

Sleeping Outside the Box: Electroencephalographic Measures of Sleep in Sloths Inhabiting a Rainforest

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Supplemental methods, results, references and figure captions

Supplemental Methods

Animals: None of the three females studied with the EEG/EMG recorders were carrying young or appeared to be obviously pregnant. Moreover, when visually checked 3 months later, they were still without young. Our subjective impression based on tooth wear was that one of the sloths was old. A different sloth had been caught in 2004 as an adult without young. She was observed carrying young in 2006, but not 2007. The remaining sloth did not seem unusually old. The 7-month activity records were obtained from two adult female sloths. Their reproductive status during this time is unknown. The sloths did not show obvious signs of ectoparasite infestation. Although sloth moths (*Cryptoses choloepi*) were present in their pelage, it is unclear whether these are parasites on sloths (Waage & Montgomery 1976).

Sloths on BCI feed primarily on the leaves of *Ficus* sp, wild plums (*Spondus lutea*), *Protium panamense*, *Piulsenia armata*, *Luhea*, and *Bombax*, although leaves from at least 28 species of trees and 3 species of lianas are included in their diet (Montgomery & Sunquist 1978).

Procedure: The hair on the top of the head was clipped, and 7 fine silver-silver/chloride wire electrodes (AG/40T, Medwire Corp., Mount Vernon, NY) were inserted through the scalp to the surface of the cranium with a 23-gage hypodermic needle. The electrodes were made and inserted following a method developed for use in humans and laboratory animals (Ives 2005). We note that

in the only previous EEG study of sleep in sloths, subdermal electrodes were also placed on the surface of the cranium (Galvão de Moura Filho et al. 1983). Consequently, trans-cranial attenuation of EEG amplitude was expected to be comparable between the two studies. Prior to working with live sloths, we examined the skull from an adult sloth and an online high-resolution X-ray computed tomographic scanner image of a skull (www.digimorph.org/specimens/Bradypus_variegatus) to determine the electrode placement that would maximize our coverage of neocortical EEG activity. Based on this assessment, the electrodes were arranged in 2 rows of 3 electrodes, each spaced 7 mm left and right of the sagittal midline and running in an anterior to posterior direction (supplemental figure 1a). For each row, the anterior electrode was placed in line with bregma and the posterior electrode was placed in line with lambda. The approximate position of bregma and lambda could be readily discerned by palpating the skin overlying the contours of the skull, which form a distinct concavity at each landmark (supplemental figure 1b). Each medial electrode was placed over the apex of the cranium, midway between the anterior and posterior electrodes. The 7th electrode, which served as a ground, was placed midway between the medial electrodes. For each hemisphere, the anterior and posterior electrodes were referenced to the medial electrode. Thus, two bipolar derivations (anterior-medial and posterior-medial) were recorded from each hemisphere. In practice, the posterior electrodes also detected EMG activity. After gluing the small insertion point closed around each wire with Histoacryl[®] (Braun, Tuttlingen, Germany), we connected the electrode wires to

the Neurologger 2 (www.vyssotski.ch/neurologger2) EEG/EMG data recorder (Vyssotski *et al.* 2006). The recorder and batteries were enclosed in a waterproof container (34 mm x 25 mm oval tube, 17 mm high) that was glued to the trimmed hair on the top of the sloth's head with superglue (figure 1a). The recorder, batteries (two CR2032) and container weighed 11 g. Each EEG/EMG derivation was digitized with a sampling rate of 800 Hz after amplification (2000x) and filtering (1-70 Hz, first order). Means of sequential groups of eight samples were stored in the non-volatile memory at a rate of 100 Hz. At this rate, 4 channels can be recorded continuously for a maximum of 5 d.

Two additional instruments were also used to monitor the location and activity of sloths. A traditional radio-telemetry collar was attached to allow locating the sloths to observe their behavior remotely, and to eventually find the individuals to remove the recorders. Finally, miniature (10g) 3-axis accelerometer recorders were affixed to the back of 2 sloths by gluing it to their fur. Acceleration was recorded every 3 min for 8.9 s at a sampling rate of 18.74 Hz. Once released, the sloths were not confined in any manner. Moreover, as completely wild animals, the sloths were vulnerable to predation by ocelots (*Leopardus pardalis*) and puma (*Puma concolor*), confirmed predators of sloths on BCI (Moreno *et al.* 2006).

The EEG/EMG was recorded from 2 sloths for approximately 5 d each between December 4 – 9, 2007. For logistical reasons, the 3rd sloth was recaptured after recording for only 3 d (December 6 – 9, 2007), but in principle could have been recorded for an additional 2 d. As a result, we obtained a total

of 12.8 d of continuous EEG/EMG recordings. The sloths were released after removing the EEG/EMG recorders. Each sloth was visually relocated again 3 months later and all appeared in good health and without young.

