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Sleep electroencephalographic characteristics of the Cynomolgus monkey measured by telemetry

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SUMMARY

Cynomolgus monkeys are widely used as models of diseases and in preclinical studies to assess the impact of new pharmacotherapies on brain function and behaviour. However, the time course of electroencephalographic delta activity during sleep, which represents the main marker of sleep intensity associated with recovery during sleep, has never been described in this non-human primate. In this study, telemetry implants were used to record one spontaneous 24-h sleep–wake cycle in four freely-moving Cynomolgus monkeys, and to quantify the time course of electroencephalographic activity during sleep using spectral analysis. Animals presented a diurnal activity pattern interrupted by short naps. During the dark period, most of the time was spent in sleep with non-rapid eye movement sleep/rapid eye movement sleep alternations and sleep consolidation profiles intermediate between rodents and humans. Deep non-rapid eye movement sleep showed a typical predominance at the beginning of the night with decreased propensity in the course of the night, which was accompanied by a progressive increase in rapid eye movement sleep duration. Spectral profiles showed characteristic changes between vigilance states as reported in other mammalian species. Importantly, delta activity also followed the expected time course of variation, showing a build-up with wakefulness duration and dissipation across the night. Thus, Cynomolgus monkeys present typical characteristics of sleep architecture and spectral structure as those observed in other mammalian species including humans, validating the use of telemetry in this non-human primate model for translational sleep studies.

INTRODUCTION

Non-human primates have been used for decades to study sleep regulation with emphasis on clinically relevant questions. The close phylogenetic relationship between non-human primates and humans enables these species to contribute significantly to sleep research by bridging the gap between knowledge gained through rodent or feline models and that obtained in humans. Thus, several sleep studies have explored sleep characteristics of non-human primates in laboratory-controlled conditions, including chimpanzees (Bert *et al.*, 1970), macaques (Reite *et al.*, 1965), baboons (Balzamo *et al.*, 1977; Bert *et al.*, 1975) and squirrel monkeys (Adams and Barratt, 1974; Balzamo *et al.*, 1977). The analysis of the electroencephalogram (EEG) in these studies was complexified by several aspects of the recording methodology. This included variations in restraint degrees as well as in recording environments. Such recording methodologies lead to difficulties during EEG recording for extended periods of time or introduced modifications in sleep architecture (Bouyer *et al.*, 1978; Holcombe *et al.*, 1979). Another limitation of most non-human primate sleep studies has been the relatively short duration of the recording period, which mainly consisted of night-time recording only, possibly masking recovery sleep that may occur during the daytime.

More recently, several studies have proved the relevance of EEG recordings using telemetry implants for sleep studies in non-human primates (Crofts *et al.*, 2001; Hsieh *et al.*,

2008). Telemetry involves surgical implantation of EEG or electrocorticogram electrodes relayed to a biosensor device (transmitter) positioned subcutaneously or intraperitoneally, enabling electronic capture of biological signals in freely-moving animals for an extended time period with a reduced infection risk (Crofts *et al.*, 2001; Pearce *et al.*, 1998), when compared with tethered methodologies. Accordingly, such a system provides a unique method to analyse the EEG in non-human primates, as it does not involve restraint protocol and the animal can be kept in its home cage, leading to a reduction in stress and in alterations of sleep characteristics.

The Cynomolgus monkey (*Macaca fascicularis*) is a non-human primate commonly used in toxicology research (Buse, 2005; Chellman *et al.*, 2009) or to model human brain disorders, such as depression (Willard and Shively, 2012), Parkinson's disease (Pearce *et al.*, 2001; Storvik *et al.*, 2010), and Alzheimer's disease (Kimura *et al.*, 2003; Podlisny *et al.*, 1991). However, most previous sleep assessments in this species were either performed without the gold standard EEG recording (Zhdanova *et al.*, 2002) or with the animal placed in a restraint chair (Almirall *et al.*, 1999; Philip *et al.*, 2005; Yao *et al.*, 2013), which presents the caveats described above. To our knowledge, the only study presenting sleep features in freely-moving Cynomolgus monkeys used a cable approach (Yukuhiro *et al.*, 2004), which may have impacted sleep behaviours. In addition, the time course of EEG activity during sleep, which indexes both sleep regulation and recovery during sleep, has never been reported in this non-human primate species.

In the present study, sleep recording from unrestrained Cynomolgus monkeys was conducted in their home cage using fully implantable telemetry transmitters and a computer data collection system, allowing continuous recording over days. By avoiding novel settings, restraint, cable and other potentially stressful apparatus and manipulations, a more accurate and complete characterization of normal sleep in this commonly used species was performed. A better description of undisturbed sleep patterns is an important step in validating the Cynomolgus monkey as a biomedical model to study the impact of various treatments and pharmacotherapies on EEG activity and sleep regulation.

