Difference in Electrical Activity During Wakefulness and the Phase of 'Sleep with Muscular Twitches' as Recorded from the Cortex of the Marsupial *Didelphis azarae* (South American Opossum)

Recent reports indicate that the sleep of cats includes a phase during which ordinary electroencephalographic technique does not show any difference in relation to the records obtained during wakefulness[1]. These observations were repeated by recordings in the monkey[2], goat[3], pigeon[4], rabbit[5], dog[6], rat[7], lamb[8], chicken[9] and armadillo[10]. We recently described in *Didelphis azarae* a phase homologous to that described in other animals, which we named the phase of 'sleep with muscular twitches'[11].

The aim of the present paper is to show that in the opossum, *D. azarae*, the records obtained from a given region of the cortex during that phase are clearly different from those obtained during wakefulness in the same region, in relation to the topographic orientation of the recording electrodes. Because of this fact (see reasons below) and because of the peculiar and almost 'schematic' brain organization[11,12], facilitating correlation of anatomical and physiological findings, this primitive mammal seems to be particularly interesting for studies on sleep physiology.

20 adult opossums weighing from 1.5–3.0 kg were used. Stainless steel screws were chronically implanted in pairs over the cerebral cortex in 2 different ways: (a) oriented on a transversal plane to the sagittal axis of the brain (transversal leads); (b) oriented on a plane parallel to the sagittal one of the brain (longitudinal leads). Different leads were made by combining these screws. The Figure shows some of the leads used (frontal and occipital ones).

The results obtained were always constant and predictable for all cortical regions from the frontal to the occipital pole of the brain. When longitudinal leads were used, no difference could be observed between wakefulness and 'sleep with muscular twitches': both showed low voltage fast rhythms (20–30/sec, 30–50 μV) (Figure A: 3 and 4). In contrast to this, in all transversal leads a great difference between the records of wakefulness and 'sleep with muscular twitches' was observed (Figure A: 1, 2, 5 and 6). During 'sleep with muscular twitches' a clear theta rhythm appeared with low voltage fast waves superimposed on it (Figure A: 1, 2, 5 and 6). The theta rhythm was seen more clearly when filters to reject fast activity were used (Figure B: 1, 2, 5 and 6). We should like to point out that sometimes, when the animal was engaged in exploring behaviour, we could observe a theta rhythm similar to that recorded during 'sleep with muscular twitches' although not so clear or so regular.

These experiments do not lead to any conclusion about the origin of the theta rhythm observed in the conditions described above. Whether they are cortical or subcortical, only further experiments can show. However, the possibility of a hippocampal origin should be kept in mind, especially if we consider: (a) the great anatomical development of the dorsal hippocampus in *D. azarae*; (b) the high amplitude theta rhythm described in other animals in the hippocampus during 'sleep with muscular twitches'[1]. This theta rhythm could be propagated to the cortex through neural pathways or could be simply 'physically' propagated, the cortical neurones being thus submitted to its fluctuating potentials.

We think that these different observations are of considerable practical importance, because they provide a rapid and simple means of recognizing the phase of 'sleep with muscular twitches' when the observation of its peripheral signs (muscular twitches, irregularity of respiration etc.) is not possible or is more difficult. Such may be

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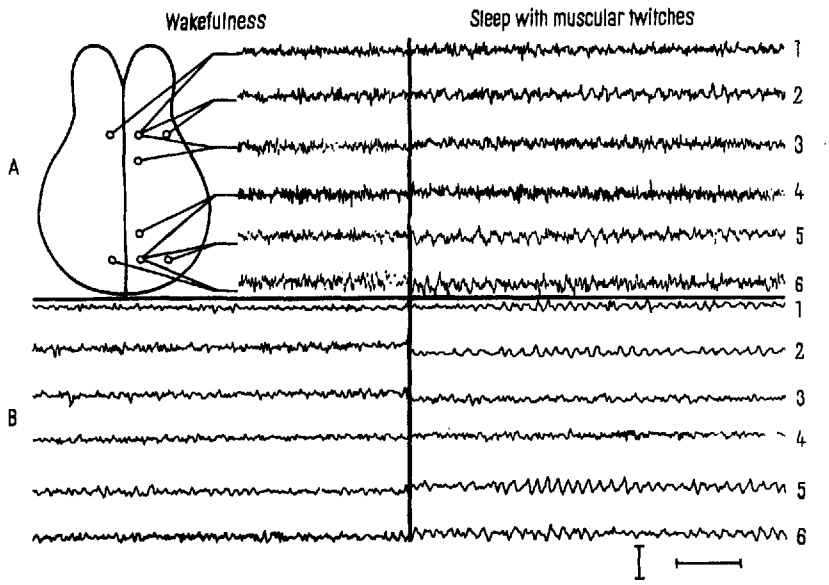

Leads shown on the schematic brain apply equally to A and B. For each stage (wakefulness and 'sleep with muscular twitches') A and B were simultaneously recorded using different filters. 1 = Frontal interhemispheric; 2 = frontal transversal; 3 = frontal longitudinal; 4 = occipital longitudinal; 5 = occipital transversal; 6 = occipital interhemispheric. Calibration: vertical bar, 100 μV; horizontal bar, 1 sec.

the case in curarized animals, or animals with different brain stem sections. The theta rhythm, together with other characteristics of 'sleep with muscular twitches' (duration, mode of onset and finishing), may help in its diagnosis.

