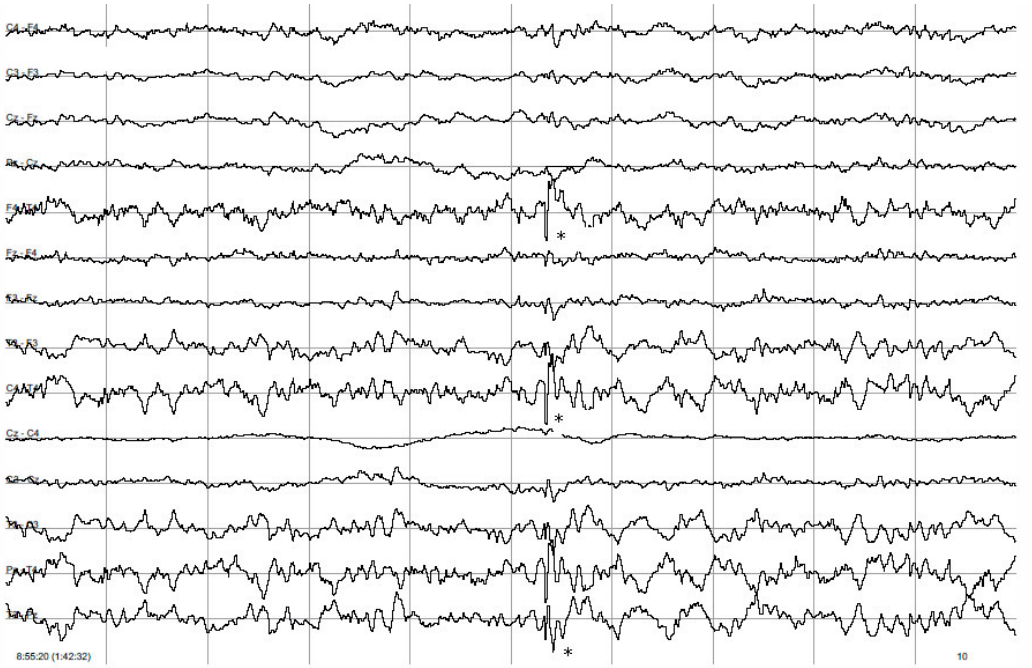
Utrecht University
Faculty of Veterinary Medicine

The possibilities of AEEG as a diagnostic tool for epilepsy in horses.



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Contents

Abstract

Introduction: (A)EEG ((ambulatory) electroencephalography) is a method to record brain waves over a period for more than forty-eight hours. The method is used routinely for diagnostics and pre surgical procedures in human patients suffering from epilepsy(Empson 1986a; Patel et al. 2009; Rose and Hodgson 2000; Shih et al. 2009; Swartz and Goldensohn 1998). In horses still insufficiently research is done to use (A)EEG as a diagnostic tool for epileptic(Aleman et al. 2006; Lacombe et al. 2001; Williams et al. 2008).
Objective: The aim of the study is test the hypothesis that AEEG can be used for longterm recordings in unsedated horses for identification of abnormalities, for instance epilepsy.
Material and methods: The control group contained 8 healthy horses. The patient group contained 7 horses. Patients showed clinical signs of abnormal behaviour of unknown origin or epileptic–like insults as judged by their own vet. Electrode placement was determined based on the human system in combination with MRI examination of a head. 9 self adhesive electrodes were positioned. The 'Porti-5' recording system was attached underneath a metal roll-band on top of a girdle. The records were analysed making montages to compare the signals of the electrodes with each other. The filter settings were 0,5 Hz high pass and 35 Hz low pass. The records were analysed off-line at an amplitude of 50-200uV and 10 seconds time scale by visual inspection. Epileptic activity was defined as records containing a spike (a sharp wave of 70-200Hz), a period of rhythmical activity (longer period of similar waves of 1-1,5Hz) or a fast rhythmic discharge (short period of high amplitude waves of 4-7Hz) ((Aleman et al. 2006; Beleza et al. 2009; Liasis et al. 2006; Tassi et al. 2009). In one patient a post-mortem MRI was made.
Results: The recording time varied from 6 until 48 hours. All the cases showed normal brain signals(Aleman et al. 2006; Empson 1986a; Williams et al. 2008). 57% of the patient group showed brain signals indicating pathology. 43% of the patient group and 12%(n=1) of the control group showed epileptic activity(Aleman et al. 2006; Beleza et al. 2009; Liasis et al. 2006; Tassi et al. 2009). One horse showed slow delta wave's indicative of brain pathology in the left side of the brain. A post-mortem MRI showed a large left-sided abscess.
Conclusions: Long-term records of good quality of horses brain waves were made with AEEG, resulting in a reasonable chance to record pathological signals indicating epileptic of pathological activity in absence of obvious abnormal behaviour.
Practical significance: Intermittent abnormal behaviour can be normal by the time the veterinarian arrives. Still, horse owners will ask for a diagnosis and therapy. For insurance issues or just for their own comfort, it is important to develop a system to do identify abnormal brain activity.

Introduction

(A)EEG ((Ambulatory) electroencephalography) is the graphic recording of rhythmic bioelectric activity which comes predominantly from the cerebral cortex (Lacombe et al. 2001). It is a method to record brain waves by placing electrodes on the scalp of a patient (Rose and Hodgson 2000; Swartz and Goldensohn 1998). The method is used for diagnostic and pre surgical procedures for patients suffering from epilepsy(Patel et al. 2009; Shih et al. 2009). It measures potential differences in the brain through the scalp. (A)EEG is used to diagnose, locate and define possible abnormalities, for instance epilepsy. (A)EEG can be used to differentiate between partial(focal) and generalized epileptic seizures and also the effect of treatment can be monitored (De Lahunta 2001; Robinson et al. 2003).

EEG in humans

In humans medicine a lot of research is performed to EEG and AEEG. A basis system for electrode placement exists(Empson 1986a; Klem et al. 1999). Normal and abnormal brain signals can be recognised(Beleza et al. 2009; Capovilla et al.; Empson 1986a; Liasis et al. 2006; Tassi et al. 2009). Both procedures are performed routinely to diagnose, locate and define possible abnormal brain signals, for instance epilepsy.

Application of the electrodes

In humane medicine an anatomical placement system for EEG electrodes was developed in 1958 by H.H.Jasper. It is called the 10/20international system for electrode placement (Empson 1986a; Klem et al. 1999; Leidse Onderwijs Instelling). This system has a fixed, reproducible structure for placement of the electrodes so that they cover the whole brain. The reference anatomical locations are the nasion(Frontal), inion(Occipitaal) and the 2 auricular points(Dexter and Sinister). From these four locations, structural bands are measured to place the electrodes on. These bands are formed by measuring the distance between the two auricular locations and from nasion to inion. Then the distances are divided in symmetrical percentages between the electrode locations.

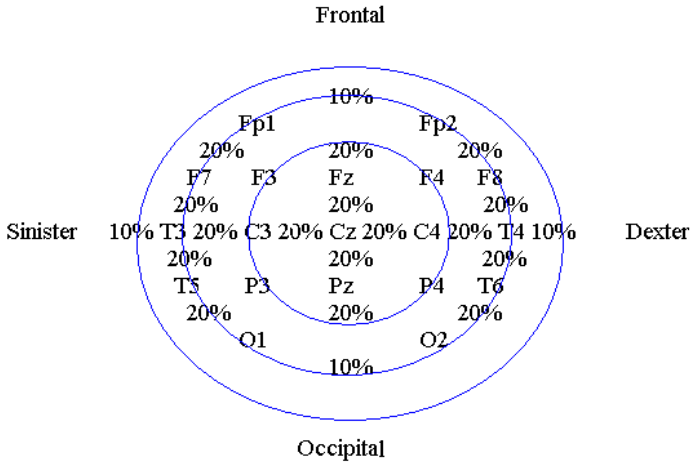


Fig.1 10/20international system for electrode placement The electrodes are named after the brain area they are located on. F=frontal, C=central, P=parietal, T=temporal. The electrodes on the left side of the horses head are odd numbered and the electrodes on the right side of the head are even numbered. The electrodes on the midline are named z.

The left hemisphere is numbered with odd numbers and the right hemisphere with even numbers. The electrodes on the midline are named z. Electrodes named with a F are placed on the frontal lobe of the brain. Electrodes named with a C are positioned centrally. Electrodes named with a P are placed on the parietal lobe of the brain. The electrodes named with a T are located on the temporal lobe of the brain.
For instance from A(auricular)1 to A2 (Fig.1):
10% of the distance from A1 →T3
20% of the distance from T3 → C3
20% of the distance from C3 → Cz
20% of the distance from Cz → C4
20% of the distance from C4 → T4
10% of the distance from T4 → A2.
Some researchers have chanced the electrode positions for several mm or use a different amount of electrodes(Binnie et al. 1982; Homan et al. 1987). Even though there are some slight differences between users over the world, the basics are the same. Such a basic system is not only simple for a practitioner to remember and use, but also has other advantages. When using the same structure for electrode placement, the EEG data at different institutes will be quite similar, thus making comparisons more reliable and consistent. The placement of the electrodes influences the outcomes of EEG data between individuals. In humans, the temporal lobe of the brain gives useful information in diagnosing epilepsy, but also produces artefacts due to ear and eye movement(Beleza et al. 2009; Noachtar and Rémi 2009; Tassi et al. 2009). To get the best signals possible from the temporal lobe the electrode needs to be placed on the scalp directly on the temporal lobe of the brain. Since seizures are unpredictable it would be beneficial to be able to diagnose epilepsy interictally. Interictal epileptic discharges (IED) are seen in humans mostly in the temporal lobe, especially in patients with Temporal lobe Epilepsy (TLE)(Beleza et al. 2009; Noachtar and Rémi 2009; Tassi et al. 2009). Epileptic seizures are unpredictable and the brain location differs. So it's desirable to obtain as much data as possible from as many locations of the brain as possible. The more data is obtained, the higher the chance is to capture epileptic signals in the EEG records.

