Sleep in an Amazonian manatee, *Trichechus inunguis*

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Abstract. For the first time, sleep was studied in a representative of the order of Sirenia. Slow wave sleep occupied 27%, and paradoxical sleep 1% of the total recording time in the Amazonian manatee, *Trichechus inunguis*. The circadian rhythmicity of sleep was pronounced. During the sleep period, the manatee woke up for a short time for each respiratory act. Interhemispheric asynchrony of the electrocortical slow wave activity was found.
Key words. Sleep; manatee.

The aquatic mammals fall into three orders: Cetacea, Pinnipedia, and Sirenia. Studies of sleep in several species of dolphins and pinnipeds have revealed a number of unusual features, such as the unihemispheric slow wave sleep in dolphins and seals of the Otariidae family, the retained locomotory activity during sleep in these animals, the absence of paradoxical sleep in dolphins, and sleep in short episodes during respiratory pauses in Phocidae seals while they sleep in water[1-3]. The structure of sleep in Sirenia, or sea cows, is of considerable interest in this respect. The present work is the first study of sleep in a representative of the order of Sirenia. Several years ago an electrophysiological polygram was recorded in a free swimming Caribbean manatee (*Trichechus manatus*) but it failed to provide a complete characterization of sleep in that animal, because the experimental time was restricted to only 2 nights[4].

All the species of the order of Sirenia are rare animals listed in the International Red Data Book. They are fully aquatic herbivorous mammals, inhabiting warm and shallow water.

The present study was conducted at Quistococha biological station of the Instituto de Investigaciones de la Amazonia Peruana near the town of Iquitos (Peru). A young female Amazonian manatee was kept for 11 months after capture in a spacious fish-breeding pond. At the time of the experiments the animal was 2 years old, 150 cm long and weighed 63 kg.

Two series of experiments were conducted. At first, the locomotory activity of the manatee in the pond was continuously monitored for 72 h. The second set of experiments was a 5-day-long electrophysiological study of the sleep-wakefulness cycle, during which the animal was kept in a small pool.

During the visual observation the manatee was in the 0.5 ha, 2 m deep pond, in which the ambient conditions were close to the natural ones. There were some plants in the pond that the animal could feed on. It is very difficult to watch a manatee under natural conditions, so a 2-m-long nylon cord with a float was attached to its tail. The float could be easily seen in daylight, and in the evening a miniature electric bulb in the float was switched on, so that night-time observation was possible. The position of the manatee in the lake was determined with a theodolite installed ashore. Horizontal and vertical coordinates of the float were recorded every 2 min. The subsequent computerized processing showed the path of the float around the water surface.

Observation of the manatee showed periods of locomotory activity and periods of immobility which, apparently, corresponded to sleep and relaxed wakefulness. Immobility was most frequently observed during the first half of the night, beginning at 18.00 h, when it got dark (fig. 1 A). The immobile phases were repeatedly confined to two locations on the lake. The main duration of a respiratory pause during immobility was 248 ± 14 s and during locomotory activity 170 ± 12 s, the difference being statistically significant ($p < 0.001$).

After the completion of the behavioral observations, electrodes were implanted into the manatee. Diazepam 0.7 mg/kg was administered intramuscularly every 2 h during the operation, with a good tranquillizing effect. Skin and muscles of the head were injected with novocaine and pierced with a steel tube 4 mm in external diameter, until the tube rested on the skull bone over the dorsal brain surface or over one of the orbits. A hole was then drilled in the skull through the tube, and a metal cylinder, with a steel electrode 1 mm in diameter glued into it, was screwed into the hole. All the cortical electrodes were epidural ones, located over anterior and posterior parts of the dorsal cortex of both hemispheres. The electrooculographic electrode was inserted inside the left orbit in front of the eyeball. The reference electrode was located inside the bone in front of the brain. Two electrodes, similar to the cortical ones in design, were implanted into the cervical muscles of both sides. The wires of all the electrodes were fixed to a steel screw 5 mm in diameter, screwed into the skull in front of the brain. The manatee's behavior was constantly observed, and respiratory acts were marked on paper. The recording of the electrocorticogram (ECoG) of both hemispheres, cervical electromyogram, electrooculogram and respiration went on continuously for 5 days after the operation. After the recording was over, the electrodes were extracted and the animal was returned to the pond.

