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THE ELECTROENCEPHALOGRAM OF THE NORMAL "GRADE" PONY IN SLEEP AND WAKEFULNESS*

L. A. HALE† & S. E. HUGGINS

Department of Biology, Program in Physiology, University of Houston, Houston, Texas 77004, and Department of Physiology, Baylor College of Medicine, Houston, Texas 77030, U.S.A.

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Abstract—1. The basic characteristics of the stages of sleep and wakefulness in the "grade" pony as found in this study are summarized as follows:

2. The waking EEG was composed of mostly fast frequency (16–25/sec) low voltage (10–50 μV) waves.

3. Drowsiness was characterized by waves of 7–16/sec (50–70 μV) and 4–7/sec (50–100 μV), interspersed with runs of alpha (8–12/sec) and sleep spindles (usually around 5–8/sec).

4. The rhythm of slow sleep was observed as having basically slow (1–4/sec), high voltage (100–200 μV) waves upon which faster waves of 10–12/sec (40–100 μV), 7–8/sec spikes (80–150 μV), and K complexes were superimposed. The heart rate and eye movement activity usually decreased from that of wakefulness. In this stage the animal was observed to be either standing with head low or sternally recumbent.

5. Paradoxical sleep was marked by a fast (18–24/sec) low voltage (10–30 μV) desynchronized EEG pattern, hardly distinguishable from that of waking except for the frequent appearance of 3–4/sec sawtooth waves in REM. Rapid eye movements, an irregular heart rate, and a greatly diminished neck muscle tone were usually seen during this period. This state was seen only in animals in the sternal recumbent position. The lateral recumbent position was never seen in these experimental animals.

INTRODUCTION

Relatively few studies of the spontaneous electrical activity of the brain of Equidae have been made. The principal ones have been those of Ruckebusch, which have included studies made on asses (1963a,b); those of Ruckebusch *et al.* on normal horses (1970a,b) and a single pony (1970a); those of Dallaire *et al.*, involving Pottock ponies (1974a,b,c); and that of Lapras *et al.* who studied both normal horses and horses infected with meningoencephalomyelitis (1968).

This study was undertaken for two reasons: first to augment the data available on normal electroencephalographic patterns of Equidae, and second to help establish basal data with the view of using the "grade" pony as a model for physiological studies as suggested by Garner *et al.* (1971a). It was also thought to be desirable to further investigate the possible existence of REM sleep during periods of "standing sleep".

MATERIALS AND METHODS

Four "grade" Shetland ponies were used in this study. The three females and one male were all 2 years of age and ranged in weight from 80.0–155.4 kg (Table 1). Animals underwent a preconditioning period to insure freedom from infectious diseases and parasites and upon arrival in the laboratory they were housed in a special facility; the treatment and housing is described in detail by Garner *et al.* (1971a,b). These animals were fed a mixed diet of hay and oats.

The first pony was studied using silver disk electrodes with a standard electrode paste. Had this proven to be completely successful, it would have been a very desirable technique because of its simplicity and safety to the pony. In practice, however, excessive muscle artifact produced by the nearly continuous chewing movements of muscles which, in Equidae, almost completely cover the cranium dictated the use of implanted stainless steel screw electrodes. Sites for electrode implantation were chosen over the anterior portion of the parietal lobe, and over the posterior portion of the parietal lobe, presumably the visual area (Skinner, 1971); placement was 12–15 mm on each side of the midline. The occipital screws were 47–55 mm anterior to the nuchal chest, depending on the size of the pony, and the frontal screws were 120–143 mm anterior to the same prominence. These sites were chosen from measurements of several pony skulls and examination of a sectioned, frozen pony head. Placement was checked by X-ray and post-mortem examination.

The implanted screw electrodes were number 6/32, 7 mm in length. These were insulated with epoxylite and oven-baked. The male component of a snap-on battery connector was soldered to the head of the screw and the female half of the connector system was soldered to the shielded lead wire. This allowed easy and rapid connection and disconnection from the recording apparatus.

