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The use of EEG and ECG Ambulatory technique in horses: Preliminary observations

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Summary

The use of the Ambulatory EEG/ECG technique in equine veterinary practice is here proposed. This technique allows the direct recording of EEG/ECG activity over a long period of time in the animal's normal environment.
The cerebral and cardiac electrical activity of twelve adult stallions was monitored during their normal daily activities, including periods of wakefulness, drowsiness and light sleep. The data were stored in magnetic cassettes of a small recorder. Subsequently these cassettes were analysed in the central unit of a computer equipped with a specific software; during the analysis biological and technical artefacts were identified and differentiated from EEG/ECG activity.
During wakefulness EEG was characterised by mixed theta, alpha and beta activity. Slower rhythms, however, mainly theta and delta in frequency, were observed during drowsiness and light sleep. These patterns were very similar to those reported in literature and obtained in traditional laboratories.
Since the animals were not restrained during recordings, several artefacts were obtained. These did not prevent any interpretation of the tracings, but on the contrary occasionally produced useful results for the analysis of animal behaviour.
Results suggest that the Ambulatory EEG/ECG technique may represent a useful tool in the investigation of the animal's clinical status. Moreover, as carrying out this technique does not require the transferral of the horse to specific laboratories, it can be used for other studies, for example concerning the behaviour and the general welfare of the horse.

Keywords: horse, electroencephalography, ambulatory EEG/ECG

Die ambulante Anwendung von EEG und EKG beim Pferd: vorläufige Beobachtungen

Hier wird die ambulante Anwendung von EEG und EKG in der Pferdepraxis vorgeschlagen. Die verwendete Technik erlaubt die direkte Aufzeichnung der EEG- und EKG-Aktivitäten über einen längeren Zeitraum in der normalen Umgebung des Tieres.
Die zerebrale und kardiale elektrische Aktivität von zwölf erwachsenen Hengsten wurde während ihres normalen Tagesablaufes aufgezeichnet, dazu gehörten Phasen des Wachseins, des Dösens und des leichten Schlafes. Die Daten wurden magnetisch aufgezeichnet und auf einem Computer mittels Spezialsoftware ausgewertet. Während der Analyse wurden biologische und technische Artefakte identifiziert und von der EEG- und EKG-Aktivität differenziert.
Während der wachen Phasen war das EEG durch eine gemischte theta-, alpha- und beta-Aktivität gekennzeichnet. Langsamere Rhythmen, meist der Frequenzen theta und delta, wurden während des Dösens und des leichten Schlafes beobachtet. Diese Muster waren den in der Literatur erwähnten und in traditionellen Laboratorien festgestellten Mustern sehr ähnlich.
Da die Tiere nicht in ihrer Bewegungsfreiheit beschränkt wurden, traten verschiedene Artefakte auf. Diese verhinderten jedoch nicht die Interpretation der Aufzeichnungen, sondern waren im Gegenteil manchmal für die Analyse des Tierverhaltens von Nutzen.
Die Ergebnisse zeigen, daß die ambulante Anwendung von EEG und EKG bei der klinischen Unersuchung der Tiere von Nutzen sein kann. Da das Tier dazu nicht in ein spezielles Labor gebracht werden muß, bietet sich zusätzlich die Möglichkeit, die Technik bei anderen Studien, zum Beispiel zum Verhalten und generellen Wohl des Pfedes, einzusetzen.

Schlüsselwörter: Pferd, Elektroenzephalographie, ambulantes EEG und EKG

Introduction

The study of a horse's performance is very important for genetic selection, but sometimes athletic proofs in themselves are not enough to demonstrate the health of the animal. In this case, clinical and laboratory investigations may provide more indicative results, particularly with regard to the functioning of the central nervous system (CNS).
Electroencephalography (EEG) is a relatively simple neurophysiological technique which can provide useful information about cerebral cortical activity and its modifications in relation to normal or pathological conditions, different behavioural states and diurnal and nocturnal cycles of sleep and wakefulness. Unfortunately, it has always been very difficult to obtain an EEG in the veterinary practice of large animals.
There are very few reports available from previous studies of equine EEG, and the existing ones are usually obtained by invasive techniques, which need to be performed in a specialised environment (*Garner*, 1972; *Dallaire* and *Ruckebusch*, 1974; *Mysinger* et al., 1985; *Otto* and *Short*, 1991) and therefore cannot be used for routine practice.
The purpose of this study has been to investigate the possibility of extending the use of a new electroencephalographic technique to horses, normally being used for humans and called Ambulatory

EEG/ECG (A/EEG-ECG). This technique allows the direct monitoring of cerebral and cardiac activity for a long time (up to 24 hours or more) in the subject's usual environment. Applied to the horse, it could be a very useful means for evaluating an animal's welfare as well as for investigating specific pathologies and/or general subclinical conditions which could affect the behaviour of the animal.

