sleep, NREM sleep, sleep deprivation, or unscorable (Wexler and Moore-Ede, 1985). Consolidated sleep and wake episodes were defined using criteria developed by Wexler and Moore-Ede (1985): a consolidated wake (CW) episode consisted of 10 consecutive minutes of wake followed by at least 50% wake in each of the next three hours. A CW episode lasted until the beginning of the next Consolidated Sleep (CS) episode, defined as 10 consecutive minutes of sleep (NREM or REM sleep) followed by at least 50% sleep in each of the next three hours. A CS episode lasted until the next CW onset. Undisturbed sleep recordings in LD and LL lasted 1-3 cycles; 19 cycles in 14 LD records and 46 cycles in 27 LL recording sessions were obtained. The recordings in LL were the baseline sleep recordings for sleep deprivations (reported elsewhere [Klerman et al., 1999]).

Special EEG Data Collection and Analysis

For each sleep recording, the tape recorder was internally calibrated at 2.5 V before recordings began. Polygraph calibration signals (50 and 500 µVolt) were recorded on paper and tape at the beginning of each recording. One channel of the tape recorder was devoted to the time signal. A once-per-second signal was recorded onto this channel and onto the paper record. At the beginning and ending of recordings, individual tapes, individual paper records, and sleep deprivations a DC voltage mark was superimposed onto this channel. This mark was used during analysis to time-match paper and tape data. Each tape was run at 15/32 speed and was changed at 12-12.5-hour intervals. Tape changes lasted approximately three minutes; during these three minutes, the paper recording was uninterrupted, but no data were recorded onto tape.

The tape output was calibrated for analog to digital conversion using the previously recorded 50 and 500 µVolt calibration signals. Digitizing began at the DC voltage time mark so that time-matched blocks of sleep state and EEG analysis could be identified and compared. The signals were played back through a 25 Hz low pass 8 pole Butterworth filter at a gain of 0 or 20 dB, digitized at 64 Hz, and stored on
computer magnetic tape in blocks of 4 seconds (256 points). For spectral analysis ("power density"), the data were smoothed with a cosine taper window and the mean was subtracted before a 256 point Fast Fourier Transform was performed. Zero-crossing analyses were performed on the same epochs of EEG data using a program supplied by Dr. B. Bergmann. The program calculates the number of half-waves ("wave incidence") and the average area under each of the half-waves per epoch ("wave amplitude"). It assigns a frequency to each of the half-waves according to the half-wave length and stores the values in the appropriate bin. For both EEG analysis methods (Fourier and zero-crossing analyses), data from 15 consecutive blocks were added to yield one minute cumulative values in the desired frequency ranges (0.5-2.0 Hz and 0.5-25.0 Hz). The results were compared with the time-matched sleep state data.

The data from both EEG analysis methods were edited to eliminate artifacts. A cutoff level for the artifacts was visually determined for each EEG analysis variable for each recording before secondary analyses were performed. Minutes with artifacts tended to occur when the animal was awake and had values greater than three times different from preceding and following minutes. Data from minutes with values above this cutoff were not used in subsequent analyses of delta activity. The same secondary analyses were performed on data from both EEG analysis methods. Data were normalized for each recording before averaging across recordings was performed.

In LD conditions, subjective day was defined as beginning at lights-on and subjective night at lights-off. In LL conditions, subjective day began, by definition, at the last undisturbed CW onset of the recording, defined as circadian time 0, and at multiples of 24 circadian hours.

Sinusoid and exponential functions were fit to group average data using least-squares methodology and the significance of the rhythms was calculated. Mixed ANOVA was used to compare the 1-hour bins relative to the first hour of CS. Statistics were performed using SAS®.

**RESULTS**

**Delta Activity During Undisturbed LD and LL Conditions**

Under LD, sleep occurred primarily during the dark period, although short sleep bouts (naps) were occasionally evident in the middle of the light period (Figure 1). Delta power density,

*Figure 1.* Sleep state and delta activity measures (one-minute epochs) during one CW-CS cycle in LD (one animal) and during one CW-CS cycle in LL (another animal). The top graph is sleep state, the lower three are delta activity measures (power density, wave incidence, wave amplitude). The dark bar indicates when lights were off during LD conditions.