Anesthesia and general surgical procedures have been described previously (Garner *et al.*, 1972). A sharpened 19 mm cork borer was used to remove skin plugs from previously prepared areas. The soft tissue was then retracted and a dental burr fitted with an aluminum stop-sleeve was used to drill through the skull. These holes were then tapped to the correct pitch for the screw electrode. Each screw was fitted with a threaded teflon sleeve which gave a more secure fit by acting as a nut between screw and skull and also limited the penetration of the screw. Any oozing blood was checked by electrocautery and screws were further stabilized with dental acrylic applied over all the exposed bone surface. A cloth hood was used to protect the electrodes when not in use.

Animals were allowed to recover from surgery for at least 36 hr before recording was initiated. EEG recordings were from bipolar transverse connections. Pin electrodes

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† Present address: Department of Nuclear Medicine and Radiation Biology, U.C.L.A.

Table 1. Data on animal subjects

	Sex	Age	Weight	EEG Electrodes
Pony I	Male	2 yrs	93.0 kg	Silver Plates
Pony II	Female	2 yrs	95.3 kg	Stainless Steel Screws
Pony III	Female	2 yrs	80.0 kg	Stainless Steel Screws
Pony IV	Female	2 yrs	155.4 kg	Stainless Steel Screws

Table 2. EEG frequencies and amplitudes for the "grade" pony in various stages of sleep and wakefulness

Pony	Awake	Drowsy	Slow-sleep	REM†
Pony I			-	
			10-12/sec	
			-	
			1-2/sec*	
Pony II	18-22/sec* (10-30 μV)	-	-	18-22/sec* (10-30 μV)
	-	7-12/sec* (50-70 μV)	10-12/sec (40-70 μV)	-
	-	4/sec* (70-100 μV)	7-8/sec (80-100 μV)	-
	-	-	1-4/sec* (100-200 μV)	-
Pony III	25/sec (10-20 μV)	-	-	18-24/sec (10-20 μV)
	-	7-12/sec (50-70 μV)	10-12/sec (50-100 μV)	-
	-	4-5/sec (80-100 μV)	7-8/sec (100 μV)	-
	-	-	1-4/sec (150-200 μV)	-
Pony IV	16-22/sec (20-50 μV)	-	-	
	-	12-16/sec (50 μV)	10-12/sec (50-100 μV)	
	-	5-7/sec (50-100 μV)	7-8/sec (100-150 μV)	
	-	-	2-4/sec (150-200 μV)	

* Dominant frequency.
† Ponies I and IV failed to show any REM sleep and were never seen to sleep in the recumbent position.

were used to record electro-oculograms (EOG) and electromyograms (EMG) and served as ground connections. No separate electrodes were used for recording the electrocardiogram (ECG), but it was usually detectable in the EMG recording when the animal was drowsy or asleep, although not in the waking condition.

All recording was done while the pony was in the stall in which it was normally housed in the laboratory. Leads from all electrodes were bound into a single cable attached mid-dorsally to a surcingle; this cable was supported from above with a spring and counter-weight to prevent tangling and unnecessary strain on the electrodes. A Physiograph (Narco-Biosystems, Inc., P.O. Box 12511, Houston, Texas, 77017), equipped with an automatic timing device which turned the paper control on for 10 min out of each half hour, was used much of the time but manual control could be used to record continuously. Two types of preamplifier systems were used: for the electro-oculogram, a Reograph (Narco-Biosystems) coupled with a Cardiac Preamplifier (Narco-Biosystems), and for electromyograms and electroencephalograms, a Hi-Gain Preamplifier (Narco-Biosystems). In order to damp the signal when the paper was not running, a 330-ohm resistor was connected across the pen motor on the EMG channel and 470-ohm resistors across the other channels.

While recording was being done with the pony in its accustomed stall, the operator was in a separate room about 4 m away and out of the pony's field of vision. The recording room was well lighted, but the room containing the pony's stall was dimly lit. A mirror placed above the stall permitted viewing the animal without entering that room or disturbing the pony. Recording was limited to 8 hr night periods, two for ponies I and III and three nights for ponies II and IV.

All records were analyzed manually with the aid of a Sheatz ruler (Sheatz, 1964).

RESULTS

A summary of EEG data is found in Table 2. The pattern of electrical activity for waking animals and three stages of diminished alertness, i.e. drowsy, slow-sleep, and REM sleep, were clearly distinguished in ponies II and III. Pony I had excessive muscle activity superimposed on the EEG, such that only when it was deeply asleep could the EEG be calibrated. Neither pony I nor IV lay down and both failed to show REM sleep.