MATERIALS AND METHODS

Animals

Four male Cynomolgus monkeys (2.5–3 years old; 2.6–2.8 kg) were used in this study. The animals were maintained in constant environment conditions (temperature 21 $\pm$ 3 °C; humidity 30–70%; 10–15 air changes per h; 12 : 12 h light–dark cycle). A standard certified commercial primate chow (Certified Hi-Fiber Primate Diet 7195C™; Harlan Teklad, Madison, WI, USA) and fresh fruits were available to each monkey twice daily, and water was available *ad libitum*. Animals were individually housed, but had visual, auditory and olfactory contact with other monkeys throughout the study. Behavioural (e.g. activity level, locomotion, coordination) and clinical (e.g. appetite) signs were evaluated daily by trained technicians before and after surgery, as well as during electrophysiological recording (see below). Monkeys had access to chew toys, and videotapes or music were played in all housing rooms to provide environmental enrichment. All experimental procedures were conducted in accordance with the Canadian Council on Animal Care (CCAC) and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (8th edn, revised 2011) at the city of Laval (QC, Canada) division of CiToxLAB North America, which is AAALAC- and CCAC-accredited. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC), and conducted as per standard operating procedures in place.

Surgery

Cynomolgus monkeys were surgically implanted with telemetry transmitters (TL11M2-D70-EEETM; DSI, St-Paul, MN, USA), as previously described (Authier *et al.*, 2009). Briefly, the telemetry transmitter was implanted between the transverses abdominis and the internal abdominal oblique muscles. The EEG electrodes were placed on the cranium to monitor one standard bipolar derivation (Cz-Oz), and relayed subcutaneously to the telemetry transmitter via a small skin incision in the neck. A linear groove was made in the cranial cortical bone to secure the electrodes with surgical glue (Vetbond™; 3M, St-Paul, MN, USA) and acrylic. The electromyographic (EMG) electrodes were positioned parallel to the longitudinal axis of the neck muscles with a distance of 10 mm between leads. Electrooculogram (EOG) leads were placed subcutaneously below the outer canthus and above the inner canthus of the right eye, and sutured in the periosteum. A period of at least 4 weeks was allowed between the surgery and the start of electrophysiological recordings. Post-surgical evaluations confirmed that all animals were normal after implantation.

Electrophysiological recordings

The EEG, EMG and EOG signals were recorded in combination with a video recording, used as a complement for interpretation of EEG traces (digital colour camera with daylight and infrared night vision), and both were connected to a computerized system (IBM Intellistation Z pro, Xeon 3.8 GHz, 3.5 TB hard drive; IBM, Markham, ON, Canada). To maximize telemetry signal quality, two receivers (DSI model RMC-1) were placed in each cage (top and side). Acquisition of the telemetry signal was done at a sampling rate of 500 Hz with the DSI software (Dataquest A.R.T. 3.01 Gold™), while the frequency range of the telemetry transmitters (D70-EEE, DSI) was 1–100 Hz. EEG, EMG and EOG were monitored continuously, and analyses presented here were performed on one full 24-h baseline recording, before exposure of animals to a complex pharmacological paradigm.

Vigilance state analysis

The identification of vigilance states was performed by visual inspection of EEG, EMG and EOG signals using the NeuroScore software version 1.1-2242 (DSI, St-Paul, MN, USA). Vigilance state identification was performed on 10-s epochs, and five different states were identified (Fig. 1a): wakefulness; non-rapid eye movement sleep (NREMS) stage 1–3; and rapid eye movement sleep (REMS). Criteria for vigilance state attribution were similar to the 2007 criteria of the American Academy of Sleep Medicine. Briefly, wakefulness was attributed when low-amplitude, rapid-frequency EEG was accompanied by high-amplitude EOG and EMG activity. NREMS stage 1 was scored when EEG showed regular theta/alpha activity (6–10 Hz) and lowered EMG activity accompanied or not with slow eye movements. Stage 2 was scored with the first appearance of specific features common to human stage 2 sleep, i.e. K-complexes and spindles, accompanied with low EMG activity and absence of eye movements. NREMS stage 3 was attributed when slow waves [high-amplitude, low-frequency waves (~1–5 Hz)] appeared on the EEG signal. REMS was scored when EEG showed a low-voltage, mixed-frequency activity with predominant theta activity (4–8 Hz) accompanied with lowest EMG activity and phasic rapid eye movements. Epochs with prominent muscular or ocular artefacts were simultaneously identified and excluded from EEG spectral analysis. The duration of each vigilance state was averaged over the full 24 h, separately for the 12-h light and dark periods, and per hour. The mean duration of vigilance state episodes (consecutive epochs not interrupted by another state) and the number of episodes were averaged over 24 h, 12-h light and 12-h dark periods.