Normal EEG findings in humans
The normal human brainwaves seen in a EEG are alfa, beta, theta and delta waves(Barlow 1993c; Empson 1986a; Visser et al.). When a person is relaxed and has their eyes closed alfa waves of 8-12 Hz and an amplitude of 25-100uV are visible in the signals. Alfa waves are blocked during a visual task. Beta waves of 14-30 Hz and an amplitude of 14-30uV are seen during sleep. During drowsiness theta waves of 4-7 Hz and an amplitude of 20-40uV appear on the records. During deep sleep delta waves of 0.5-3.5 Hz and an amplitude of 10-20uV dominate the brain signal. Lambda waves of 8 Hz and an amplitude of 10-20uV appear when a person concentrates on a visual task. Sleep spindles are intermittent seen during sleep as irregular waves of 14 Hz and an amplitude similar to the stage of sleep (Tab.1) (Barlow 1993a; Barlow 1993c; Empson 1986a; Empson 1986b; Noachtar and Rémi 2009; Visser et al.; Visser et al.; Visser et al.).

Normal human EEG signals			
Waves	**Appearance**	**Amplitude(uV)**	**Occurrence**
Alfa	8-12 Hz	25-100	Relaxation with closed eyes
Beta	14-30 Hz	5-30	Sleep
Theta	4-7 Hz	20-40	Drowsiness
Delta	0.5-3.5 Hz	10-20	Deep sleep
Lambda	125ms	10-20	Visual task
Sleep spindles	12-15 Hz	Similar to the stage of sleep	Intermittent during all stages of sleep

Tab.1 Normal human EEG signals awake and during sleep(Barlow 1993a; Barlow 1993c; Empson 1986a; Empson 1986b; Noachtar and Rémi 2009; Visser et al.; Visser et al.).

Certain movements such as chewing and eye movements are recognisable on the EEG chart. Chewing is seen as EMG(Electromyogram) activity and eye movements are seen as big loops in the records(Leidse Onderwijs Instelling; Visser et al.).

EEG signals indicating pathology in humans, for instance epilepsy
In patients suffering from epilepsy spikes, complexes and multi-spike activity are seen(Tab.2)(Visser et al.; Visser et al.). They can occur local or generalized, which is visible on the EEG in respectively a small amount of electrodes or on all the electrodes. Spikes of <70 and slower spikes of 70-200ms and an increase in amplitude of 2uV/ms can occur solitaire or multiple in an EEG recording of epileptic patients. Sharp wave-slow wave complexes of 2Hz and spike - wave complexes of 2-3Hz and an amplitude of 100-300uV occur in fixed rhythmic recurring combinations. Interictally spikes and spike-wave complexes are the most common epileptic finding. Multi-spikes of 15-30Hz and an amplitude of > 100uV is often seen during the tonic stage of a seizure. The amplitude increases during the attack. During the clonic stage slow uncoordinated waves appear of variety in frequencies and amplitudes (Barlow 1993b; Noachtar and Rémi 2009; Visser et al.; Visser et al.).

Pathologic EEG signals indicating epilepsy in humans			
Waves	**Appearance**	**Amplitude(uV)**	**occurrence**
Spike	<70ms	Increase in amplitude 2(uV/ms)	Solitaire or multiple
Slower spike=sharp wave	70-200ms	Increase in amplitude 2(uV/ms)	Solitaire or multiple
Sharp wave-slow wave complex	2 Hz	100-300	Groups of rhythmic waves
Spike - wave complex	2-3 Hz	100-300	Groups of rhythmic waves
Multi-spike complex	15-30 Hz	>100	Groups of rhythmic waves

Tab.2 Pathologic EEG signals indicating epilepsy in humans(Barlow 1993b; Noachtar and Rémi 2009; Visser et al.; Visser et al.).

Intermittent spikes are seen in several forms of epilepsy in humans. For instance in Benign focal epilepsy and in Rolandische epilepsy in children (Fig.2) (Liasis et al. 2006; Overvliet et al.; Visser et al.). Children diagnosed with Benign focal epilepsy in show centro-temporal spikes. Rolandische epilepsy is a form of epilepsy which also shows spikes in the EEG. The spikes are mostly centered in the central and temporal part of the brain. The children usually have normal

brain signals again after a period of 6 months till 15 years. Also not al of the patients have symptoms. Some epileptic EEG signals are subclinical (Capovilla et al.; Liasis et al. 2006). Epileptic activity concentrates itself in the diversions of the Temporal lobe when a patient has TLE(Temporal Lobe Epilepsy) (Beleza et al. 2009; Tassi et al. 2009), but mostly the activity is seen over all the diversions(Fig.3).

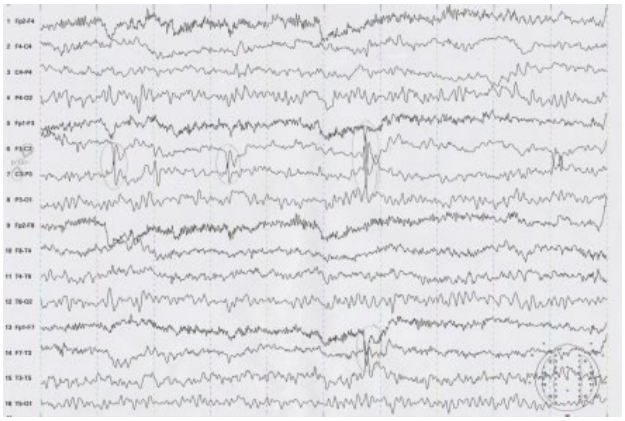


Fig.2 An EEG of a child with rolandische epilepsy. The spikes are marked by circles. The maximum(location with the most spikes) is on the C3 electrode.

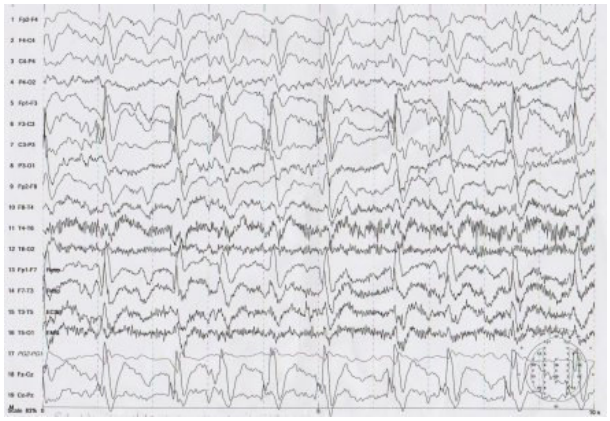


Fig.3 Spike and wave complexes seen as rhythmical activity in the brain of a human epileptic patient.

AEEG

In humans AEEG is performed routinely on neurophysiology departments for diagnostic and pre surgical procedures for patients suffering from epilepsy (Ebersole 1987; Marsan and Zivin 1970; Patel et al. 2009; Sheridan and Sato 1985; Shih et al. 2009). The advantages found are numerous. The device can be worn for several days and the method is cheaper than using EEG. The patient does not have to be monitored by a person during the recording. The equipment is light of weight so it is comfortable to wear during a longer period of time. The patient doesn't have to be hospitalised for the recording and normal day activities can be recorded. Most important the chances of recording abnormal brain signals are much larger when the recording takes longer, since a seizure is unpredictable. It enlarges the chance to detect a seizure at the time of onset. AEEG could predict a diagnose seizures more accurately (Patel et al. 2009; Sheridan and Sato 1985; Shih et al. 2009). For instance, J.S. Ebersole(1987) did a research in 500 patients. The 24 hour AEEG records of the patients showed a 64% increase in interictal epileptiform abnormalities and a 21-fold increase in seizure recording compared with the routine EEG of thirty minutes(Ebersole 1987). Also Ajmone-Marsan and Zivin(1970) found in their research an increase of 27% of detecting epilepsy after several EEG recordings in comparison with the results of one routine EEG recording(Marsan and Zivin 1970). Also University Medical Centrum Amsterdam reports in a document about clinical neurophysiology that longterm recordings increase the amount of positive epileptic findings(Bridgers and Ebersole 1985; Marsan and Zivin 1970; Sheridan and Sato 1985; Visser et al.):

- 1 EEG = 55,5% positive
- > 1 EEG = 82,5% positive
- long-term AEEG = 89-95% positive

Even though the increase in detecting epilepsy by AEEG is not the same in al the researches, it is clear that AEEG in humans definitely increases the chance of diagnosing epilepsy in comparison to a routine EEG.

EEG in horses

In horses EEG has just been performed a few times before(Aleman et al. 2006; Lacombe et al. 2001; Williams et al. 2008). AEEG is not performed at all in horses. There isn't a routinely used method to diagnose epilepsy other than by symptoms.

Application of the electrodes
In horses the application of the electrodes was based on the human system(Aleman et al. 2006; Lacombe et al. 2001; Williams et al. 2008). The EEG was made placing 9-13 electrodes on the forehead, between the eyes and ears, in symmetrical rows. The electrodes on the left side of the head were also numbered uneven numbered and the electrodes on the right side were even numbered. The electrodes on the midline were named with a z.

Normal EEG findings
Similar waves to those in humans are seen on the EEG of horses during drowsiness and sleep (Tab.3) (Aleman et al. 2006; Williams et al. 2008). During drowsiness and REM(Rapid Eye Movements) sleep some rhythmical activity was seen of 4-30Hz and an amplitude of 5-40uV. During SWS(slow wave sleep), waves of 1-8Hz and an amplitude of 30-80uV were seen. Also sleep spindles of 10-14Hz and an amplitude of 40-90uV were recognisable on the EEG. Alfa waves, seen in humans when they are relaxed and awake with their eyes closed, are not noticed in EEGs of horses. Eye movements are also recognisable in horses, as in humans. Eye movement is seen as several big loops in the EEG recordings (Williams et al. 2008).

