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Sleep in the tortoise *Kinosternon* sp.

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Summary. Individuals of *Kinosternon* sp., previously confined to laboratory conditions, were chronically implanted with electrodes for electroencephalogram, electro-oculogram and electrocardiogram recording. Behavioral states of waking and sleep were clearly observed. Two sleep stages were present: quiet sleep and REM or active sleep. Electrical cerebral activity was polymorphic and irregular. EEG frequencies declined and amplitudes diminished with sleep. Arrhythmic spikes occurred during behavioral sleep and declined with waking. Heart rate decreased when passing from wakefulness to quiet sleep. It was slightly but consistently higher during active sleep compared with quiet sleep.
Key words. Behavioral sleep; quiet sleep; active sleep; REM sleep.

In sleep studies carried out in mammals and birds, scanty attention has been given to the separation of behavioral sleep from electrophysiological sleep. These two parameters generally correlate well enough, so the electrophysiological signs are used as indicators of behavioral sleep.

Sleep in mammals is accompanied by two alternating electro-

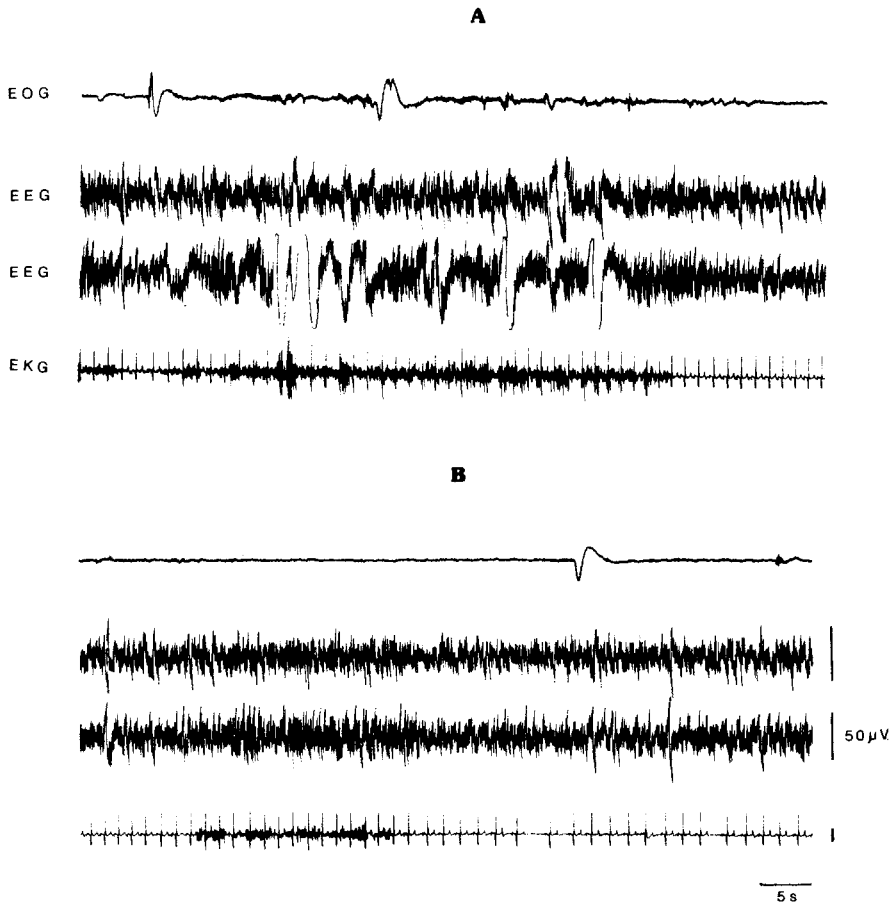

Figure 1. Polygraphic characteristics of active (A) and quiet (B) waking. During active waking, there are frequent eye movements; on the EEG some slow waves, produced by the animal's movements, can be observed; heart rate is elevated. During quiet waking, the eye movements become less frequent and heart rate decreases. EOG, electro-oculogram; EEG, left and right cerebral hemispheres respectively. Cal: 100 μv.