In the alert, awake ponies the EEG showed a dominant fast frequency, low voltage, desynchronized rhythm varying slightly from individual to individual, but in the 16–25/sec and 1–50 μV ranges. Pony III had the fastest frequency and the lowest voltage (Fig. 1). The neck muscle myograms of waking ponies were of high voltage (50–100 μV), and the EOG showed a high frequency of eye movements. Heart rates were not available in this awake condition (Fig. 1). Table 3 presents a review of EMG, EOG, and heart rate as they accompanied the various sleep stages.

As the ponies became drowsy, EEG frequencies decreased and amplitudes increased. Two rhythms of approximately equal importance were seen, both of which might be described as midrange in frequency and amplitude; one was in the 7–16/sec and 50–70 μV range, and the other was in the 4–7/sec and 50–100 μV range (Fig. 2). In addition, runs of waves having an alpha frequency, 8–12/sec, lasting a few sec were seen in all three ponies, as well as spindle-like formations of slower, 5–8/sec, frequencies. Both the alpha and spindle activities were more prominent from the occipital lead (Fig. 3). The heart rate differed in the two drowsy ponies from which it was recorded: 51/min for pony III, which was the smallest of the animals, and 32/min for pony IV, which was the largest. The EMG had dropped in amplitude to 30–50 μV in the drowsy state and eye movement was diminished.

Animals in what was judged to be a deep stage of "slow-sleep" showed a dominant slow, high voltage rhythm of 1–4/sec and 100–200 μV with mid-range patterns in the 7–8/sec, 80150 μV and 10–12/sec, 40–100 μV groups superimposed, the former often appearing spike-like (Fig. 4). There were also what appeared to be K-complexes consisting of one or two high voltage (150 μV), sharp waves followed by spindles of 10–12/sec activity (Fig. 5). These were more prominent from the posterior electrode placements. During slow sleep the heart rate did not

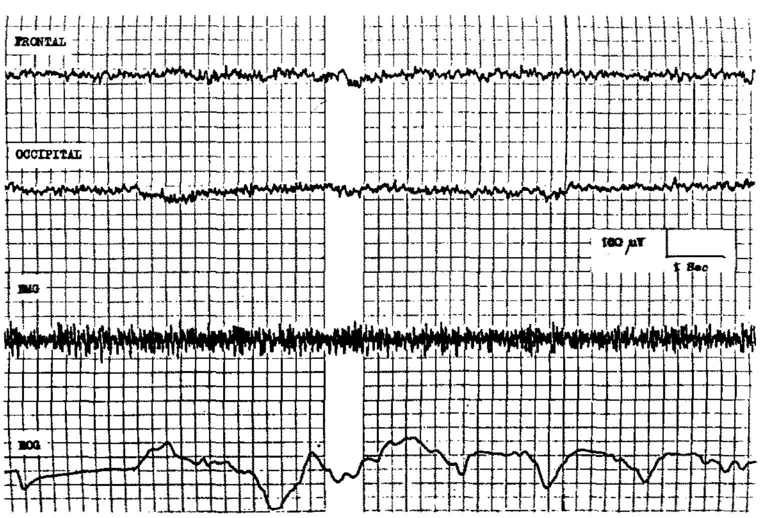

Fig. 1. EEG of pony III when fully awake showing dominant 25/sec waves with an amplitude of 10–30 μV. Also shown are the high degree of muscle activity and the frequent eye movements.

Table 3. Heart rate, EMG, and EOG in relation to sleep

	Awake	Drowsy	Slow-sleep	REM
Heart rate pony III	-	51	50-51	49-53*
Heart rate pony IV	-	32	30*	-
EMG (av.)	50-100 μV	30-50 μV	20-40 μV	0-10 μV
EOG (av.)	High frequency	Moderate frequency	Low frequency	High frequency

* Incomplete heart block and dropped beats were conspicuous features.

change materially from that seen in the drowsy animal, but animal IV showed considerable irregularity with R–R intervals varying from 3.5–5.3 sec, usually appearing as two or three beats with reduced intervals followed by one beat of a longer interval. The amplitude of the EMG of the neck and the frequency of eye movement both diminished. Animals might be either standing with the head very low, sometimes nearly reaching the floor, or they might be lying down when this pattern was displayed.