Normal EEG signals in horses			
Waves	**Hz**	**Amplitude(uV)**	**Occurrence**
Theta	4-20	10-40	Drowsiness
Rhytmhic activity	4	30-40	REM sleep
Rhytmhic activity	20-30	5-10	REM sleep
Slow waves	1-8	30-80	Slow wave sleep
Sleep spindles	10-14	40-90	Intermittent during all stages of sleep

Tab.3 Normal EEG signals awake and during sleep in horses(Aleman et al. 2006; Williams et al. 2008).

EEG signals indicating pathology in horses, for instance suffering from epilepsy
In research to epilepsy in horses several specific brain waves are visible on the EEG. Spikes <70ms, sharp waves of 70-200ms and spike-and-wave discharges of 6Hz are seen. Most of them are located in the central electrodes (Tab.4) (Aleman et al. 2006; Williams et al. 2008).

Pathologic EEG signals indicating epilepsy in horses	
Waves	**Appearance**
Spike	<70ms
Sharp wave	70-200ms
Sharp wave-slow	6 Hz

discharges	
Spike and wave discharges	6 Hz
Multiple-spike complex	unknown

Tab.4 Pathologic EEG signals indicating epilepsy in horses(Aleman et al. 2006; Williams et al. 2008).

AEEG

AEEG is not performed in horses, but could be a big step towards a more accurate and certain way to diagnose epilepsy in horses. Since the advantages it shows in human medicine, the goal of this research is to try and diagnose epilepsy in horses, which were suspected of having epilepsy due to abnormal behaviour of unknown origins. The first part of the experiment will be to develop a basic system for electrode placement in horses. Because of the advantages a basis structure for electrode placement has in humans, as mentioned above, and the growing interest in EEG as well as AEEG(ambulatory electroencephalography) in horses, it is now time to develop such a system for horses.

The second part is to record AEEG in healthy and in horses who were suspected of having epilepsy based on clinical signs of abnormal behaviour of unknown origin, as judged by their own vet. Intermittent abnormal behaviour can be normal by the time the veterinarian arrives. Still, horse owners will ask for a diagnosis and therapy. For insurance issues or just for their own comfort, it is important to develop a system to do identify abnormal brain activity and a basic system for electrode placement is a good start.

The aim of the study is test the hypothesis that AEEG can be used for longterm recordings in unsedated horses for identification of abnormalities, for instance epilepsy.

Material and methods

Horses

For this research fifteen horses were used(Tab.5). Two groups were made, a control group and a patient group. The control group contained healthy horses of the University of Veterinary medicine in Utrecht. The patient group contained 7. Patients showed clinical signs of abnormal behaviour of unknown origin or epileptic–like insults as judged by their own vet.

Case information					
	Case	**Gender**	**Age**	**Breed**	**Symptoms**
Controle group	1	Female	15	KWPN	-
	2	Female	15	KWPN	-
	3	Female	7	KWPN	-
	4	Female	12	KWPN	-
	5	Female	12	KWPN	-
	6	Female	9	KWPN	-
	7	Male	10	Friesian	-
	8	Female	17	KWPN	-
	Mean	*12,5% Male*	*12.13*		
	±SD		*3.4*		
Patient group	9	Female	11	Selle Francais	Fell down one time after arterial blood tapping and had a crooked eye and a excitated lip.
	10	Female	19	KWPN	Frequent spontaneous and stimulated partial seizures in the nose area.
	11	Female	15	Tinker	Several seizures in the last 5 years during the horse suffered from uncontrolled muscle spasms and fell down.
	12	Male	19	KWPN	The horse had frequent attacks his whole life during which it crashed into a wall.
	13	Male	4	Falabella	Had a half a year ago and the week before the experiment had several seizures. The horse walked in circles with neck, head and nose cramped. Sometimes fell down.
	14	Male	15	Welsh	Had the last weeks frequently attack during which the legs weakened and the horse sometimes fell down.
	15	Female	31	Haflinger	Had a had attack for the last 2,5 years during which the horse has uncontrolled muscle spasms and fell down.
	Mean	*42,9% Male*	*16.29*		
	±SD		*8.3*		
	Total mean	**26.7% Male**	**14.07**		

	Total ±SD		**6.3**		

Tab.5 Case information. CG= control group, PG= patient group.

The control group

The control group contained 8 healthy horses. The control group contained 1 gelding and 7 mares. The age of the horses varied between 7 and seventeen years. The mean age was 12.13 years. The ±SD was 3.4. The gelding was ten years of age. The mean age of the mares was 12.43 years. The ±SD was 3.6. The breeds of the horses were all KWPN mares and a Friesian gelding (Tab.5).

Two of the horses were sedated during the application of the equipment, because they behaved agitated. Case 1 had been given 0,5 ml IV Domosedan[1] and case 3 had been given 0,4ml IV and 0,25 ml IV Domosedan[1] (Tab.6). The horses weren't included in any other experiment during the time they participated in this research.

The patient group

The patient group contained 7 horses, which showed clinical signs of abnormal behaviour of unknown origin or epileptic–like insults as judged by their own vet. The age of the horses varied between 4 and thirty-one years, giving a mean age of 16.29 years. The ±SD was 8.3. The patient group contained 3 geldings and 4 mares. The age of the geldings varied between 4 and nineteen years, giving a mean age of 12.67 years. The ±SD was 7.8. The age of the mares varied between eleven and thirty-one years, giving a mean age of 19 years. The ±SD was 8.6. In the patient group the following horse breeds were present: 1 Selle Français, 2 KWPN, 1 Tinker, 1 Falabella, 1 Welsh and 1 Haflinger(Tab.5).

Case 9 had 1 attack the week before the experiment took place, more then seventy hours before. After arterial blood tapping she fell down, had a crooked right eye and excitated lips during 2 minutes. The attack resulted in some wounds on her legs. This was the only attack ever noticed in this horse.

Case 10 was admitted. in the hospital one month before the experiment with partial epileptic seizures, which was expressed by facial muscle spasms. The seizures could be stimulated by a touching the face, especially in the nose area. During the following months including during our experiment the horse was treated with Rapidexon[2] 6 ml IV per day(Tab.6). Since the treatment had started the seizures became less until no seizures had been noticed at least two weeks before the experiment. Also no visible seizures could be aroused by touching the nose or other facial areas.

Case 11 had had generalised seizures. The horse started to walk circles in a counter-clockwise direction with her mouth wide open and all the facial muscles strained. Her head moved and cramped. Her hind legs became weak and the horses head kept flipping backwards. The horse sweated extensively and fell unconscious on the ground. After the attack the horse was disorientated, was shaking and didn't recognise the owner. These seizures resulted in wounds on her legs and head. Sometimes the horse had 2 attacks consecutively. The patient experienced frequent seizures in the last 5 years. On average the horse had had 5 seizures a year. The horse hadn't had a seizure in the 4 months before the experiment took place. Most of the seizures took place in the autumn and spring, especially in march.

[1] Domosedan 10 mg/ml, Orion pharma, Espoo(FI)
[2] Rapidexon 2 mg/ml, Eurovet animal health, Bladel(NL)

The Case 12 had been having partial seizures. The attack always started with loud snoring and an empty look in his eyes. Then his head and front legs would start beating. The horse leaned and crashed against the wall. He fell on the floor one time and then his whole body was tensed. The muscles tightened and his tongue would hang out of his mouth. The most violent part of the seizure would take about 2 minutes and then for 5 to ten minutes after shocks occur. The attack often resulted in a wounded tongue and head. The horse remained disorientated and depressed for approximately one hour after the attack. The patient had had seizures his whole life. Attacks had become more frequent and severe during the last years. In the beginning the horse had one till three attacks a year. Last year the horse had one attack every 2 or 3 months.
Case 13 had had partial and generalized seizures. During the attacks the horse would walk circles in a counter-clockwise direction. He sometimes leans against the wall. His head turns to his left side and even touches his rump. His nose distorts to the left. His head beats upwards and with his mouth wide open. Left front and hindleg cramps aside and upwards. It looks like if the horse yawnes. His breathing is accelerated and his tongue and gum are grey colored. When having a generalized seizure, the horse falls down on his right side and lost his conscience. After the attack the horse is disorientated and lets his head hang down. The patient had several attacks half a year before, in July 2010, and also the week before the experiment. The horse has always been slower and behind in growth, when compared to its brother. He is not well formed and suffered from several illnesses, like wound infections and increased liver values. The day before and during his hospital stay he was slow. The horse had been given Rapidexon[2] 1 ml IV 20 hours before the recording started. During his hospital stay the horse had five noticed attacks. These attacks did not happen during our recordings. On day 2 of his admission the horse was euthanized because of his overall bad condition. The day after the euthanasia a MRI of his head was made.
Case 14 had had partial seizures. The horse rears, his legs become weak and he falls down without losing his consciousness. After and during the attack the horse shivers. The horse doesn't want to be touched after the attack. This sometimes could result in a bloody mouth. He had several attacks in the 3 weeks before the experiment took place. Mostly 2 attacks a week, 1 every half week. The attacks were becoming more frequent. They mostly occur after stimuli like a truck driving by or at feeding or brushing time. The day before the experiment and the day before that the horse was put on 180 mg Metacam[3] PO(Tab.6). During its hospital stay the horse had 3 attacks consecutively. He reacted as if he was scared when the electrodes were taken off his head. He reared, his legs weakened and he fell against the wall. After the attack he behaved normal again within a few minutes. The attacks did not happen during the recording.
Case 15 had been having partial and generalized seizures. When having a partial seizure, the symptoms were limited to shocks in the head and neck. These attacks would last for approximately 10 minutes. The more severe attacks could last for up to 1,5 hours. First her head and neck starts to shock. She would walk uncoordinated and after a while her legs would begin to pull away from underneath her. She would try to lean into something, but after a while she would fall down. Her muscles contracted uncontrollably, her legs beat and her mouth and neck would cramp. She began to sweat extensively and even foam would develop between her legs. When the contractions stopped, the horse would lie down exhausted, breathing heavily for several minutes. Then she has difficulty standing up and starts walking around disorientated and often in circles. She doesn't react to the environment or to the owners. This can continue for more than twenty minutes. Sometimes she would get a second attack directly following the previous one. The horse had attacks for the last 2,5 years. The frequency of the seizures could vary from a few days to a

[3] Metacam 15 mg/ml, Boehringer, Ingelheim(DE)

few months. The horse had had 15 noticed attacks in the last 2,5 year. A year ago the horse was tested positively for Cushing's disease and has been treated with Pergolide[4] 0,5 mg PO per day(Tab.6). Since the medication for Cushing has started the seizures stopped, until 1,5 months before the experiment the head and neck shocks came back.