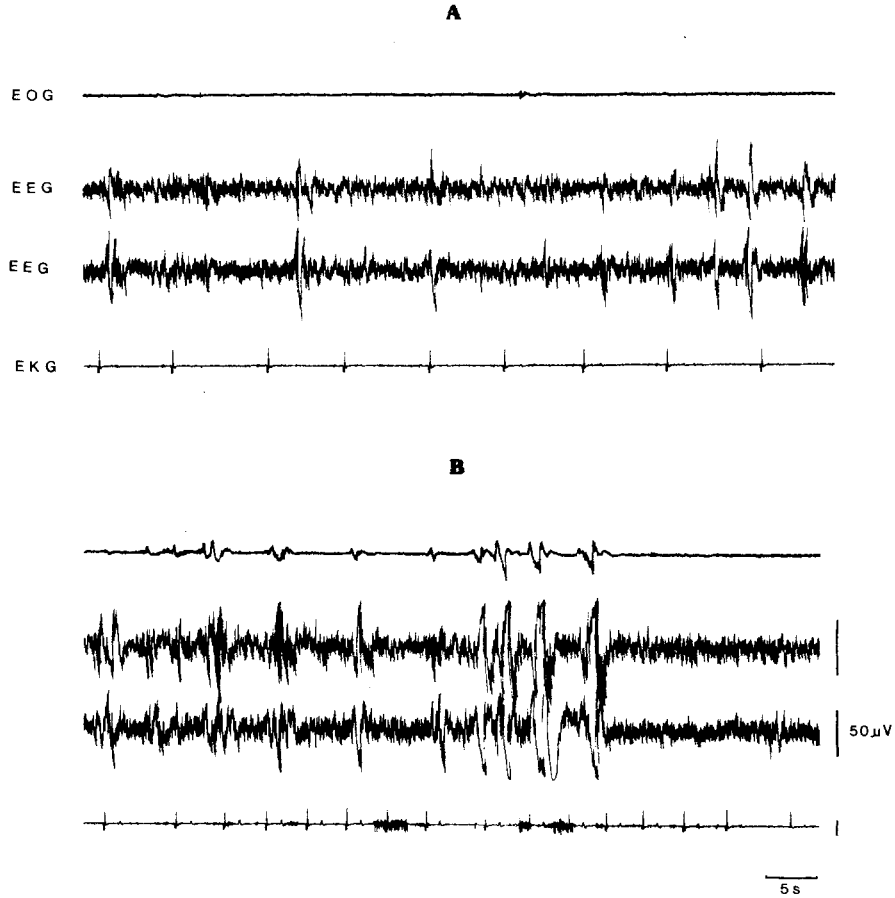

Figure 2. Polygraphic characteristics of quiet (A) and REM (B) sleep. During A, there are no eye movements, some spikes stand out over the basic cerebral rhythm, and cardiac frequency is minimal. REM sleep shows several eye movements coinciding with spikes and an increase in heart frequency. Cal: 100 μv.

physiological patterns: 1) Light or slow wave sleep (SWS), characterized by a cerebral activity of relatively low frequency and large amplitude; 2) Rapid eye movements sleep (REM) or paradoxical sleep (PS), characterized by a relatively high frequency and low voltage cerebral activity. This stage of sleep is typically accompanied by eye movements, lowered neck muscle tone, myoclonic twitches of the extremities and phasic variations in heart and respiration rates. Behavioral and electrophysiological sleep has also been reported in birds. Behavioral sleep has been normally observed to be correlated with an electoencephalographic pattern of high voltage slow waves. Furthermore, short epochs of mammalian-like PS have been described too. Sleep has been investigated in three major groups of reptiles: Chelonians[1-3], lizards[4,5] and crocodilians[6]. Excepting three species[3,7,8] all reptiles studied up to date exhibit behavioral sleep. In the species that were reported not to sleep, additional studies would be advisable; reported observations vary widely and this probably reflects not only species differences, but also variations in the experimental approach. Reptilian behavioral sleep is not accompanied by the electrophysiological characteristics of avian-like or mammalian-like SWS or PS. Due to the contradictory and inconclusive results concerning SWS and PS and other possible manifestations of sleep in reptiles, the present investigation was undertaken to determine whether the tortoise *Kinosternon* sp. (a representative of Chelonia order) exhibits behavioral sleep and, if so, the electrophysiological correlates of this behavior.

Methods. Four adult specimens of *Kinosternon* sp. of undetermined sex were used in this study. The animals were anesthetized with Nembutal (35 mg/kg) administered i.p. between the neck and forelimb. Under aseptic conditions stainless steel screw electrodes for chronic EEG bipolar recordings were placed bilaterally on the dura overlying the anterior and posterior dorsolateral cortex. Electrode placement was guided by comparison with a prepared and preserved specimen. Heart rate was recorded from stainless steel electrodes threaded into the carapace and the electro-oculogram was obtained from electrodes placed in the supraorbital bone. Flexible wires from the electrodes were soldered to a connector mounted on the carapace and fixed with acrylic cement. Recordings were made with the animals located in a sound-attenuated chamber, electrically shielded and constantly illuminated by a 60-W white bulb to facilitate observation through a one-way glass window. The recording chamber was kept at a temperature of 23 ± 3 °C. Several 24-h recordings were obtained at least two weeks after implantation of the electrodes. Recordings were made on a Grass Model III D electroencephalograph. Paper speed was 3 mm/s, with some samples at other speeds. The animals were continuously watched during these recording periods. Arousal thresholds to cutaneous stimulation were evaluated. Cutaneous stimulation involved touching the head of the animal with a rod. Subjective notations of relative response were made. Heart rate of each animal was measured in 60 s epochs during each state of vigilance. During PS this parameter was measured only in those periods longer than 5 s. Heart rate was then computed and averaged.