Activity: The automated radio-telemetry system (ARTS) in place on Barro Colorado Island was used to estimate the activity of sloths wearing radio-collars (see Crofoot *et al.* 2008). Specifically, automated receivers operated by built-in microcontrollers collected the activity data. These automated receivers (Sparrow Systems, Fisher, IL) are portable (15 cm³, 600 g), precisely timed (Cochran 1980) and powered continually with a solar charger. The receiver output is proportional to the logarithm of the peak value of a received signal. These receivers are convenient but not unique; any other receiver that produces an output that is proportional to the log of the input signals (i.e., dBm) can be used. The receivers detected radio signals by switching through a circular array of horizontally polarized directional antennae. Receivers repeatedly scanned through a list of frequencies of multiple transmitters at set time interval of 1 min. Data were saved in receiver memory before analysis.

Activity data can be obtained from radio signals because any change in position of a radio transmitter causes a change in received signal strength (Cochran & Lord 1963; Cochran 1980). Transmitter movements occur both when the animal changes position on the landscape and when it changes posture, thereby rotating the orientation of the transmitting antenna. By recording the receiver output as a proportion of the log of the input signal, we eliminated problems of observer bias caused by distant signals being weak (Cochran 1980).

We compared signal strength level between time $t+1$ and t and used the change in signal strength to determine whether the animal was active or inactive during the particular time period. Because small movements can cause large changes in signal strengths, the signal strength amplitudes cannot be used as a continuous measure of the magnitude of an animal's activity. Instead, we used changes in signal strength to judge an animal as active (large changes in signal strength, coded as "1") or inactive (small changes in signal strength, coded as "0") between two readings. This analysis produced a high-resolution time series of activity values that was plotted as an actogram.

To obtain an index of activity from the acceleration data recorder, we calculated the standard deviation (SD) of acceleration (m/s^2) for each axis for each 3-min period. The SD was then averaged across all 3 axes for each 3-min period.

Weather: Ambient temperature during the EEG/EMG recording period was recorded 40 m above ground in the forest canopy, and rainfall was measured in a nearby clearing.

Supplemental Results

Post-release sleep: Supplemental figure 2 shows the percent time spent in each state for consecutive 12-h intervals following release. Time (mean + s.e.m.)

spent in non-REM and REM sleep were reduced following release for 12 and 24 h, respectively. Because time spent in non-REM and REM sleep stabilized 24 h after release, only data after the first 24 h was used to calculate the time spent in each state, as shown in figure 2a.

Activity: Only one of the 2 accelerometer recorders provided useful data. Although one failed after 2 d, the other recorded data for 35 d. Supplemental figure 3a shows activity levels during the 35-d period. The first 5 d encompassed the period during which the EEG/EMG data was recorded from the sloth. The sloth's activity pattern during this period was similar to that occurring during the 30-d period following removal of the EEG/EMG recorder.

As in figure 2c, supplemental figure 4 shows the long-term activity pattern of another three-toed sloth recorded via ARTS. As in the three-toed sloth presented in figure 2c, a period of inactivity also occurs around 0600 in this three-toed sloth.

Weather: The minimum temperature occurred between 0415 and 0645 and varied between 22.7°C and 23.4°C across days (mean \pm s.e.m., 23.1 \pm 0.1°C). The maximum temperature occurred between 1045 and 1345 and varied between 26.0°C and 29.4°C across days (27.6 \pm 0.7°C). Rain occurred every day (3.24 \pm 1.44 mm/d).

Supplemental References

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Supplemental Figure Captions

Supplemental Figure 1. High-resolution X-ray computed tomographic (CT) scanner images of a sloth (*Bradypus variegatus*) skull showing the landmarks

used to determine the placement of the electrodes. (a) Dorsal surface of the cranium with bregma and lambda marked by arrows, and the placement of the electrodes (red dots). (b) Lateral image showing the distinct anterior and posterior concavities in the surface of the cranium used to approximate the location of bregma and lambda. See www.digimorph.org/specimens/Bradypus_variegatus for a 3-D movie of the CT scan. The CT images were provided with permission from DigiMorph.org.

Supplemental Figure 2. Percent time (mean + s.e.m.) spent in wakefulness (green), non-REM sleep (blue), and REM sleep (red) for each 12-h interval following release. Because the sloths were recorded for different durations, the 7th – 9th intervals were based on data from 2 animals, and the 10th interval was based on data from 1 animal.

Supplemental Figure 3. (a) Activity calculated from the acceleration recorder for a sloth during the 35-d recording period. The activity level is plotted for each 3-min period starting from the time of release. The first 5 d encompassed the period during which the EEG/EMG was recorded (blue bar). The sloth was recaptured on day 5 to remove the EEG/EMG recorder. The sloth was then released and left undisturbed for the remaining 30 d. A representative 24-h period is expanded in (b) and plotted with brain state (blue) and feeding (red). The area shaded in gray shows the time between sunset (1800) and sunrise (0620).

Supplemental Figure 4. Long-term activity plot recorded with ARTS from a 2nd three-toed sloth. Each line represents data from one 24-h period plotted twice, with black lines indicating periods of activity plotted every 4 min.