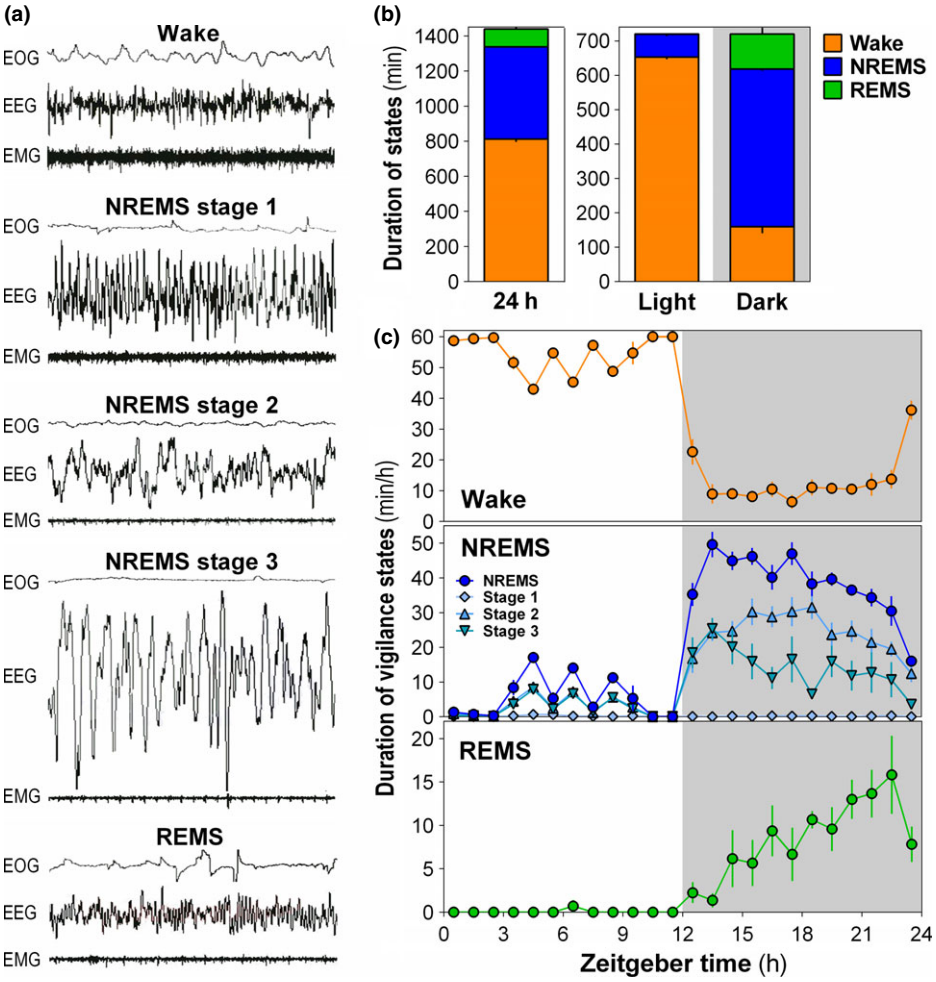

Figure 1. Duration and distribution of vigilance states in Cynomolgus monkeys. (a) Representative 10-s traces of electrooculogram (EOG), electroencephalogram (EEG) and electromyogram (EMG) signals observed during wakefulness, non-rapid eye movement sleep (NREMS) stages 1–3 and rapid eye movement sleep (REMS). (b) Total duration of wakefulness, NREMS and REMS for a 24-h period (left), a 12-h light period (middle) and a 12-h dark period (right). NREMS represents the total of NREMS stages 1–3. (c) Hourly distribution of wakefulness, NREMS (stages 1–3 and their sum) and REMS. A significant effect of hour was observed for wakefulness (24 h: $F_{23,69} = 109.7$, $P < 0.0001$), NREMS (24 h: $F_{23,69} = 56.2$, $P < 0.0001$) and REMS (12 h dark: $F_{11,33} = 3.9$, $P < 0.01$). Grey backgrounds indicate the dark period.