Administrated medication			
	Case	**Weight(kg)**	**Administration**
Control group	**1**	573	Domosedan 0,5 ml (IV)
	3	575	Domosedan 0,4 & 0,25 ml(IV)
Patient group	**10**	510	Rapidexon 6 ml(IV)
	13	82	Rapidexon 1 ml(IV)
	14	293	Metacam 180 mg(PO)
	15	±500	Pergolide 0,5 mg(PO)

Tab.6 Administrated medication for cases 1,3,10,13,14 and 15. ± means an estimated value.

Environment of the experiment
Except for case 15 all the horses were admitted in the equinehospital of the Univercity of Utrecht for the recordings. They weren't aloud to leave there stable during the recordings. Case 15 was taped at the owners home, because of health and safety aspects. This horse walked freely in the riding arena during the recording.

Electrodes
For the experiments shielded cables, re-usable connector leads, of Medi Factory BV Nieuwkoop NL. The electrode stickers were from Disposable Center Snap Rectangle Electrodes of Nicolet VIASYS healthcare Madison USA were used.

Computer system
For the recording and processing a computer box and a software system called 'Porti-5' of TMS international Enschede NL, was used. Before the EEG recording the memory card was initialised with patient name, patient number, patient gender, birth date, measure date of initialising, measure number. Also storage, name, reference, rate and solution were initialised (Tab.7).

Computer settings				
storage	**name**	**reference**	**rate**	**resolution**
2 bytes	1 (n)	unipolar	100 Hz	0.0175 uV

Tab.7 Computer settings. The name of the electrode is dependent on the number of electrodes used.

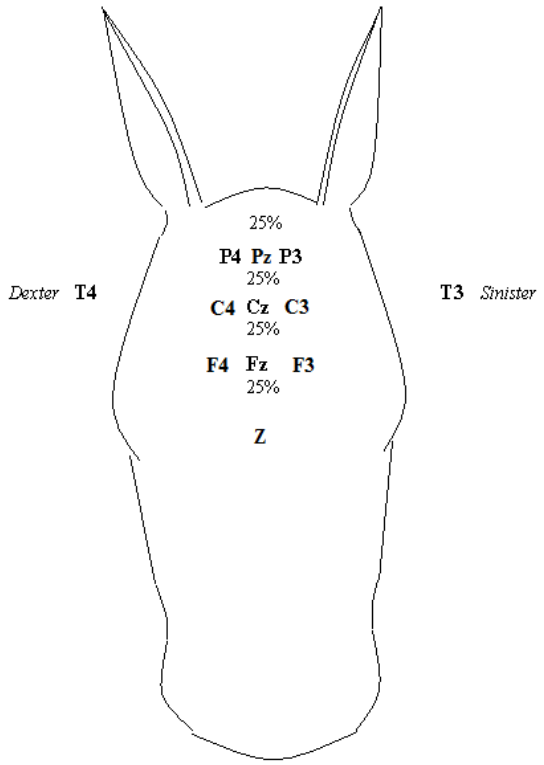
Montages
After recording at first all the rough signals were saved on the computer. Dependent of the number of electrodes used, montages were made. To localise a focus/abnormality in the EEG signals, diversions between the electrodes were made to compare the brain signal from one electrode with the one next to it. In every montage 6 diversions were made manually according to the instructions of the computer system (Tab.8). Three vertical rows of electrodes and three

[4] Pergolide 05mg/day for a average horse(Toribio 2004), Sandoz, Almere(NL)

horizontal rows of electrodes were compared. Every electrode was compared to the one on the left, on the right, above and underneath itself (Fig.4). For instance F4 was compared with C4, which was placed above F4, and C4 was compared with P4, which was placed above C4. This made the first diversion (Tab.8 & Fig.4). Each comparison was drawn underneath each other on the computer screen, starting with the first diversion until 6. The first line on the screen was the compared signal of electrodes named C4 and F4. The last line that was drawn on the computer screen was T3 compared with Pz.

Montage					
Number of diversion	**Electrode names**				**Direction on the horse head**
1	C4-F4	P4-C4			Vertical
2	C3-F3	P3-C3			Vertical
3	Cz-Fz	Pz-Cz			Vertical
4	F4-T4	Fz-F4	F3-Fz	T3-F3	Horizontal
5	C4-T4	Cz-C4	C3-Cz	T3-C3	Horizontal
6	P4-T4	Pz-P4	P3-Pz	T3-Pz	Horizontal

Tab.8 The 6 montages made according to te instructions of the computer system. The electrodes are named after the brain area they are located on. F=frontal, C=central, P=parietal, T=temporal. The electrodes on the left side of the horses head are odd numbered and the electrodes on the right side of the head are even numbered.



Determination of the position of the electrodes on the brain by MRI

To find out the best positions for the electrodes a MRI(magnetic resonance imaging) scan of the horses head was made. First the probable right position of the electrodes on the head was determined using anatomy books and some preparations of a horse head(Budras et al. 1994a; Budras et al. 1994b; König and Liebich 2007; König et al. 2007; Nickel et al. 1975a; Nickel et al. 1975b). Then 9 garlic pills were taped to a dead horse head. Because the different density of the pills in comparison with the scalp and brain they appear as lighted spheres on the MRI. 6 pills were placed on the forehead in three rows of two electrodes. The distance of the beginning of the manes until the supra-orbital bone was measured and divided in to 4 areas of 25%.

Fig.4 Electrode placement in the horse. The electrodes are named after the brain area they are located on. F=frontal, C=central, P=parietal, T=temporal. The electrodes on the left(sinister) side of the horses head are odd numbered and the electrodes on the right(dexter) side of the head are even numbered. The electrodes on the midline are named z. 25% is the distance between the horizontal electrode rows on the forehead. Electrodes F 3 and 4 are the

placed just inside the edges of the location of the brain underneath the forehead(Budras et al. 1994a; Budras et al. 1994b; König and Liebich 2007; König et al. 2007; Nickel et al. 1975a; Nickel et al. 1975b).

Between the manes and the first row, between the pills rows itself and between the last row and the supra-orbital bone 25% of the distance was measured. All the pills were placed in rows between the ears on the places F4, F3, C4, C3, P4 and P3 (Fig.4). Seen from lateral view two pills were placed in the midline of the ears and eyes. One pill was placed on the upper part of the temporal bone and the other one on the lower part of the temporal bone. A MRI scan of the head was made. (Fig.5 & 6)
The MRI of the dead horse showed the positions of the placed spheres by a light spot. It showed that al the placed spheres were located on the scalp above of the cerebrum (Fig.5). Only the sphere placed on the lower part of the temporal bone was placed on the scalp above the cerebellum (Fig.6). The MRI determined the right electrode positions.

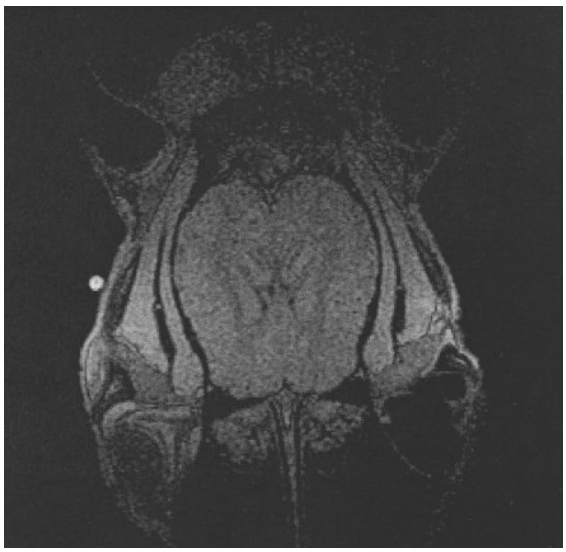


Fig.5 Sphere placed on the upper part of the temporal bone, longitudinal view.

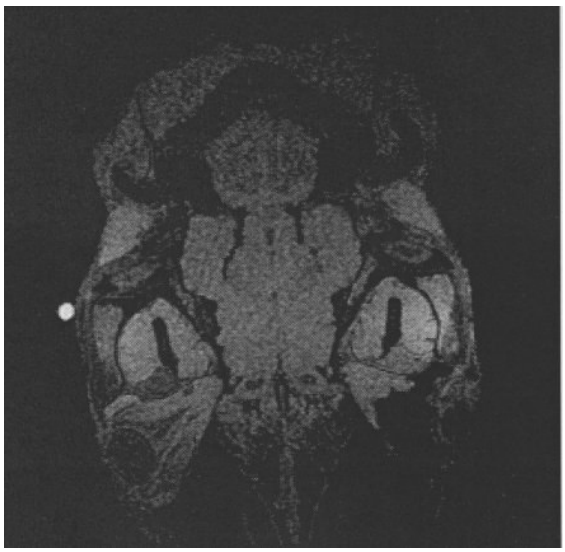


Fig.6 Sphere placed on the lower part of the temporal bone, longitudinal view.

Performance of the experiment
By placing electrodes at several locations on the scalp the electrical signals are passed through wires to a small computer box. The memory card inside this small computer box can be put into a normal computer and this one converts the signals into graphic recordings, which then can be analysed by neurologists.