Materials and methods

For the experiments, twelve adult stallions of the maremmana breed, aged 3.5 to 3.9 years, were used. These animals were kindly provided by the Associazione Nazionale Allevatori Cavallo Maremmano during the annual performance test. Before any EEG/ECG recording began, the animals underwent a complete clinical, radiological and laboratory examination in order to establish their state of health.

During the entire recording period the animals were housed in single boxes (3m × 3m) and were not restrained in any way. The food and water rations which they received were balanced according to their body weight.

Recordings were obtained using silver disk electrodes applied with collodion to the skin. To increase the electrode adhesion and to improve the conduction of the electrical signals, a bentonite paste was used (Nihon Kohden Co., Tokyo, Japan). The skin was scrubbed with isopropyl alcohol but not shaved. The proper attachment of the electrodes was checked before each recording session and an electrode resistance of less than 5000 ohm was ensured.

Six electrodes were used for recording EEG, and three for ECG.

Cephalic electrodes were placed over the right frontal (RF), left frontal (LF), right occipital (RO), left occipital (LO) and vertex (V) regions, according to *Mysinger's* suggestions (*Mysinger* et al., 1985). The ground electrode was placed on the nasal bone. The positioning of the electrodes on the head of the horse is shown in Fig.1.

Cardiac electrodes were positioned as follows: the first one along the Vogel's line (at the level of the 13th rib); the second along the same line but 6 cm more ventrally, and the third at the level of the 12th rib in the middle vertical point, between the other two electrodes.

As only three electrodes were used, only the frequency and the regularity of the ECG rhythm could be evaluated. Any other considerations about the morphology of the single components of the PQRST complex were not investigated.

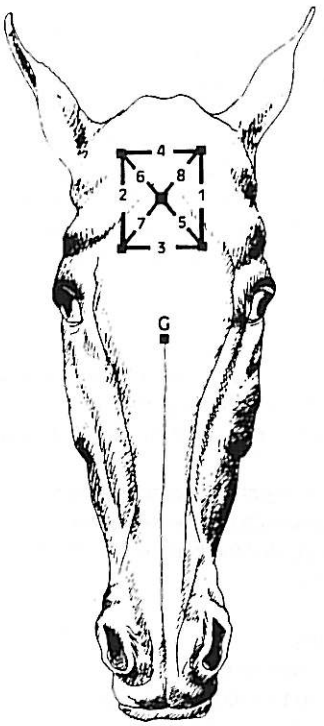

Fig.1: Electrode positioning on the scalp of the horse. Electrical signals are derived connecting together two electrodes (bipolar montages). Numbers represent the sequence of the connections and correspond to the numbers of the EEG channels (see Tab.1).

Using a 10 direction shielded cable (Micromed, Italia), electrodes were connected to a specially developed miniature recorder (Micromed, Italia) provided with a magnetic cassette (D-120-TDK, Japan) which stored the signals and which was attached to the horse's withers.

Before recording began, the EEG and ECG signals were checked on the monitor of a portable PC (Compaq, USA), equipped with appropriate software (Micromed, Italia). Subsequently, the signals were stored in digital form on the magnetic tape and then analysed in the central unit of the apparatus for A/EEG-ECG (Brain Spy System, Micromed, Italy). During the review process, suitable

Tab.1: Lead system, symbols, filters, gain and area of cortex monitored by EEG.