In both pony II and pony III, a condition comparable to the REM sleep of other mammals was seen, but only during the periods when the animals were recumbent. At this time the dominant activity was desynchronized high frequency (18–24/sec) and low voltage (10–30 μV) with intermittent slow waves and notably a rather regular sawtooth wave with a frequency of 3–4/sec and a moderate amplitude of 50 μV (Fig. 6). When this general pattern was present, pony III showed a very irregular heart rate, averaging 49–53/min, but with individual R–R intervals varying between 0.75 and 1.25 sec. The EMG of the neck muscles of the ponies displaying this stage of sleep was low in amplitude (0–10 μV) and both animals then displayed greatly increased ocular activity.

In ponies II and III, REM sleep accounted for a significant portion of the time spent sleeping in the recumbent position. In pony II this amounted to 44% of the recumbent sleeping time with individual REM periods lasting from 3.0–5.8 min. In pony III the percentage was less, 33%, but the average periods were longer, 3.8–8.0 min. Both animals slept only in the sternal recumbent position with the head resting on either the ground or on a forelimb; neither adopted the lateral recumbent position. Ponies I and IV did not lie down during any recording session, and actually lay down very little during the entire period during which they were kept in the laboratory under observation.

DISCUSSION

The electroencephalographic sleep-waking patterns in the "grade" pony and other concomitant physiological events agree substantially with those found by Ruckebusch, Dallaire & Ruckebusch, and Ruckebusch *et al.* (1963a,b; 1970a,b; 1974a,b) and therefore stages of the sleep-waking continuum in this study are those used by Ruckebusch, i.e. waking, drowsy, slow wave sleep, and paradoxical sleep. The EEG patterns described in this study are in part similar to those outlined by Dement & Kleitman (1957) for man,

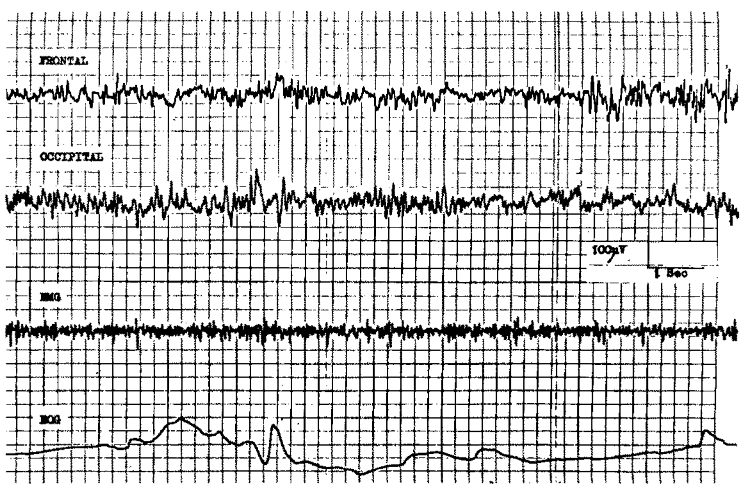

Fig. 2. Drowsiness as seen in pony III with some runs of alpha frequency (10–12/sec).