SWISHER[13] also reported the existence of a theta-like rhythm of 6–8/sec waves differentiating wakefulness from what he called 'activated sleep'. However, in some of his rats (3 out of 5) he also reported the existence of 6–8/sec rhythm during wakefulness, although it was barely recognizable. This theta rhythm described for 'activated sleep' was never seen in symmetrical leads and was seen only in asymmetrical ones. In contrast to this, our opossums showed the theta rhythm only in transversal combinations, including the symmetrical ones (interhemispheric).

According to our observations, the name of 'rapid' or 'paradoxical sleep' applied to other animals is justified in the opossum only for the longitudinal position of the electrodes. As in this animal other electrode positions show definite advantage for the reasons mentioned above, these terms do not seem to be the best ones. We think that it would be better to refer to this phase as 'sleep with muscular twitches' or 'theta sleep'.

Resumen. Se ha observado una neta diferencia entre los registros electroencefalográficos de la vigilia y la fase de «Sueño con sacudidas musculares» del Marsupial *Didelphis azarae* según sea la posición de los electrodos sobre la corteza cerebral.

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Das hypoxiebedingte EEG-Arousalstadium unter dem Einfluss von Pentobarbital und Urethan

Nach Inhalation eines Gemisches von N_2 + 4% O_2 tritt bei erwachsenen Laboratoriumstieren nach symptomfreier Latenzzeit ein EEG-Stadium auf, das durch niedrige Amplitude der corticalen Wellen und eine Frequenz um 20/sec gekennzeichnet ist. Die dieser EEG-Veränderung zugrunde liegende aufsteigende Aktivierung wird durch Mittelhirndurchtrennung verhindert[1,2] und kann daher pontinen Teilen der Formatio reticularis zugeordnet werden. Eine pharmakologische Beeinflussung des hypoxiebedingten EEG-Arousalstadiums wurde unserens Wissens bisher nicht unternommen und war Gegenstand der vorliegenden Untersuchung.

10 erwachsene Ratten und 5 Kaninchen wurden nach Implantation corticaler Elektroden über den Areae praec. agr. und striata in einer mit konstantem Stromzeitvolumen (N_2 + 4% O_2) durchflossener Kammer zunehmender Hypoxie ausgesetzt. Bei den unbehandelten Tieren kam es im Mittel nach 39 sec zur Arousalreaktion. Am folgenden Tage wurde den gleichen Tieren 30 mg/kg Pentobarbital i.p. injiziert. $^1/_2$ h nach der Injektion wurde in gleicher Weise wie am Vortage der Hypoxieversuch ausgeführt. Es trat bei keinem Tier das Arousalstadium auf. 2 Tage später bekamen die Tiere 1,0 g/kg Äthylurethan i.p. injiziert. Unter Hypoxie kam es zur EEG-Arousal wie beim unbehandelten Tier. Diese Reaktion trat aber im Gegensatz zum wachen Tier im Mittel erst nach 69 sec auf. Der Unterschied der Latenzzeiten ist (t-Test) signifikant ($\alpha = 2\%$).

Die hypoxiebedingte EEG-Arousal wird durch Reizung der Receptoren des Carotissinus einerseits und direkten Sauerstoffmangel an den reticulären Neuronen andererseits ausgelöst[1,3]. Sie wird durch Pentobarbital und

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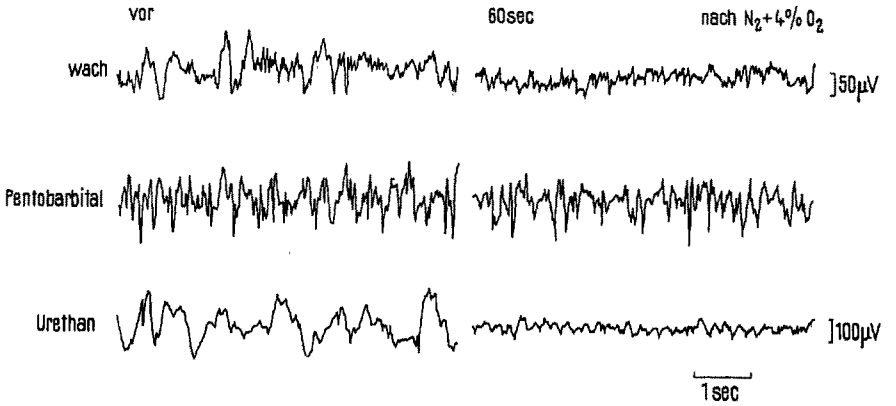

Bipolare fronto-occipitale Elektrogramme der erwachsenen Ratte vor und 50 sec nach Inhalation von N_2 + 4% O_2. Unter Pentobarbital tritt keine Arousalreaktion auf.