Application of the electrodes
First the forehead of the horse and the upper part of both temporal bones was shaved using a electric shaver and a razor of Bic medial with Palmolive shaving cream until the skin was completely hairless(Photo.1a). Then the skin and surrounding hairs were cleaned with paper and water. The cleaned skin was treated with alcohol 70% denaturized, 2,5% isopropanol of Orphifarma twice. When the skin had dried the head was marked with a vertical stripe with a red

glass pencil. First a midline in between the ears, starting from the manes to the eyes, was marked. The distance between the beginning of the manes, between the ears and the margin of the supra-orbital bone was measured. The distance was divided into 4 pieces of 25%. Between the beginning of the manes and the first row, between the pills rows itself and between the last row and the supra-orbital bone 25% of the distance was measured and marked as horizontal stripes with the pencil. Just below the margin of the supra-orbital bone the z electrode was placed. Seen from a lateral view the temporal electrode were placed on in the middle of the distance between the ears and the eyes on the upper part of the temporal bone. The electrodes are named to the parts of the brain they were placed on. F = frontal, C= central, P= parietal, T=temporal Z= ground electrode. The electrodes on the midline are named with a z, the electrodes on the right hemisphere with even numbers and the electrodes on the left hemisphere with odd numbers. The locations of the electrodes were made smooth by sandpaper P600. In the first 3 horses of the control group and the first 2 horses of the patient group Collodium 4%, 40mg/ml pyroxiline of dispencery of AZL was used to fix the electrodes on the horses head(Photo.1b). Before the electrode was placed on the head the concave side was filled with electrode paste, skin conductance gel of Discount disposables, with a needle. Then the electrode was held on the right position on the head with the concave side on the head. Then an already small cut gauze, 4mx8cm KLINION of utermohlen medical care, was hold over the electrode. The Collodium was injected over the gauze with a needle. In the other horses sticker electrodes were used instead of the glued ones(Photo.1c en d). The electrode stickers were cut to fit in right and then placed on the head. Then the electrodes, shielded cables, were clicked on the stickers. Next the resistance was measured with a XI-1 electrode impedance tester OXFORD. The impedance should be smaller then 10 kΩ. When the impedance was higher then 10 kΩ the relevant electrode was filled with extra electrode gel with a needle again(in case 1, 2, 3, 9 and 10), a new sticker was placed(in case 4, 5, 6, 7, 8, 11, 12, 13, 14 and 15) or the whole electrode was placed on again. When the impedance was correct Leucoplast, 5cmx9.2m BSN Medical Hamburg Germany, was taped over the electrodes. Dependant on the sizes of the horses head it wear a eye-healing cap(Photo.1e), a cap for trotters or a bandage over there head to protect the electrodes. The bandage was used in the small horses. The eye-healing cap was used in the bigger horses. Next the cables were put between braids in the manes and then put in the small EEG recording computer on the back of the horse.
Case 1, 2 and 3 were measured with eleven electrodes (Fig.4). Case 9 and 10 were measured with 8 electrodes on their head. The Pz, Cz and Fz were nit used. Case 4 was measured with 10 electrodes. The Pz was not used. The rest of the cases were measured with 9 electrodes. The Pz was not used.

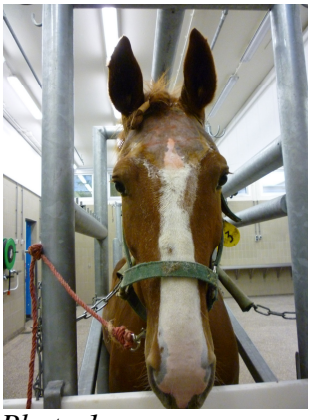


Photo.1a

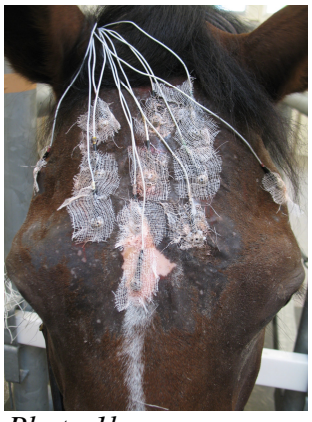


Photo.1b

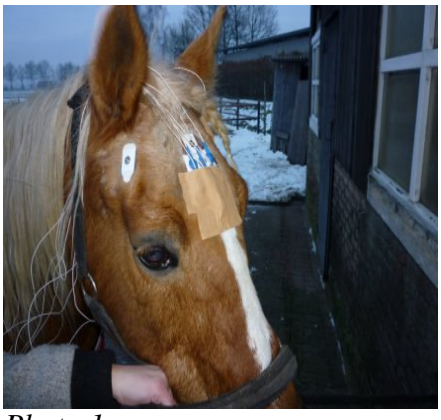


Photo.1c

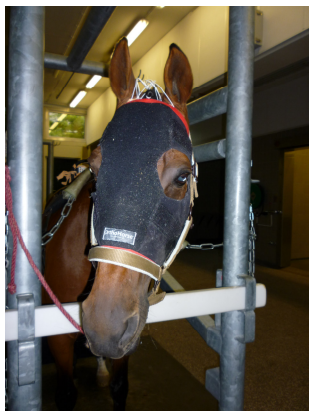


Photo. 1a,b,c,d,e
a Shaved fore head case 3,
b Electrodes glued on with Collodium case 9,
c Electrodes stickers placed on the foreheadand and on the temporal bona case 15
d Electrodes stickers placed on the forehead case 14
e Case 10 with the eye healing mask on

Photo.1d *Photo.1e*

Application of the small computer box
The small computer was put inside a leather cover and attached underneath a metal roll-band on top of a girdle in case 1, 2, 3, 9 and 10. This was all connected with Leucoplast bands, 2,5cmx9,2m BSN Medical Hamburg Germany. Underneath the girdle laid a blanket.
The small computer was put inside a metal cover and attached underneath a metal roll-band on top of a girdle incase 4, 5, 6, 7, 8, 11, 12, 13, 14 and 15(Photo.2). This was all connected with Leucoplast bands. Underneath the girdle laid a gelpad, a blanket and between the abdomen and the girdle lied also a blanket.
In the last 5 horses of the control group the metal box was connected to the roll-band with tie ribs, black 280x4,8 mm, and Leucoplast bands. When everything was attached, the computer box was put on. During the recordings the horse and equipment was checked on every hour and all the activities of the horse and people visiting the stable were written down in a logbook.

Photo.2 'Porti-5' recording system was attached underneath a metal roll-band on top of a girdle.

Analysing of the results and statistics
To estimate the percentages EEG/EMG/Artefact of the records the first half hour of the records was scored on these 3 categories. In case 1 and 3 the 5th half hour was scored, because these horses were clinical sedated for the first 2 hours. The signals were scored per 10 seconds. The variable that was present the most in the 10 seconds was scored. Artefact means signals that can not be analysed, because the result was unrecognisable. The signals were scored on EEG when there was an assessable part in the 10 sec. If there wasn't an assessable part of EEG in the 10 sec than it was scored on EMG and artefact which was the most in the 10 sec. To estimate the significance in the results the one way ANOVA test was used. A $P<0,05$ would be considered significant.

The EEG records were visually interpreted by a chief lab assistant from the neurology department of LUMC(Leiden University Medical Centrum), a veterinary specialist in internal medicine from the equine department of UU(University of Utrecht) and a veterinary student.
The filter settings were 0,5 Hz high pass and 35 Hz low pass. The records were analysed off-line at an amplitude of 50-200uV and 10 seconds time scale by visual inspection.

Definitions
The EEG results were analysed by the following definitions, based on human and equine EEG literature.

- Drowsiness = theta waves of 4-7Hz and an amplitude of 10-40uV.
- Sleep= beta waves of 14-30 Hz and an amplitude of 14-30uV
- Deep sleep= delta waves of 0.5-3.5 Hz and an amplitude of 10-20uV
- Sleep spindle= an irregular wave of 10-15Hz with an amplitude similar to the stage of sleep. They occur during all the stages of sleep.
- Epileptic activity= records containing a spike, a period of rhythmical activity or a fast discharge.
- Spike= a very sharp wave of 70-200ms and an amplitude of 20-130uV.
- Rhythmical activity= long period, about fifty seconds or longer, of similar waves with a frequency of 1-1,5 Hz and an amplitude of 30-150 Hz. This can be slow waves, sharp wave-slow wave complexes or spike – wave complexes.
- Fast rhythmic discharge or multi-spikes= a short period, of 0,5-2 seconds, with a frequency of 4-30Hz and an amplitude >100uV.
- EMG activity= fast and dense electrical activity originating from muscles.
- Artefact= Signals that can not be analysed, because the result was unrecognisable.
- Eye movements= big loops of high amplitude in the EEG recordings.

Results

Records

The recording time varied from 6.2 untill 48.4 hours. The mean recording time of the control group was 26 hours and the mean recording time of the patient group was 20.9 hours. The ±SD of the control group was 5.4. the ±SD of the patient group was 13.15. The overall mean hours recorded was 23.6 hours. The overall ±SD was 10(Tab.9).

EEG data			
	Case	**Recording time (hours)**	**Disconnected electrodes (hours)**
CG	1	29.6	F4=6.09, P4=1.3, Pz=4.4
	2	24.2	F4=20.4, F3=15.3, P4=15.2
	3	23.2	F4=14.2, Fz=1.0, F3=21.3, Pz=22.1, P3=0.6
	4	23.4	Fz=Cz=T4=T3=3.4, C4=C3=P4=P3=15.2
	5	24.2	C4=18.3, Cz=16.2, C3=17.3
	6	38.2	-
	7	23.2	Cz=10.4, C3=11.3
	8	22.1	-
	Mean	26	
	±SD	5.4	
PG	9	19.4	-
	10	48.4	C3=4.6, P4=1.1, T4=1.1, T3=22.0
	11	22.2	-
	12	17.1	F3=7.1
	13	6.2	-
	14	23.2	-
	15	11.1	-
	Mean	20.9	
	±SD	13.5	
	Total mean	23.6	
	Total ±SD	10	

Tab.9 EEG data. CG= control group, PG= patient group. The electrodes are named after the brain area they are located on. F=frontal, C=central, P=parietal, T=temporal. The electrodes on the left side of the horses head are odd numbered and the electrodes on the right side of the head are even numbered. The electrodes on the midline are named z.