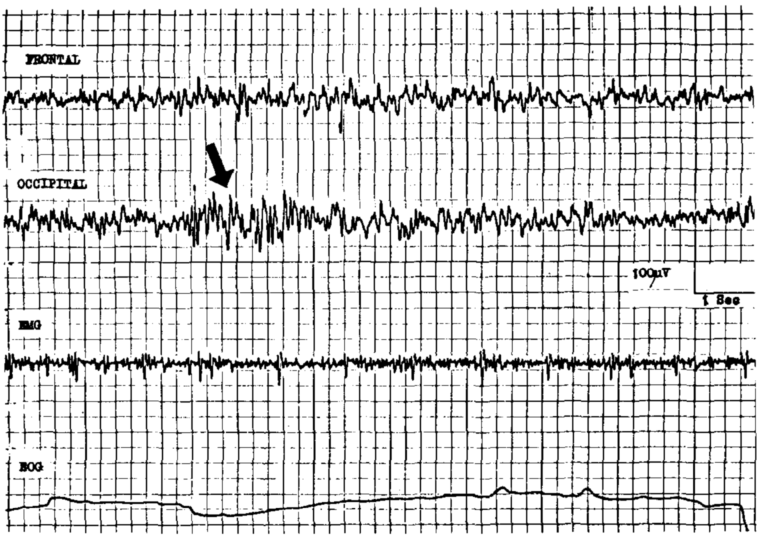

Fig. 3. More drowsy than in Fig. 2, waves of 7–12/sec (50–70 μV). The arrow ↓ marks a high voltage sleep spindle. EMG is of low voltage and ocular activity is much reduced.

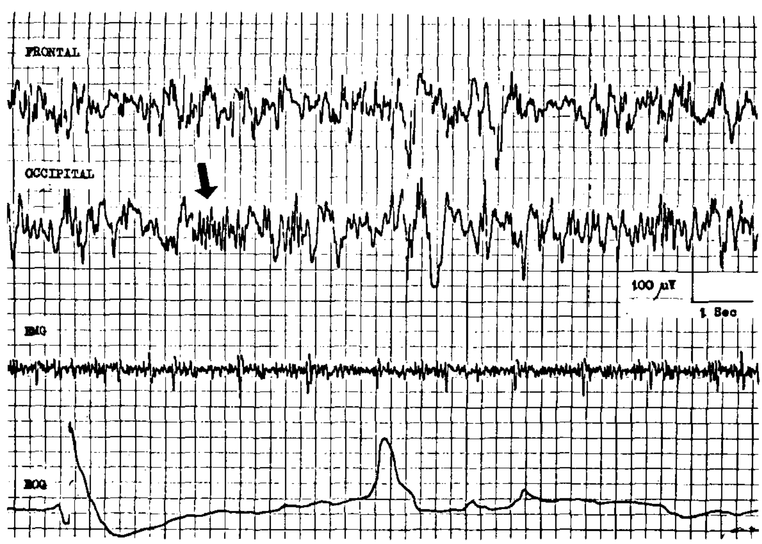

Fig. 4. Slow sleep as seen in pony III with dominant 1–4/sec (150–200 μV) rhythm and superimposed 7–8/sec (100 μV) and 10–12/sec (50–100 μV) rhythms. Arrow ↓ marks a K-complex. EMG voltage and eye movement are reduced.

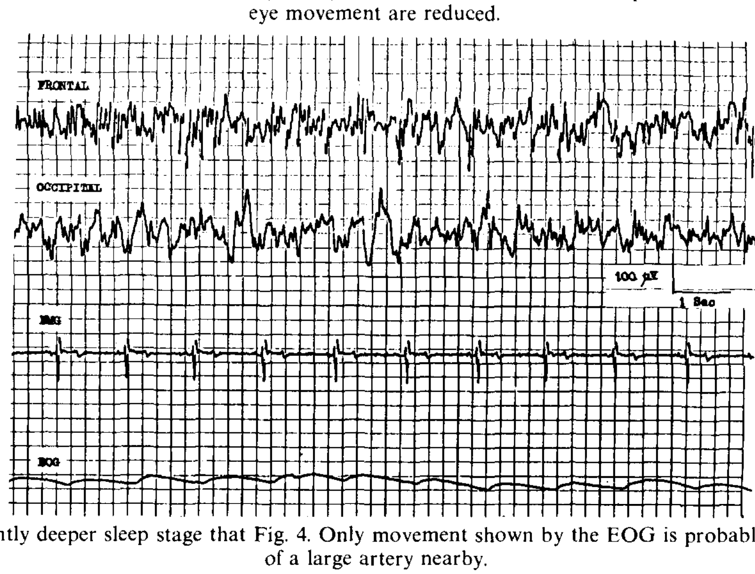

Fig. 5. Slightly deeper sleep stage that Fig. 4. Only movement shown by the EOG is probably the pulse of a large artery nearby.