EEG spectral analysis

The EEG of artefact-free 10-s epochs was subjected to spectral analysis using Discrete Fourier Transform to calculate absolute power density between 1 and 50 Hz per Hz-bin separately for wakefulness, combined NREMS stages 2 and 3, and REMS. Spectral power computed for each Hz-bin was then averaged for all 10-s epochs over the full 24 h recording. Relative spectral activity (activity in one band relative to total activity in all bands) was also calculated for six different frequency bands [delta (1–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), sigma (12–16 Hz), beta (16–24 Hz), gamma (24–50 Hz)], and averaged per 12-h light and 12-h dark periods separately for wakefulness, combined NREMS stages 2 and 3, and REMS. To evaluate the time course of delta activity during the full 24 h recording, delta activity was averaged per six equal intervals to which a similar number of epochs contributed during the light period, and per five equal intervals during the dark periods. The time course of individual Hz-bin activity during the night period was assessed using 2-min means of normalized activity (full variation spectrum of all Hz-bins of each animal fixed between 0 and 100).

Statistical analysis

Vigilance state durations, episode durations, number of episodes and EEG activity in frequency bands were compared between the light and dark periods using Wilcoxon matched-pairs tests. The time courses of vigilance state duration per hour and that of delta activity were analysed using repeated-measures analysis of variances (ANOVA). Significance levels were adjusted for repeated measures using the Huynh–Feldt correction when appropriate. Analyses were performed using Statistica (StatSoft Inc., Tulsa, OK, USA). Data are reported as mean ± SEM.

RESULTS

Duration of wakefulness and sleep during the light and dark periods

Cynomolgus monkeys spent more than a third of the 24-h day asleep, as sleep occupied 43.6% of the 24-h recording (Fig. 1b; Table 1). Diurnal preference of Cynomolgus monkeys was confirmed with 90.7% of the 12-h light period spent awake. Naps during the 12-h light period were mainly composed of NREMS (99.1% of 12-h light total sleep). During the 12-h dark period, NREMS prevailed and occupied an average of 63.7% of the total 12 h. Most of this NREMS was composed of stages 2 and 3 (respectively, 51.1% and 30.7% of total sleep time), whereas stage 1 represented only a small fraction of total sleep during the dark period (<1%). REM sleep occupied 18% of total sleep time, which represented 14.2% of the 12-h dark period. The remainder of the 12-h dark period was spent in wakefulness (22.2%). Although long wakefulness episodes could be observed (>3 h), mean episode duration was <5 min for all three vigilance states when measured over the 24-h recording (Table 1).

Table 1 Durations and percentages of vigilance states in Cynomolgus monkeys (n = 4)

	24 h	*12-h light*	*12-h dark*
Wakefulness			
Minutes	812.5 ± 16.4	653.0 ± 6.6	159.6 ± 19.1*
%	56.4 ± 1.1	90.7 ± 0.9	22.2 ± 2.6*
Number of episodes	262.7 ± 29.2	61.5 ± 7.9	201.2 ± 29.5*
Mean episode duration (min)	3.2 ± 0.3	11.3 ± 1.7	0.8 ± 0.1*
TST			
Minutes	627.5 ± 16.4	67.0 ± 6.6	560.4 ± 19.1*
%	43.6 ± 1.1	9.3 ± 0.9	77.8 ± 2.6*
NREMS stage 1			
Minutes	3.5 ± 0.8	2.0 ± 0.8	1.5 ± 0.5
%	0.2 ± 0.05	0.3 ± 0.1	0.2 ± 0.1
% TST	0.6 ± 0.1	3.0 ± 1.0	0.2 ± 0.1
NREMS stage 2			
Minutes	322.1 ± 33.5	33.8 ± 4.7	288.3 ± 32.8*
%	22.4 ± 2.3	4.7 ± 0.7	40.0 ± 4.6*
% TST	51.0 ± 3.9	50.0 ± 3.4	51.1 ± 4.1
NREMS stage 3			
Minutes	199.0 ± 34.7	30.5 ± 2.1	168.6 ± 33.1*
%	13.8 ± 2.4	4.2 ± 0.3	23.4 ± 4.6*
% TST	32.2 ± 6.2	46.2 ± 3.7	30.7 ± 6.6
Total NREMS			
Minutes	524.7 ± 2.8	66.3 ± 6.2	458.3 ± 5.1*
%	36.4 ± 0.2	9.2 ± 0.9	63.7 ± 0.7*
%TST	83.8 ± 2.3	99.1 ± 0.9	82.0 ± 2.5*
Number of episodes	256.2 ± 30.6	60.2 ± 7.9	196.0 ± 30.9*
Mean episode duration (min)	2.1 ± 0.2	1.2 ± 0.3	2.5 ± 0.5*
REMS			
Minutes	102.8 ± 17.6	0.7 ± 0.7	102.8 ± 18.0*
%	7.1 ± 1.2	0.1 ± 0.1	14.2 ± 2.5*
% TST	16.2 ± 2.3	0.9 ± 0.9	18.0 ± 2.5*
Number of episodes	24.2 ± 2.1	0.5 ± 0.5	23.7 ± 2.1*
Mean episode duration (min)	4.2 ± 0.5	0.3 ± 0.3	4.2 ± 0.5*

NREMS, non-rapid eye movement sleep; REMS, rapid eye movement sleep; TST, total sleep time.
*P < 0.07 compared with the 12-h light period (Wilcoxon matched-pairs tests).