**LD**

- Wake
- REM
- NREM

**CS onset**

**LL**

- Wake
- REM
- NREM

**CS onset**

**Power Density (µV²/1000)**

- 125
- 100
- 75
- 50
- 25
- 0

**Wave Incidence (# half waves)**

- 125
- 100
- 75
- 50
- 25
- 0

**Wave Amplitude (µV)**

- 125
- 100
- 75
- 50
- 25
- 0

**Time relative to CW onset (hours)**

sr0049

sr0046
wave incidence and wave amplitude were higher during nocturnal sleep than during naps. The daily patterns of sleep (both consolidated nocturnal and short nap) and delta activity were also apparent in animals in LL (Figure 1).

Group average waveforms of delta activity, normalized by number of NREM sleep minutes, revealed a circadian rhythm of delta power density, wave incidence and wave amplitude within NREM sleep ($p<0.002$ for all measures) in both LD and LL (Figure 2). In both LD and LL, delta power density and wave incidence within NREM sleep began to increase during the latter half of the subjective day, reached a maximum around mid-subjective night, and gradually declined thereafter. Thus,

Figure 2. The number of minutes of NREM sleep and of the delta activity per minute of NREM sleep (average and standard deviation) in 30-minute bins across the cycle in LD and LL. The dark bar at the bottom of the LD plot indicates when lights were off. For the LD condition, the curve was aligned by lights off; for the LL conditions, they were aligned by the last undisturbed Consolidated Wake onset. Two time bins during the subjective day in LD conditions, bins number 7 and 21, had no one-minute epochs scored as NREM sleep; measures of delta activity could not, therefore, be obtained for these bins.
both Fourier and zero-crossing analysis of the EEG demonstrated circadian rhythms in delta activity independent of the amount of NREM sleep.

To quantify the phase and amplitude of circadian variations in delta activity, a best fitting sinusoid was obtained for each delta activity variable. In both LD and LL, the maxima of the fit sinusoid occurred several hours after average Consolidated Sleep (CS) onset. Plotted relative to circadian phase (not CS onset), the acrophase of sinusoids fit to the NREM sleep and power density and wave incidence data in LD was at approximately mid-subjective night (CT 18). For LL conditions, the sinusoid fit to the NREM sleep, power density and wave amplitude measure also had its maximum at approximately CT 19. The time of the peak of the wave incidence curve was slightly more than one hour earlier, at approximately CT 18; however, its amplitude was ~1/3 that of the other analyses. Based on visual inspection, NREM sleep reached a plateau in both LD and LL shortly after subjective night began, several hours before the acrophase of the sinusoids fit to the delta activity measures. When an exponential was fit to data only from CT 12-24 (subjective night), only delta power had a significant decline during subjective night in both LD and LL.

The timing of delta activity during NREM sleep was further evaluated by collapsing the data into 1-hour time bins and expressing these as ratios against the first 1-hour bin of CS (Figure 3). The average value of NREM and delta activity measures was higher in the second hour of CS than in the first. There was no significant monotonic decline in cumulative power density from the values in the first hour of CS in LD but there was an overall linear decline in power density ($F_{1,437}=9.78, p=0.0019$) and wave amplitude ($F_{1,437}=7.87, p=0.0053$) in LL. When the data were normalized by number of minutes of NREM sleep, there was no significant linear decline in any delta activity measure during CS. There was a significant linear decline beginning in the 2nd hour of CS in

**Figure 3.** NREM sleep (line), delta power density (filled squares), delta wave incidence (open circles) and delta wave amplitude (filled triangles) in successive one-hour bins starting at CS onset relative to the first one-hour bin of CS in LD (left two graphs) and LL (right two graphs) conditions. Both total values (top two graphs) and values per minute of NREM sleep (bottom two graphs) are plotted for the three delta activity measures. NREM sleep data are not included in the bottom two graphs because those data have been normalized by the number of NREM minutes. The dashed horizontal line indicates 1.0 (the value in the first hour bin).
only power density ($F_{1,152}=8.96, p=0.0032$) and power density normalized by the number of minutes of NREM sleep ($F_{1,152}=4.21, p=0.0419$) during LD conditions and in power density ($F_{1,39}=11.97, p=0.0006$), wave incidence ($F_{1,39}=9.12, p=0.0027$) and wave amplitude ($F_{1,39}=5.14, p=0.0239$) during LL conditions.