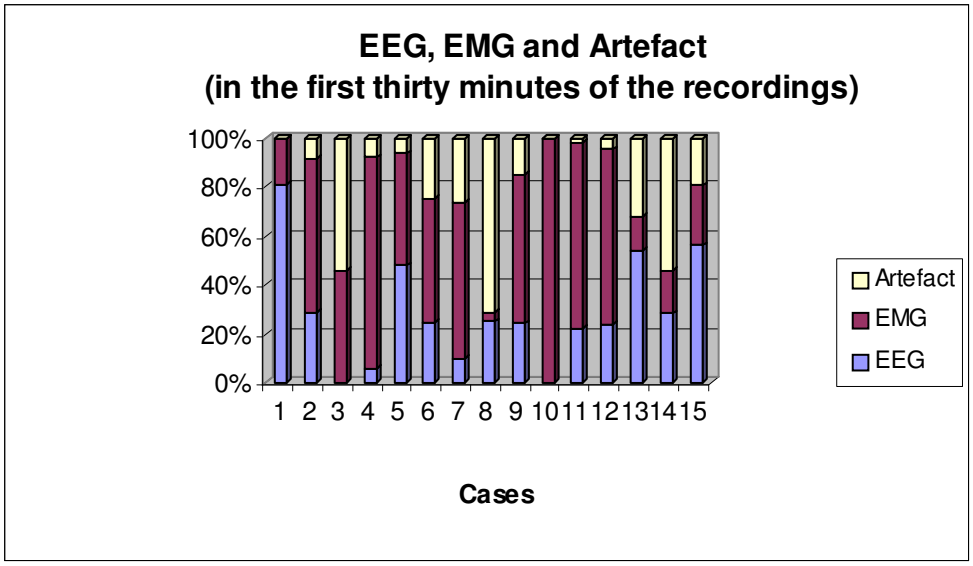
EEG, EMG and Artefact

In 8 of the fifteen cases and in the means EMG, total mean 49,6%, had the highest percentage in the first half hour(without clinical sedation) of the records. In 4 of the fifteen cases EEG, total mean28,8%, had the highest percentage in the first half hour(without clinical sedation) of the records. In 3 of the fifteen cases Artefact, total mean21,6%, had the highest percentage in the first half hour(without clinical sedation) of the records(Tab.10). The one way ANOVA test used on the control group gave P=019 and on the patient group gave P=0,06. Both P>0,05, and not significant. Case 10 showed 100% EMG activity in the first half hour and >95% EMG activity in

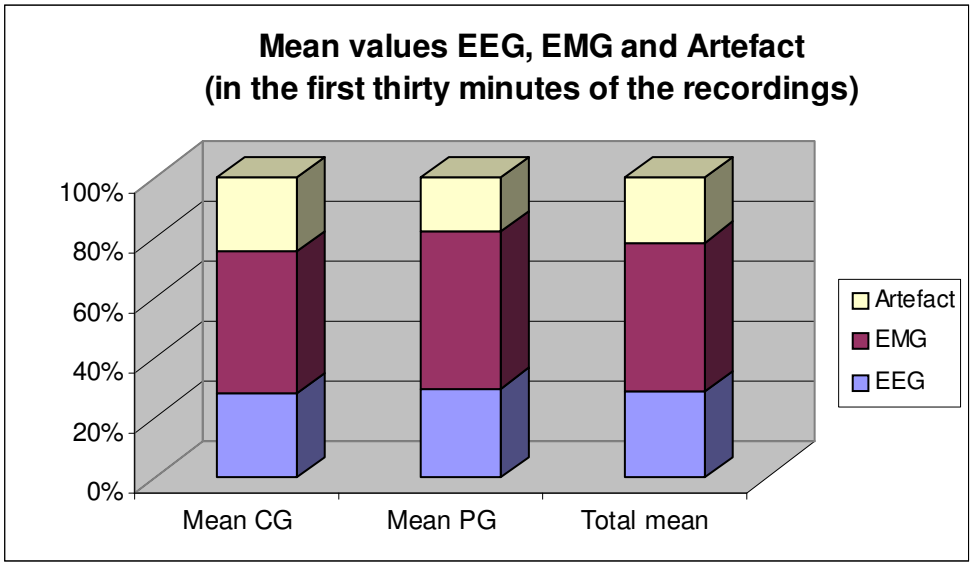
the whole recording. Also case 2, 4, 11 an 12 showed >60% EMG activity. Case 1, 13 and 15 showed >50% EEG activity. Case 3, 8 and 14 showed >50% Artefact.

% EEG, EMG and Artefact in the first half hour recording without clinical sedation				
	Case	EEG%	EMG%	Artefact%
CG	1	81.1	18.9	0
	2	28.9	62.8	8.3
	3	0	46.1	53.9
	4	5.6	86.7	7.8
	5	47.8	46.1	6.1
	6	24.4	51.1	24.4
	7	10	63.9	26.1
	8	25	3.9	71.1
	Mean	*27.9*	*47.4*	*24.7*
	±SD	*26.3*	*26.1*	*25.4*
PG	9	24.4	60.6	15
	10	0	100	0
	11	22.2	76.1	1.7
	12	23.9	71.7	4.4
	13	53.9	13.9	32.2
	14	28.3	17.8	53.9
	15	56.1	24.4	19.4
	Mean	*29.8*	*52.1*	*18.1*
	±SD	*19.5*	*33.5*	*19.5*
	Total mean	**28.8**	**49.6**	**21.6**
	Total ±SD	**22.6**	**28.8**	**22.3**

Tab.10 Percentages EEG, EMG, Artefact in the recordings. CG= control group, PG= patient group. The electrodes are named after the brain area they are located on. F=frontal, C=central, P=parietal, T=temporal. The electrodes on the left side of the horses head are odd numbered and the electrodes on the right side of the head are even numbered. The electrodes on the midline are named z.



Graphic.1 Percentages of EEG, EMG and Artefact in the first 30 minutes of the recordings. CG= control group, PG= patient group.



Grafic 2. Mean values of the percentage EEG, EMG and Artefact in the first 30 minutes of the recordings. CG= control group, PG= patient group.

Normal EEG findings

In all the horses periods of normal brain activity were found. This occurs when the horse stands calm and awake.(Fig.7)

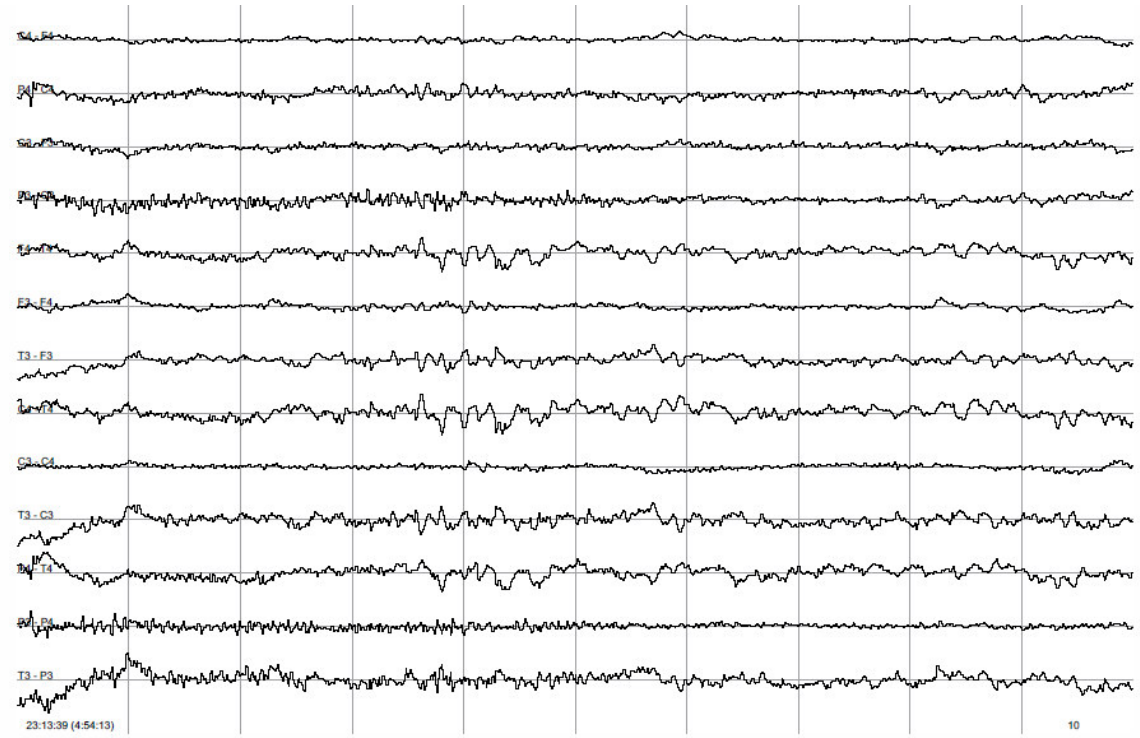


Fig.7 EEG 50uV. Normal brain activity in case 1 from the control group.

In the horses, chewing and eye movements are, as in humans, also recognisable on the EEG (Fig.8 & 9) Chewing shows rhythmical dense EMG activity and eye movement shows several loops in the EEG recording.