Detailed temporal distribution of wakefulness and sleep states

Vigilance state duration was averaged per hour for the full 24-h recording to evaluate in greater detail their distribution as a function of time of day (Fig. 1c). The time course of vigilance state occurrence confirmed the predominance of wakefulness during the 12-h light period, with naps taking place around the middle of the light period. When lights went off,

animals quickly fell asleep. NREMS predominated during the first half of the 12-h dark period, and showed a maximum 2 h after lights off, which was followed by a progressive decrease across the 12-h dark period. These observations were also true for NREMS stage 3 specifically. In contrast, REMS incidence increased over the course of the 12-h dark period, peaking during the second to last hour of the night. The majority of REMS episodes were followed by wakefulness.

Spectral characteristics of the EEG

Fig. 2a shows the power spectra of each vigilance state calculated over the full 24-h baseline recording. Power spectra showed the expected pattern of change between vigilance states, with wakefulness showing higher beta and gamma EEG activities compared with sleep states, NREMS (stages 2 and 3) showing a predominance of delta activity over other vigilance states, and REMS presenting an elevated contribution of theta activity. The relative contribution of the different frequency bands showed a strong tendency to be affected by the 12-h period for each vigilance state (Fig. 2b). More precisely, delta activity tended to contribute more to total EEG activity during the 12-h dark period compared with the 12-h light period for wakefulness (47.5% versus 36.7%) and NREMS (68.5% versus 53.5%). In contrast, the contribution of the theta band tended to be lower during the 12-h dark period compared with the 12-h light period for NREMS (20.9% versus 29.9%). Light–dark differences in REMS relative activity in frequency bands could not be quantitatively tested because only one animal expressed REMS during the 12-h light period.

Time course of EEG activity during sleep

The temporal pattern of power spectra during NREMS (stages 2 and 3), normalized within each animal, was computed by averaging Hz-bin activity per 2 min to assess the evolution of sleep spectral activities over the course of an undisturbed night (Fig. 3a). As anticipated, low-frequency activity dissipated in the course of the night. Overall, this gradual dissipation seemed to apply to the full-power spectrum, as activities in the second half of the night were mostly lower than those in the first half of the 12-h dark period. EEG delta activity was quantified in more detail as it represents the main marker of recovery sleep known to closely track the duration of previous wakefulness (Dijk and Czeisler, 1995; Franken *et al.*, 2001). In mice, the time course of delta activity during the rest period is often analysed over 12 intervals having equivalent duration of NREMS (El Helou *et al.*, 2013; Franken *et al.*, 2001), whereas it is commonly averaged per NREMS–REMS cycle, usually four, in humans (Feinberg and Campbell, 2003; Mongrain *et al.*, 2006). To evaluate the most appropriate methodology to compute delta dynamics in Cynomolgus monkeys, the time course of delta power across the 24-h recording was plotted using each 10-s epoch for each individual animal (Fig. 3b). The time course of