Lead	Symbol	Filters (Hz) (low – hi)	Voltage (gain)	Area measured
1	LF–LO	1.4–020	200 μv	Left occipital – left frontal
2	RF–RO	1.4–020	200 μv	Right occipital – right frontal
3	LF–RF	1.4–020	100 μv	Left frontal – right frontal
4	LO–RO	1.4–020	50 μv	Left occipital – right occipital
5	LF–V	1.4–020	100 μv	Left frontal – vertex
6	V–RO	1.4–020	200 μv	Vertex – right occipital
7	RF–V	1.4–020	100 μv	Rigt frontal – vertex
8	V–LO	1.4–020	200 μv	Vertex – left occipital
9	ECG	0.2–010	400 μv	Electrocardiography
10	REFERENCE			Time and ampl marker

software (Micromed, Italia) allowed the connection of the electrodes to each other according to different configurations (reformatting process) and the modification, if necessary, of the high-low filters and the single channels a gain.
Recordings lasted 18–20 hours, including day and night time. A fast review of the recordings was possible, so that a minimum of 18 minutes was required to examine data collected over a 20 hour period. The tracings were reviewed on a monitor and significant samples were transferred onto paper.

Results

Cerebral, cardiac and artifactual activities were obtained:
1) cerebral activity: electrical potentials from the brain recorded during a) wakefulness, and b) reduced alertness (drowsiness and light sleep);
2) cardiac activity: electrical potentials from the heart;
3) artefacts: signals not cerebral or cardiac in nature, which could modify, change and/or cancel the underlying cerebral and/or cardiac activity.

1) Cerebral Activity
A) wakefulness: During periods of wakefulness at rest the EEG was characterised by irregularly mixed alpha (8–13 Hz), theta (4–7 Hz) and beta (> 14 Hz) activity (Fig.2, A and B).

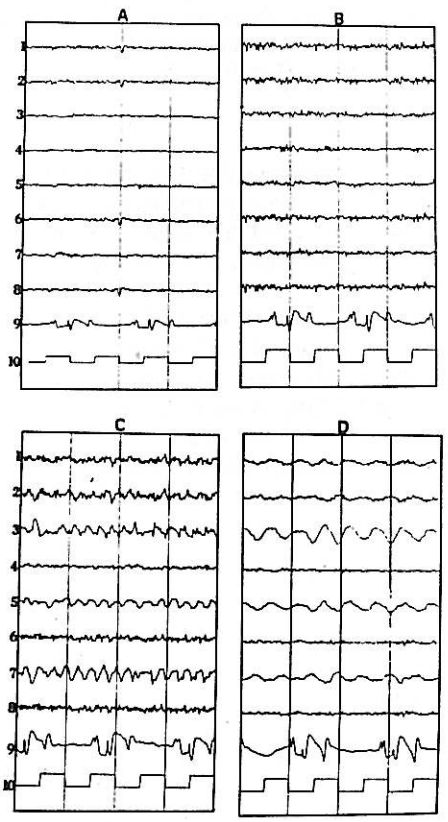

Fig.2: Examples of EEG/ECG tracings:
A and B: tracings obtained during wakefulness, at rest, with different gains.
C: tracing obtained during drowsiness.
D: tracing obtained during light sleep.
1–8: EEG channels; 9: ECG channel; 10: time/amplification marker.

B) reduced alertness: when the animal was relaxed (principally during drowsiness), sequences of monomorphic theta potentials were observed, synchronous in the frontal areas of both the left and right hemispheres (Fig.2, C). Monomorphic anterior activity of lower frequency (up to 2-3 Hz) and higher voltage was occasionally recorded, probably correlated to a state of light sleep (Fig.2, D). That activity was interrupted by the horse's arousal due to environmental stimuli, thus re-establishing the typical EEG pattern observed during wakefulness.

2) Cardiac activity
All the animals, with the exception of one, demonstrated a rhythmic activity of 30–40 beats/min whilst at rest. As a result of environmental stimulation and physical exercise, the cardiac frequency increased reaching 60–70 beats/min.
One horse, which was judged to be healthy after the clinical and laboratory examination, experienced some periods of sinus arrhythmia and also a period of sinus tachycardia, lasting about 20 seconds, during which EEG frequency increased up to 60 beats/min. It was impossible to correlate these features with any environmental stimulation but a reduced athletic performance was noted.

3) Artifacts
Technical and biological artifacts could be differentiated.