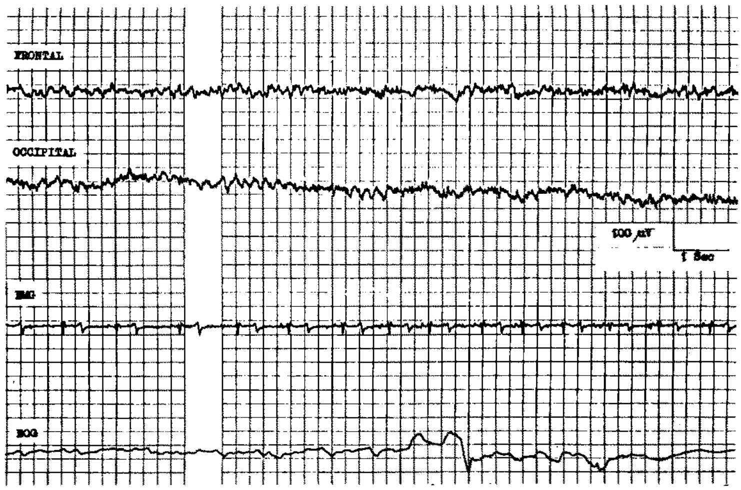

Fig. 6. REM sleep as illustrated by pony III showing 18–25/sec desynchronized waves of low voltage (10–20 μV). Some "sawtooth" waves. The EMG is almost nonexistent, and therefore the ECG, which is quite irregular, comes through clearly. There are fine, rapid eye movements.

although it was not possible to distinguish the subtleties of the four human sleep stages, and there was no stage in which there was a conspicuous, nearly continuous alpha rhythm, such as is seen in the relaxed but awake human subject.

The stage described here as drowsiness has characteristics described by Dement & Kleitman as occurring in both stage I and stage II human sleep, namely, an intermittent alpha rhythm and scattered spindle-like activity. Slow sleep in the pony is similar to stage III sleep in man with scattered spindles interspersed with waves in the delta frequency range. There is not, either in this study or in the published records of Ruckebusch, or of Dallaire, a pattern truly resembling human stage IV sleep with its almost pure delta rhythm. It may be that Equidae need a long period of adjustment to their surroundings before they sleep profoundly. The Equidae studied by Dallaire & Ruckebusch as well as the two ponies in this study did, however, display typical REM sleep, which is usually considered to indicate profound relaxation.

Ruckebusch *et al.* (1970) were able to determine from continuous recordings that 4.1–9.7% of the 12-hr night recording consisted of REM sleep and that the duration of individual REM periods was 2.1–13.4 min. Dallaire & Ruckebusch later found that in Pottock ponies REM sleep accounted for from 8–15% of the 12-hr night recordings or 20–33% of the time in the recumbent position, depending on the food, i.e. hay or oats. In the present investigation, REM periods were similar in length to those seen by others varying from 3.0–8.0 min.

REM sleep occupied 44% of the time that pony II was recumbent and 33% for pony III. The mixed hay and oat diet of the present group did not, as might be expected, lead to a time duration of REM intermediate between the separate hay and oat diet seen by Dallaire. Ruckebusch & Dallaire found that animals lay in lateral recumbency for about 20% of the total lying down time. This position was not observed in the present study but the animals exhibiting REM sleep were always found in the sternal recumbent position. In neither study was paradoxical sleep observed in standing animals. Presumably standing is not compatible with the profound muscle relaxation of the REM complex.

As has been stated by Dallaire & Ruckebusch, each animal has its own pattern of sleep and this is nowhere better illustrated than in cardiac activity. In the earlier papers Ruckebusch & Dallaire did not comment much on cardiac activity but in their 1974 paper (Dallaire & Ruckebusch, 1974b) they observed irregularly during paradoxical sleep of about the same degree as was seen here in pony III. In pony IV of the present study there was a very conspicuous cardiac irregularity including incomplete block and dropped beats during SWS. Possibly it was this cardiac "problem" which prevents recumbency and therefore REM sleep in pony IV. As was observed by Dallaire & Ruckebusch the cardiac irregularity of pony III did not alter the overall heart rate and the changes in overall rate seen in pony IV were not substantial.

The fact that the present study used female ponies for the main body of data and used animals of uniform age make the general agreement with the work of Dallaire & Ruckebusch more significant because they used primarily males ranging in age from 6 months to 6 years.

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