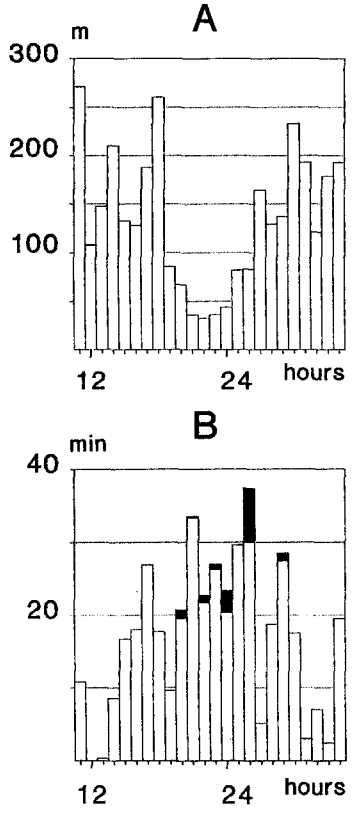

Figure 1. *A* Locomotory activity of the manatee in the lake during 24 h. Abscissae: time (hours), ordinate: distance (m) moved by the animal during the respective hour. The values are means of three 24-h periods. *B* Sleep time of the manatee during 24 h. Abscissae: time (hours), ordinate: sleep time (min) during the respective hour. The values are means of two 24-h periods. The light parts of the bars correspond to slow wave sleep, the dark parts to paradoxical sleep. During all the experiments it got dark at 18.00–18.30 h and dawn was at 05.00–05.30 h.

During the polygraphic recording the manatee was swimming in a pool 2.2 × 1.4 × 0.6 m in size, to which it had been well adapted in advance. The electrodes were connected to an electroencephalograph input with artefactless cables of sufficient length. The pool was situated inside a building. The illumination was natural, except for a low-power electric bulb used to illuminate the pool at night. The manatee was fed in the morning and in the evening.

Wakefulness, slow wave sleep and paradoxical sleep were identified in accordance with the conventional behavioral and polygraphic criteria. Both stages of sleep were characterized by complete immobility; the manatee lay at the bottom of the pool. Sleep mainly occurred during the first half of the night (fig. 1 B), which agreed well with the observations of the manatee's behavior in the lake. Therefore, the Amazonian manatee has a circadian rhythmicity of sleep and wakefulness.

No paradoxical sleep was observed during the first two days after electrode implantation, apparently owing to the adaptation to postoperative factors. The main criterion of paradoxical sleep was a zero record on the neck electromyogram, associated with desynchronized ECoG. All the episodes of paradoxical sleep occurred after long periods of delta sleep. 13 episodes of paradoxical sleep, 20–253 s long, were identified. No phasic components of paradoxical sleep (rapid eye-movements, head and trunk muscle twitches, jerks of fins) were found in the manatee used in this study. However, further evidence from other individuals of this species is needed, to differentiate between individual and species-specific features.

The paradoxical sleep observed in the Amazonian manatee was also found in all the pinnipeds that have been examined. However, we failed to identify paradoxical sleep in dolphins [5]. This is possibly due to the fact that the dolphins, unlike the manatees and pinnipeds, have to move continuously to maintain their posture and to breathe.

Two 24-h periods at the end of the polygraphic recording session were selected for quantitative characterization of different behavioral stages in this animal. The onset of slow wave sleep was defined as the appearance of slow waves in the ECoG of one or two brain hemispheres with an amplitude at least 50% higher than the mean amplitude of desynchronized ECoG. 72% of the total recording time was occupied by wakefulness, 27% by slow wave sleep, and 1% by paradoxical sleep.

The sleep of the Amazonian manatee, as well as that of the Caribbean manatee examined before, was characterized by short arousals of the animal which accompanied each respiratory act and were followed by a rapid return to the interrupted stage of sleep (fig. 2). Each of the 13 identified periods of paradoxical sleep was confined to one respiratory pause. Thus, similarly to Phocidae seals while they are in water, the manatees sleep in short episodes during respiratory pauses. Short arousals during respiratory acts are apparently due to the need to move for breathing. Respiratory pauses during wakefulness (172 ± 7 s) and during sleep (222 ± 6 s) were significantly different ($p < 0.001$) and correlated perfectly with the values obtained during observations of the manatee's behavior in the pond.