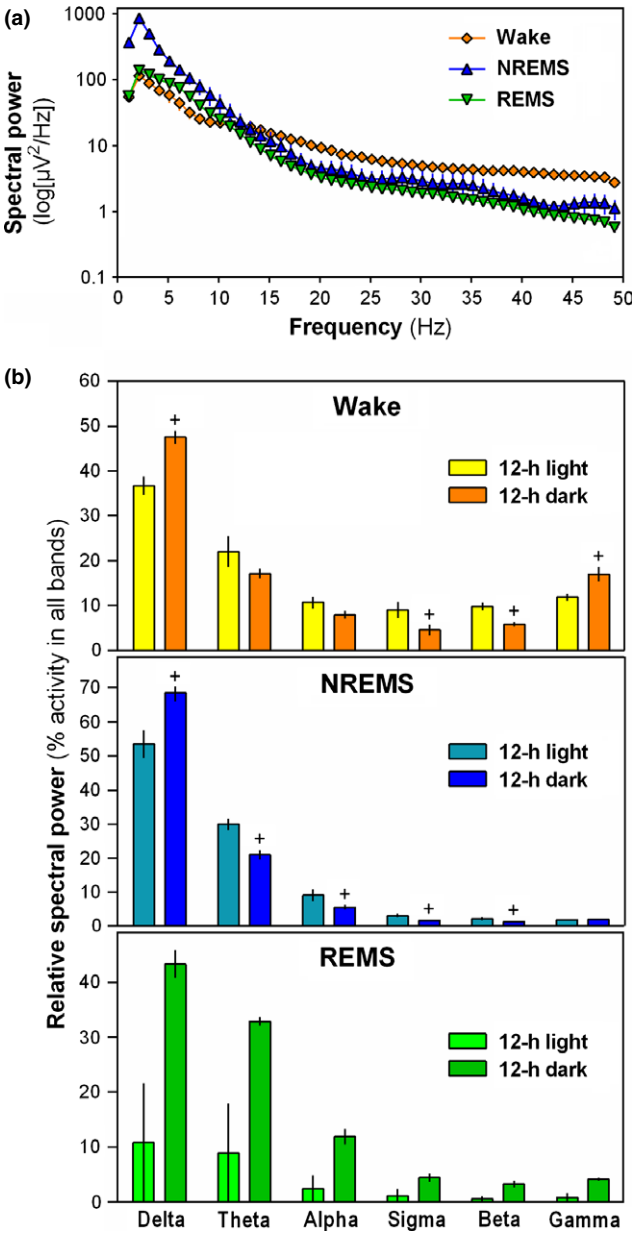

Figure 2. Absolute electroencephalographic (EEG) power spectra and relative activity of frequency bands in Cynomolgus monkeys. (a) Averaged 24-h power spectra calculated for wakefulness, non-rapid eye movement sleep (NREMS) stages 2 and 3 together, and rapid eye movement sleep (REMS). (b) Mean spectral bands relative contribution during the 12-h light and the 12-h dark periods calculated separately for wakefulness, NREMS (stages 2 and 3) and REMS. Statistical comparisons of light and dark periods could not be performed for REMS because only one animal expressed REMS during the light period. $^{+}P < 0.07$ compared with the 12-h light period (Wilcoxon matched-pairs tests).

delta activity per 10 s emphasized that Cynomolgus monkeys present approximately six small naps during the 12-h light period, during which delta activity was generally similar. During the 12-h dark period, NREMS–REMS alternations were obviously shorter than in humans. Nevertheless, for most animals, the pattern of delta activity dynamics seemed

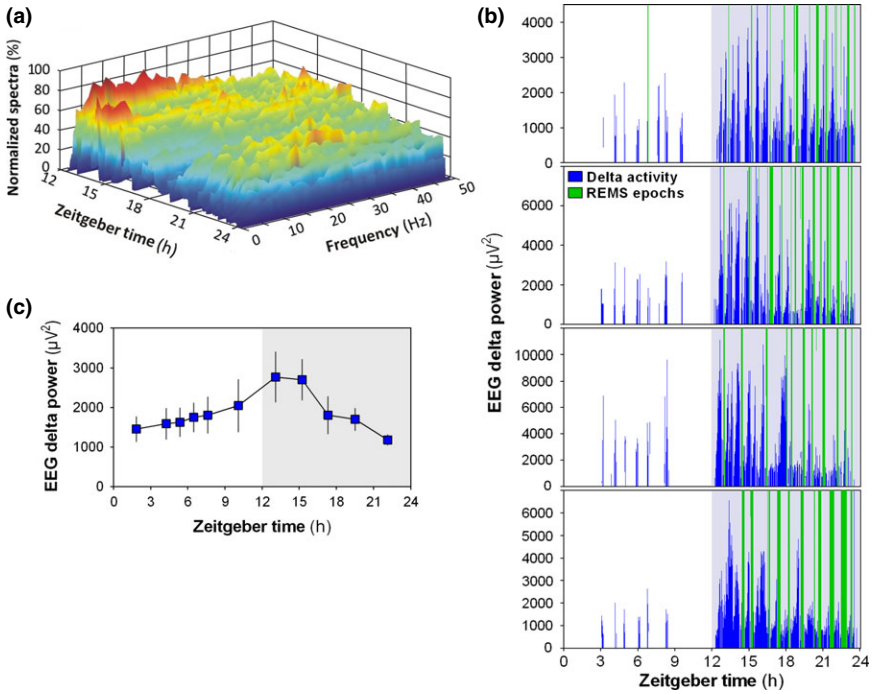

Figure 3. Time course of electroencephalogram (EEG) activity during sleep in Cynomolgus monkeys. (a) Heatmap of the time course of the power spectrum during non-rapid eye movement sleep (NREMS; stages 2 and 3) across the 12-h dark period. (b) Individual time courses of delta activity across the 24-h recording and position of rapid eye movement sleep (REMS) epochs. (c) Time course of delta activity average for all animals calculated using six equal intervals during the 12-h light period and five equal intervals during the 12-h dark period. A significant effect of interval was observed ($F_{10,30}$ = 6.8, P < 0.05). Grey backgrounds indicate the dark period.