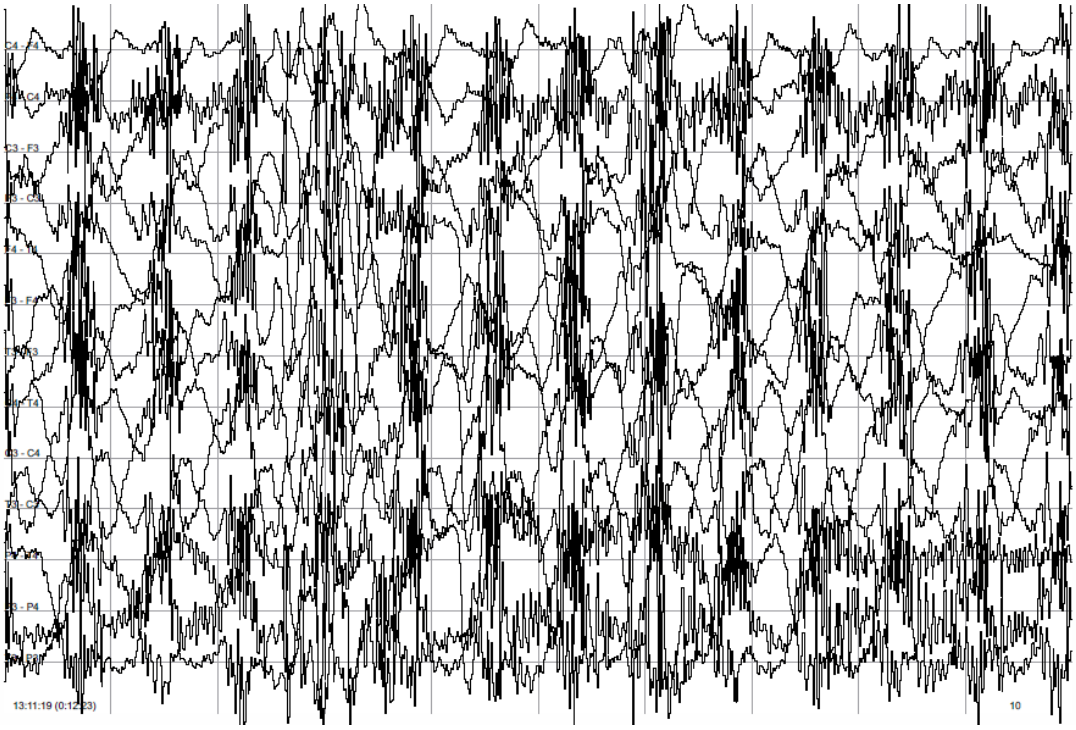


Fig.8 EEG 200uV/cm. EMG activity by chewing artefact. Case 10.

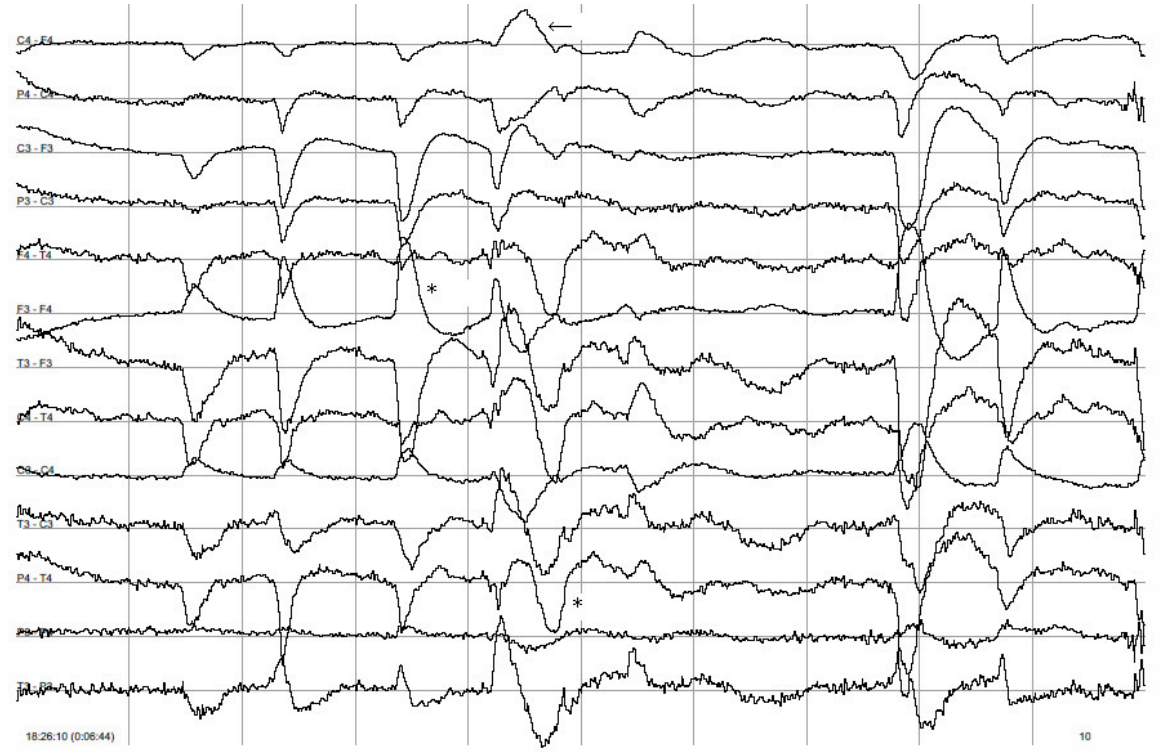


*Fig.9 EEG 50uV/cm. Eye movements. The big loops downwards are big eye movements, like looking up or to the right, marked with a *. The smaller bumps are eye blinks marked with a ←. Case 1.*

Also sleep/delta waves and some sleep spindles were recognized in the EEG records(Fig.10-12). Fig.10 shows an example of drowsiness, recognized by a period of approximately 7-10 seconds of theta waves of 5-6 Hz with an amplitude of 10-40uV. Fig.11 shows an example of deep sleep, recognized by a period of approximately twenty seconds of delta waves of 2 Hz with an amplitude of 10-30uV. Fig.12 shows an example of sleep spindles, recognized by single waves of 10-12 Hz with an amplitude of 30-60uV.

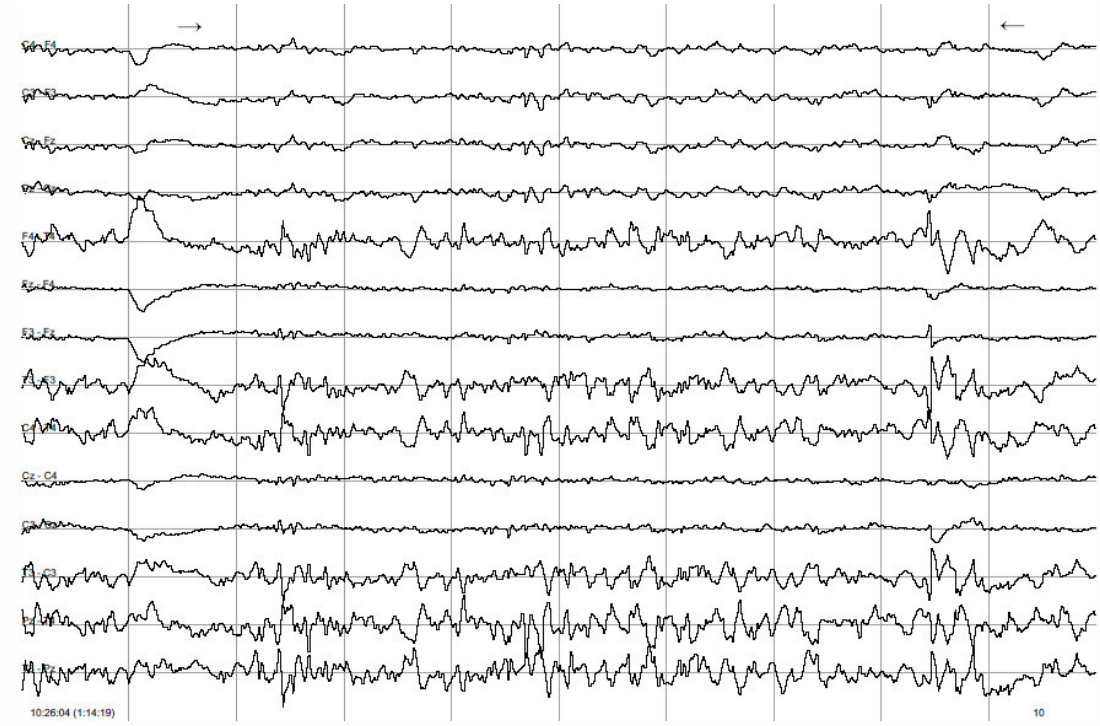


Fig.10 EEG 50uV/cm. Theta waves, seen during a period of drowsiness, are visible as irregular waves of 5-6 Hz with an amplitude of 10-40uV. The theta waves are seen between the → ← over al the electrodes . The waves occur for a period of approximately 7 seconds Case 15.