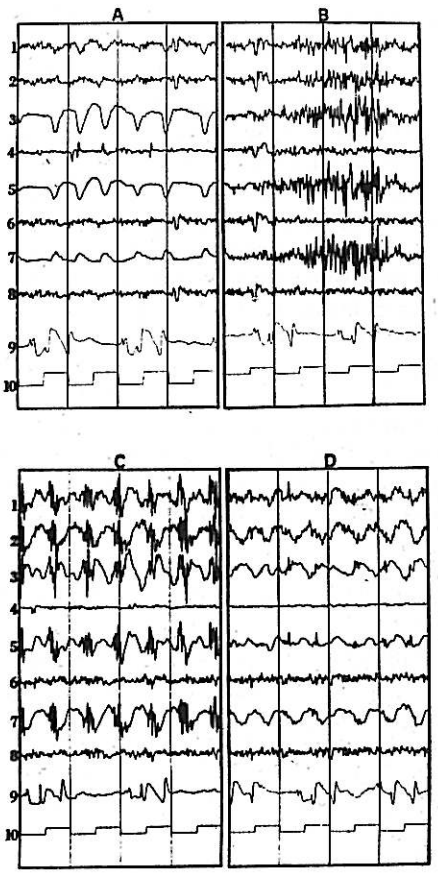

Fig.3: Examples of biological artifacts:
A: blinking (channels 3,5,7).
B: muscular activity (channels 1,2,3,5,7).
C: vigorous masticatory movements (channels 1,2,3,5,7).
D: weak masticatory movements (channels 1,2,3,5,7).
1–8: EEG channels; 9: ECG channel; 10: time/amplification marker.

A) Technical artifacts. These included:
1) disconnection of an electrode from the skin, characterised by periods of flat EEG;
2) pulling of one or more wires, characterised by fast and/or slow activity of high voltage;
3) drying out of the conductive paste, characterised by the appearance of a 14 Hz sinusoidal activity.

B) Biological artifacts. These included:
1) blinking, characterised by slow and /or sharp waves of high voltage and positive polarity in the frontal regions (Fig.3, A);
2) eye movements (vertical and/or horizontal), characterised by slow activity of medium/low amplitude in frontal regions, resembling theta and delta waves;
3) muscular activity (EMG), characterised by sequences of 18–23 Hz potentials, their amplitude being directly correlated to the degree of muscular contraction (Fig.3, B). This was the most common artifact observed on the recordings;
4) masticatory movements, characterised by rhythmic activity resembling polyspike-wave complexes. The fast activity (polyspikes) corresponded to the contraction of the masticatory muscles, while the slow waves corresponded to their relaxation. This artifact was very easily recognised and could not be confused with physiological and/or pathological cerebral activity.
It must be noted that masticatory movement artifacts varied in relation to the type of feed: in the case of long fibres (such as hay and straw) the artifacts were more noticeable, due to the more effective and rapid masticatory muscle contraction, than in the case of oats, cereals and grain (Fig.3, C and D).
5) Animal movements, characterised by fast and slow potentials of high and very high voltage (300–700 μV), which completely covered the underlying cerebral activity. As the horses were not restrained during the recording period, these artifacts occurred when the animals made large movements. ECG recording was also usually contaminated due to the high amplitude of these artifacts.

Discussion

Our results show that: 1) Ambulatory EEG technique may be applied to horses for recording EEG/ECG, without the necessity of moving them to specialised sites; 2) the cerebral activity recorded on cassette is very similar in frequency, amplitude and morphology, to that obtained in laboratory EEG, during either wakefulness or drowsiness and light sleep (*Dallaire* and *Ruckebusch*, 1974; *Mysinger* et al., 1985; *Andrews* and *Fenner*, 1987); 3) it is possible to prolong the period of registration (up to 24 hours or more), without reducing the wellbeing of the animal which can continue its normal daily activities. For this reason a very interesting application of A/EEG could be the study of the circadian rhythms of horses, as well as an investigation of their sleep cycles. Both of these studies could supply useful information about the welfare of the animal.
Other noticeable advantages of the A/EEG technique appear to be the possibility of taking recordings in an environment which is familiar to the animal, and the possibility of studying the role of environmental factors in inducing some neurological and/or behavioural pathological patterns.
Although recordings were taken from freely moving animals, their quality was satisfactory and the artifacts not so many as to prevent the visualisation of the cerebral signals. It must also be considered that artifacts may sometimes give helpful information about certain aspects ofanimal behaviour, such as motor activities, masticatory movements, etc.
Even if the technique should be improved, the satisfactory, even encouraging, results obtained suggest that A/EEG-ECG may broaden the use of the EEG as a means of diagnosis of a wide range of physiological and pathological conditions in veterinary practice.

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