to resemble that of humans in part because of a general build-up of delta in the first sleeping hour (similar to the build-up typically observed at the beginning of a NREMS–REMS sleep cycle in humans). Therefore, delta activity dynamics was averaged for all animals using six equal intervals during the light period and five equal intervals during the dark period to which an equal number of epochs contributed (Fig. 3c). This analysis revealed a progressive build-up of delta activity during the 12-h light period followed by a gradual dissipation of delta activity in the course of the 12-h dark period.

DISCUSSION

In this study, we validated the use of telemetry in Cynomolgus monkeys to evaluate the 24-h sleep–wake distribution as well as the EEG spectral activity profile of the different vigilance states. At the same time, we reported that, when studied in their home cage, undisturbed and uncabled freely-moving Cynomolgus monkeys present a diurnal pattern of activity, as the majority of their wakefulness occurred during the light period, with few daytime naps. Importantly, we also describe for the first time that Cynomolgus monkeys display typical patterns of EEG spectral activity specific to wakefulness, NREMS and REMS, and that the time course of delta activity during NREMS shows the expected build-up with wakefulness duration and decay in the course of sleep. Overall, our results support that sleep assessed by telemetry in Cynomolgus monkeys represents a potent model to study physiological and molecular regulatory mechanisms of sleep.

As described above, sleep studies in non-human primate models, including those in Cynomolgus monkeys, commonly used restraint chair for sleep assessments (Almirall *et al.*, 1999; Bouyer *et al.*, 1978; Holcombe *et al.*, 1979; Philip *et al.*, 2005; Yao *et al.*, 2013). However, to overcome limitations associated with restraint, studies have more recently began to use telemetry methodologies to record EEG in freely-moving unrestrained monkeys (Crofts *et al.*, 2001; Hsieh *et al.*, 2008). To our knowledge, this study is the first to make use of such methodologies for the characterization of the sleep macro- and micro-architecture in the Cynomolgus species. The telemetry implants permitted recording of EEG, EOG and EMG channels in physiological conditions by limiting potential stress and allowing the animal to stay in its home cage while interacting with other monkeys in the same room. The fact that the time course of wakefulness and sleep stages was very similar between animals, which is indexed by low between-animal variations, and that sleep occupied more than 40% of the total 24-h recording session may represent good indexes that stress was maintained low using these recording conditions.

Baseline characteristics of sleep in other non-human primates that have previously been described by numerous research groups (Adams and Barratt, 1974; Balzamo *et al.*, 1977; Bert *et al.*, 1970, 1975; Breton *et al.*, 1986; Crofts

et al., 2001; Hsieh *et al.*, 2008; Yu *et al.*, 2004) are somewhat comparable to our observations in Cynomolgus monkeys. For instance, the Rhesus monkey (*Macaca mulatta*) is another commonly used non-human primate that was previously suggested as one of the best models of human sleep (Balzamo *et al.*, 1977). Sleep architecture in this species was previously described both in restrained (Balzamo *et al.*, 1977; Yu *et al.*, 2004) and freely-moving (Hsieh *et al.*, 2008) conditions, and only the latter will be compared with the present study. The total proportion of time during the 24-h day occupied by sleep in the Rhesus monkey, also assessed by visual identification (Hsieh *et al.*, 2008), is very similar to what we observed in our study (46.4% versus 43.6%). However, the distribution of naps during daytime differed in this species compared with Cynomolgus, in that naps mainly occurred in the hours before lights off (Hsieh *et al.*, 2008) compared with in the middle of the day for Cynomolgus monkeys. This difference might have emerged given the different light–dark schedule imposed in this study compared with the present study (i.e. 16 h light : 8 h dark), or could result from different feeding schedules. Other differences between the two studies concern the durations of NREMS stage 1 and REMS, which appeared higher in the Rhesus monkey (Hsieh *et al.*, 2008) compared with Cynomolgus monkeys. However, these differences need to be interpreted with great caution as they might be due to differences in vigilance state identification performed by differently trained technicians.