Obvious interhemispheric asynchrony of ECoG slow waves, similar to that observed before in different dolphins and Otariidae seals, was found in the Amazonian

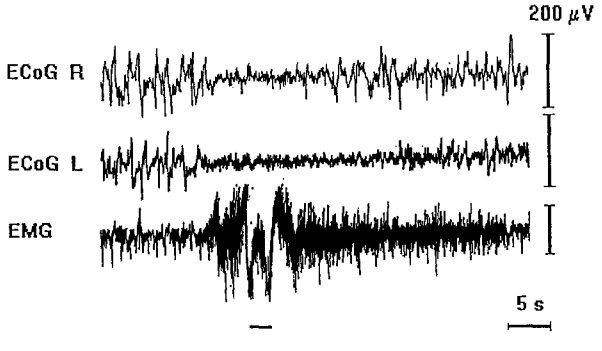

Figure 2. Arousal of the manatee during the respiratory act. ECoG R, the electrocorticogram of the right hemisphere; ECoG L, the electrocorticogram of the left hemisphere; EMG, the electromyogram of the neck muscles. Horizontal mark below: the time of the opening of the nostrils.

manatee. For example, interhemispheric asymmetry of slow waves occupied approximately a quarter of the total slow wave sleep time in a 24-h period. In contrast to dolphins and Otariidae seals, in which, as we suggested previously[1-3], interhemispheric asymmetry of ECoG slow waves is due to retained locomotory activity during sleep, no movements were associated with the slow wave interhemispheric asymmetry in the Amazonian manatee. The functional significance of this ECoG pattern therefore remains unknown.

The first study of sleep in a representative of the order of Sirenia showed that some features of its sleep were similar to those found before in other aquatic mammals. Thus the aquatic mode of life resulted in considerable changes of sleep structure in Cetacea, Sirenia, and Pinnipedia as compared to terrestrial mammals.

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Pheromone binding proteins of the mouse, *Mus musculus*

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Abstract. Proteins of the Major Urinary Complex of the adult male mouse (*Mus musculus*) selectively bind the male pheromones 2-(*sec*-butyl)thiazoline and dehydro-*exo*-brevicomin, and concentrate them in urine.
Key words. Pheromone; binding; proteins; urine; male mouse.

The ecology of the mouse is very dependent of olfactory cues. Chemical stimuli for intra-species communication (pheromones) are released with the urine by sexually mature – but not by impuberal nor castrated – male mice, and influence hormonally driven functions of female conspecifics, e.g., the induction of estrus (Whitten effect)[1] and the male-induced block of embryo implantation (Bruce effect)[2], as well as the behavior of conspecifics[3]. Some pheromones of the urine of the male mouse have been isolated and characterized; 2-(*sec*-butyl)thiazoline (**I**), 2,3-dehydro-*exo*-brevicomin (**II**), α- and β-farnesene[4,5]. The urine of the sexually mature – but neither impuberal nor castrated – male mouse is characterized by a high concentration of small proteins, the Major Urinary Protein Complex[6,7], whose function is not known. It seems probable that these proteins do have an important function, for three reasons. Firstly, the urinary loss of roughly 1 mg protein per day is a high cost for a mouse which in the wild state often ekes a life on the brink of starvation. Secondly, the genomic representation of the protein complex is highly amplified. Thirdly, the genes expressing the urinary proteins are highly conserved within the species, indicating a phylogenetic pressure against change in amino acid sequence[8]. An olfactory function seems plausible[9,10]. The present study showed that the proteins of the Major Urinary Complex selectively bind 2-(*sec*-butyl)thiazoline (**I**) and 2,3-dehydro-*exo*-brevicomin (**II**).

Methods and results

Major Urinary Proteins (MUP), separated from the very great variety of organic compounds present in urine by means of dialysis and chromatography, still retained bound pheromones. The gas chromatography and mass spectroscopy of the CH_2Cl_2 extract of purified MUP (fig.) showed almost exclusively pheromones **I, II**[11] and 4-(ethyl)phenol (**III**) in the ratio 17/3/4, and a non-identified compound (**IV**) with the mass spectrum of a terpene different from α- and β-farnesene. About 40% of the proteins had pheromone selectively bound to them, assuming complete extraction in CH_2Cl_2.

The approximate values of the binding constant of synthetic pheromones **I**, the two enantiomers (+)- and (−)-**II** and **III**, were assessed. The equilibrium binding of pheromones to the second isoform of MUP isolated by DEAE liquid chromatography from mouse and Wistar rat urine was studied in dialysis by displacement of 2-