Results. Four states of vigilance were observed: Active Wakefulness (AW), Quiet Wakefulness (QW), Quiet Sleep (QS) and Active or REM Sleep. During AW extended limbs supported the body; the neck was partially extended with head slightly ele-

vated, eyes were open and some movements were observed. Heart rate throughout this stage oscillated between 23 and 49 beats/min ($\bar{X}$ = 40). EEG activity (fig. 1A) was of relatively high voltage and fast frequency (20–90 μv; 10–25 Hz). When animals were passing on to QW, the limbs were flexed and the shell rested on the cage floor; the neck was partially extended; eyes were usually open, with periodical closure preceding behavioral sleep. Heart rate slightly declined to an average of 32 ± 8 beats/min. EEG activity (fig. 1B) was similar to that of the preceding state. Each animal characteristically spent most of the 24-h period quietly with its eyes closed in one corner of the recording chamber. This constituted the behavioral position for QS. During this stage the animal's extremities were relaxed, its shell rested on the cage floor, its eyes were closed and ocular movements were abolished. The neck was extended, the head rested on the plastron or the floor. Heart rate was reduced to a minimum ($\bar{X}$ = 11 ± 3 beats/min), it was statistically significantly lower compared with waking ($p < 0.001$). EEG frequencies slightly declined and amplitudes decreased with sleep. Arrhythmic and intermittent spikes (50–125 μv) occurred monophasically or polyphasically in both cerebral hemispheres (fig. 2A). These electrophysiological signs reached peak levels during behavioral sleep and vanished or declined upon waking. After extended periods of behavioral sleep, short phases of active sleep or REM sleep were observed (fig. 2B). These phases had a mean duration of 19.2 ± 8.5 s. They were accompanied by motor automatisms which consisted of limbs and neck stretches, jaw and ocular movements (single or conjugated). The animals continued sleeping throughout these automatisms. During this sleep phase heart rate was significantly faster than that observed during QS (mean 21 ± 5, $p < 0.005$).

Stimulus presentations during AW and QW elicited head and limbs withdrawal and shell closure. Subjectively it was noted that when the animal was in QS, there was a higher threshold for response to stimuli.

In fact, no clear distribution of the vigilance states throughout the day was seen, but in two animals a slight increase in PS frequencies was observed between midnight and 06.00 h; however, this increase was not significant.

Discussion. It is always precarious to compare the behavior of species phylogenetically distant from one another; similar behavior may actually serve different functions in different species. As we do not yet understand the functional significance of sleep, we remain fixed essentially at a behavioral level. Proceeding at this level, there is no question that behavior observed in *Kinosternon* sp. bears a definite resemblance to what is called sleep in mammals and birds: total immobility, a characteristic posture, continuous eyelid closure, diminished responsiveness to stimulation, etc.

On the other hand, it has been suggested that mammalian slow wave sleep and reptilian spikes are functionally similar[1]. In mammals the elaboration of slow waves and spindles during sleep depends on the integrity of the neocortex[9]. Since reptiles have only a rudimentary cortex[10] it is unlikely that the electrophysiological expression of the spikes during behavioral sleep depends on the neural activity of this thin cellular layer. Furthermore, the thalamo-cortical system responsible for EEG rhythmicity is poorly developed in reptiles[11]. However, studies in the cat have shown that spikes recorded from the ventral hippocampus during SWS are similar to spikes recorded from the reptilian brain during behavioral sleep[12].

When studying the states of vigilance, motor automatisms may be observed during the sleep of poikilotherm vertebrates, such as fish[13], amphibians[14], and reptiles[2,4,15]. These automatisms, also observed in *Kinosternon* sp., were accompanied by ocular movements. A similar behavior is displayed by birds and mammals during typical states of REM or paradoxical sleep. REM sleep has been reported in the chameleon, since ocular movements, either isolated or grouped, were observed during behavioral quiescence[16]. Moreover, this sleep phase has been reported also in the iguanids *Ctenosaura pectinata*[5] and *Ctenosaura similis*[4] as well as in the desert iguana *Dipsosaurus dorsalis*[17]. From the present facts, it seems plausible to conclude that the tortoise *Kinosternon* sp. displays behavioral characteristics of REM sleep.

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Carnosine-like immunoreactivity in the primary olfactory neuron of the rat[1]

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Summary. Using the peroxidase-antiperoxidase immunohistochemical technique, carnosine-like immunoreactivity was demonstrated to localize specifically within the primary olfactory neuron.
Key words. Carnosine; neurotransmitter; olfactory bulb; primary olfactory neuron; immunohistochemistry.

Carnosine (β-alanyl-L-histidine) was shown biochemically to be present within the primary olfactory neuron[2,3]. The concentration of this dipeptide in the olfactory epithelium and olfactory bulb was 10–20-fold higher than that in any other region of the