In general, Cynomolgus monkeys presented a more fragmented and polyphasic sleep pattern in comparison to humans, which can be appreciated when looking at the distribution of wakefulness during the 12-h dark period (approximately 10 min per hour; Fig. 1c) and the time course of delta activity and REMS in individual animals (Fig. 3b). Higher durations of wakefulness during the main rest period in non-human primates are commonly described as a survival adaptation given that constant vigilance may be needed in natural environments to lessen the risks of predation. Thus, the usual sleep cycle definition applied in humans (Feinberg and Campbell, 2003) cannot be adequately applied in monkeys given their less consolidated sleep architecture. In contrast, NREMS proportion, generally including that of NREMS stage 2 representing approximately half of the time spent asleep, was similar in our model to that typically recorded in human adults (Carskadon and Dement, 2011), as well as other non-human primates (Breton *et al.*, 1986; Crofts *et al.*, 2001; Daley *et al.*, 2006; Hsieh *et al.*, 2008). In addition, NREMS in Cynomolgus monkeys predominated at the beginning of the night and was gradually replaced by REMS similar to the time course observed in humans, which strongly suggests that sleep in Cynomolgus monkeys is regulated by circadian and homeostatic influences in a manner analogous to humans (Dijk and Czeisler, 1995).

Importantly, spectral profiles of the different vigilance states measured by telemetry in Cynomolgus monkeys were consistent with observations in other mammals, including humans. Indeed, as in humans, wakefulness was characterized by higher EEG activity in fast frequencies (>16 Hz) compared with NREMS and REMS; NREMS was dominated by low-frequency activities, and more particularly in the delta range (1–4 Hz); and REMS showed a noticeable contribution of theta activities (4–8 Hz). However, and similar to what has been reported for Rhesus monkeys (Hsieh *et al.*, 2008), a peak in the spindle frequency range was not observed during NREMS despite the observation of spindles on EEG traces. This may represent a limitation from the telemetry methodology (also used by Hsieh *et al.*, 2008), or masking by elevated spectral activity lower than spindle frequencies. Nevertheless, day–night differences seemed to occur in specific frequency bands during both wakefulness and NREMS, which are reminiscent of the significant circadian variations reported for EEG activity in many frequencies of the different vigilance states for both humans and rodents (Dijk and Czeisler, 1995; Dijk *et al.*, 1997; Yansekov and DeBoer, 2011). Alternatively, these differences may also emerge from the lighting condition because light was specifically shown to change EEG spectral activity during all three vigilance states (Alföldi *et al.*, 1991). Of course, elevated delta activity during the dark phase in comparison to the light phase is also expected to reflect the pressure for sleep, which, according to our observations, may even extend to wakefulness.

Accordingly, another relevant characteristic of Cynomolgus monkey sleep common to human sleep was the predominant distribution of the spectral activity in low frequencies in the first hours of the 12-h dark period, and a decreasing pattern over the course of the dark period (Dijk and Czeisler, 1995; Feinberg and Campbell, 2003; Mongrain *et al.*, 2006). We have focused our analysis on delta activity as an indicator of sleep homeostasis/need in our model, and reported that the dissipation of NREMS delta activity during the 12-h dark period followed a time course similar to the one assessed using NREMS–REMS cycle averages in humans (Feinberg and Campbell, 2003; Mongrain *et al.*, 2006). In addition, when delta activity was plotted across the 12-h light period, it showed a gradual build-up that seemed to reflect wakefulness duration. These last observations are similar to those that have been reported in Rhesus monkeys (Hsieh *et al.*, 2008). Therefore, telemetry-assessed delta activity dynamics in non-human primates seems to represent an appropriate tool to evaluate the properties of homeostatic sleep regulation. Of course, quantification of the delta activity response to sleep deprivation, which was not possible in the present study due to the primary pharmacological objectives of the project, will be important in future studies to further confirm the suitability of the presented species as a translational model of sleep regulation.

In summary, we demonstrated through a 24-h quantification of wakefulness and sleep distribution, and of spectral EEG activity, that the use of telemetry in Cynomolgus monkey is an applicable methodology that makes this non-human primate a relevant model for translational sleep

studies. Indeed, our data revealed that this model presents the typical characteristics of the proportion and distribution of sleep macro- and micro-architecture inherent to sleep regulatory mechanisms extensively described in humans. Further validation of the model should include measurements of the impact of various pharmacological treatments known to affect human sleep structure and EEG activity during sleep.

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AUTHOR CONTRIBUTIONS

AR and VM performed vigilance state identification, data analyses and wrote the manuscript. SA, LB and MP developed the Cynomolgus telemetry model, conceived and performed the experiments, assisted data analyses and reviewed the manuscript. GT performed EEG analyses and figure panels.

CONFLICT OF INTERESTS

SA, LB and MP are employees of CiToxLAB North America.

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