**DISCUSSION**

This study examined the pattern of EEG delta activity during NREM sleep in undisturbed sleep recorded across the circadian cycle in squirrel monkeys. Both cumulative delta activity and delta intensity (delta activity per minute of NREM sleep) exhibited a circadian rhythm with higher levels during subjective night than subjective day under both entrained and free-running conditions. Within a CS episode, NREM sleep and delta activity peaked, not at sleep onset as would be expected in a purely homeostatic system, but several hours after CS episodes began. While some individual recordings (e.g., Figure 1) showed a non-monotonic decline in delta power over the entire CS episode (lasting 8-12 hours), most recordings did not show any decline. Instead, the average delta activity increased during the first hours to a maximal level 2-4 hours after CS onset in both LD and LL. In another series of studies of squirrel monkeys in LD, Slow Wave Sleep 2 ("deeper" NREM sleep) was not observed until after the first 60-90 minutes of consolidated sleep (Edgar, 1986). This feature of the data was also observed after sleep deprivations ending at different circadian phases (Klerman et al., 1999).

There are a number of factors which may account for the discrepancy between our results and those of other studies of human and nocturnal rodent sleep which report maximal power density, wave incidence and/or wave amplitude values at the beginning of the consolidated sleep episode (for humans, see Agnew, Jr. et al., 1967; Borbely et al., 1981; Church et al., 1975; Dijk et al., 1987; Feinberg et al., 1978, 1982; for rats, see Borbely and Neuhaus, 1979; M斯特尔berger et al., 1987). While squirrel monkeys were chosen because their consolidated nocturnal sleep patterns are more similar to that of humans than rodents, these monkeys may take longer to "build-up" or become "efficient" in their sleep. This may result in a delayed expression of the homeostatic influence. Alternatively, a circadian influence on NREM sleep, and delta frequency EEG kinetics may be stronger in squirrel monkeys than in other species. Other reasons for the difference between this and most studies of other species may be related to methodology. Most studies of human sleep analyze delta activity by NREM-REM cycle, which is not fixed in length or in number of minutes of NREM sleep. Therefore, changes in delta activity could be obscured by larger or opposing changes in time spent in NREM sleep or could be secondary to the amount of NREM sleep within a NREM-REM cycle.

In accordance with our results, a few studies have reported maximal delta activity at times other than at the beginning of the sleep episode. In a study of rat sleep by Dijk and Daan (1989), a pattern suggesting a circadian modulation of delta activity was observed as the proportions of light and dark were varied. In one study in humans, neither linear nor exponential declines in period-analyzed delta activity during sleep were found (Armitage and Roffwarg, 1992). A disentrainment protocol in humans showed maximal amounts of SWS at the time of the maximum body temperature rhythm, not at sleep onset (Campbell, 1984; Campbell and Zulley, 1987). Since delta activity is a significant element in the definition of SWS (Stages 3 and 4), the suggestion of a circadian rhythm in delta activity observed here may support evidence that SWS may be influenced by the circadian cycle as well as by wake duration (Akerstedt and Gillberg, 1981; Campbell and Zulley, 1987; Gagnon et al., 1985; Webb, 1986; Weitzman et al., 1980).

A limitation of this study is that no independent measure of circadian phase was available. Therefore, averaging needed to be done relative to sleep measures. Analyses were therefore performed both by circadian phase (Figure 2) and relative to CS onset (Figure 3).

This study of undisturbed sleep in squirrel monkeys was not designed to address directly the question of circadian and homeostatic influences on delta activity, since circadian phase and wake duration were allowed to covary. To fully test the predominantly homeostatic models of the role of delta activity, studies are required of sleep at times when circadian and homeostatic processes are opposed (c.f. Edgar et al., 1993). We have performed a protocol designed to test the relative influence of circadian and homeostatic processes using sleep deprivations of different lengths and ending at different circadian phases in squirrel monkeys (Klerman et al., 1999). We found that the amount of sleep and delta activity after sleep deprivation in squirrel monkeys was weakly dependent on prior wake duration. Instead, circadian factors appeared to dominate homeostatic processes in determining the timing, duration and content of sleep. The interaction of circadian and homeostatic influences on scheduled sleep in humans has been studied in a forced desynchrony protocol (Dijk et al., 1997). Our study also was not designed to test the many possible manifestations of a homeostatic effect on delta activity; rather it was designed to characterize the temporal distribution of delta activity during normal, undisturbed sleep in a diurnal primate, under daily LD cycles and in constant conditions. Other significant homeostatic processes may exist within the sleep state.

The present results confirm the importance of studying the sleep of a variety of animal species with different sleep-wake patterns. In one model of sleep regulation, delta activity is assumed to reflect a homeostatic process that accumulates during wake and dissipates during sleep. Such a model would predict that the level of delta activity should be maximal at sleep onset and decline monotonically during the sleep episode. Our results from a diurnal primate with consolidated sleep and wake cycles failed to confirm either of these predictions. Delta activity in the EEG during NREM sleep may not be a pure marker of homeostatic drive in all mammalian species.
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