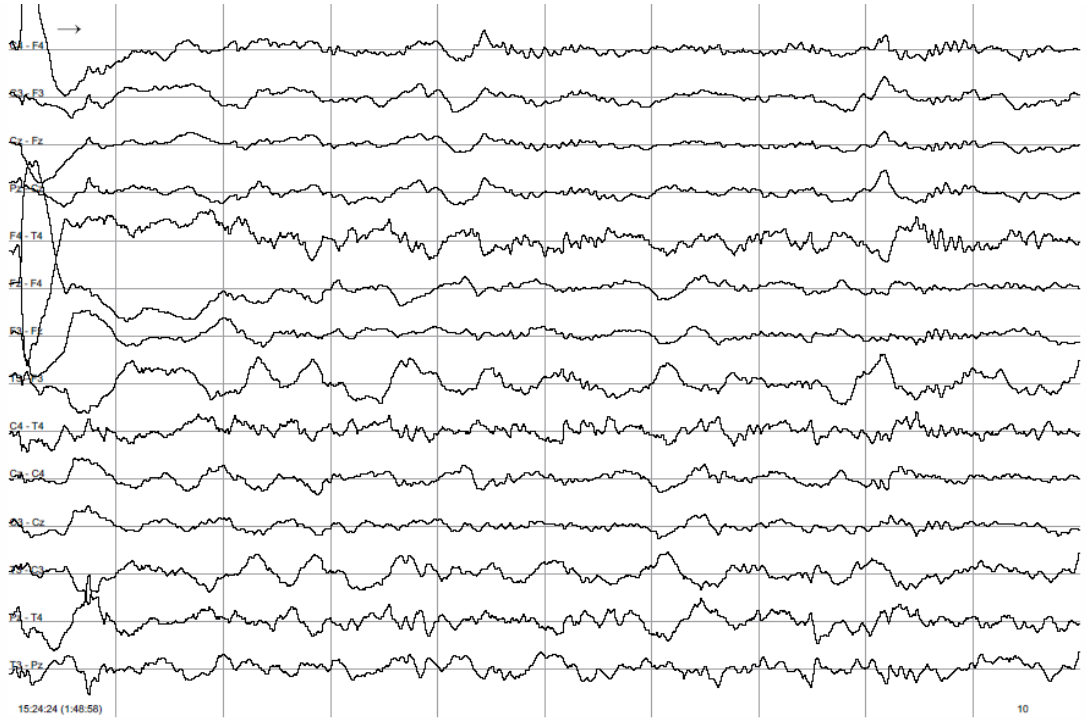


Fig.11 EEG 100uV/cm. Deep sleep waves are seen as delta waves of 2 Hz with an amplitude of 10-30uV. The delta waves are seen from the → entirely to the right of the figure over al the electrodes . They occur for a period of approximately twenty seconds. Case 13.

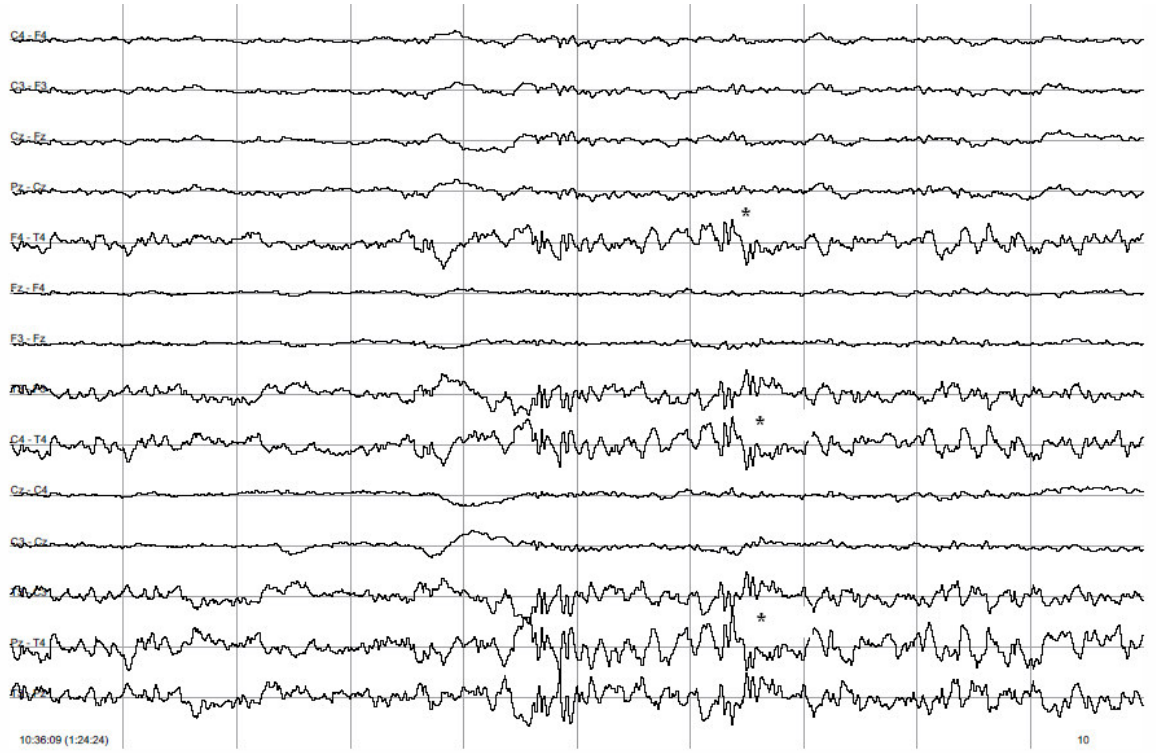


Fig.12 EEG 50uV. Sleep spindles are seen as single waves of 10-12 Hz with an amplitude of 30-60uV. Case15.*

Abnormal EEG signals indicating pathology

In 5 of the horses abnormal EEG signals indicating pathology were seen. This contained rhythmical activity, spikes and sharp waves and hypo-activity.

The patient group
The patient group contained 4 horses with abnormal EEG signals.
The recordings of case 11 showed several spikes of 100-200ms. The amplitude differed from 120uV on the diversions with the temporal electrodes till 20uV on the others (Fig.13). This spikes showed random and repeatedly in the recording with a maximum of 4 spikes in 1 minute.
Case 12 showed fast discharges and spikes. The spikes, of 70ms with amplitude of 20-40uV and with a maximum of 3 per minute, showed only on the diversions with the temporal electrodes (Fig.14). The fast discharges, of 4-7 Hz, showed randomly the whole recording with a maximum of 2 per minute. They were recorded on all the electrodes with amplitude of 600-800uV (Fig.16).
Case 15 showed fast discharges and spikes. The spikes, of 150ms, showed only on the diversions with the temporal electrodes with amplitude of 50-130uV and with a maximum of 10 per minute (Fig.15). The fast discharges, of 4-7 Hz, showed randomly the whole recording with a maximum of 11 per minute. They were recorded on all the electrodes with amplitude of 600-800uV (Fig.17).
The control group contained one horse with abnormal EEG signals. Case 1 had two parts with abnormal brain signals during the 24 hours of recording. The rhythmical activity was seen on all

the electrodes with a frequency of 1-2 Hz and an amplitude of 30-70uV(Fig.18). The first period of rhythmical activity lasted for 54 seconds and the second lasted for 68 seconds. After the rhythmical activity the EEG showed large loops in a chaotic manner (Fig.19).
The results of case 13 showed hypo-activity of 1 Hz. This hypo-activity showed for 5 seconds on all the electrodes with amplitude of 50-100uV and later for 17 seconds on all the electrodes with amplitude of 20-70uV (fig.20).
57% of the patient group showed brain signals indicating pathology. 43% of the patient group and 12% (n=1) of the control group showed epileptic activity.

Pathology indicating epilepsy
Horse 11, 12 and 15 showed spikes of 70-150ms (Fig.13-15). Horses 12 and 15 also showed large discharges of 4-7 Hz (Fig.16 & 17) in their EEG records. Case 1 showed rhythmical activity on all the electrodes with a frequency of 1Hz (Fig.18). The maximum spike amplitude lied on the temporal electrodes.

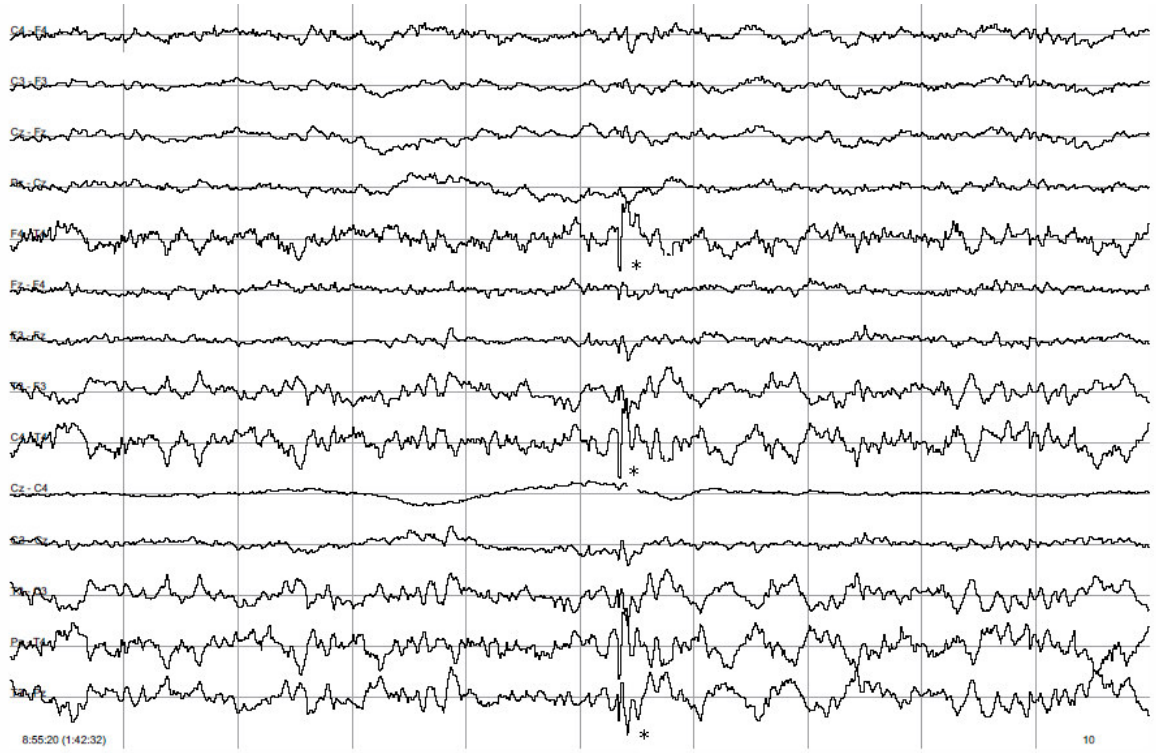


Fig.13 EEG 50uV/cm. Spikes are visible as sharp waves with an amplitude of 20-120uV and a frequency of 100ms. The maximum amplitude lies on the temporal electrodes. Case 11.*

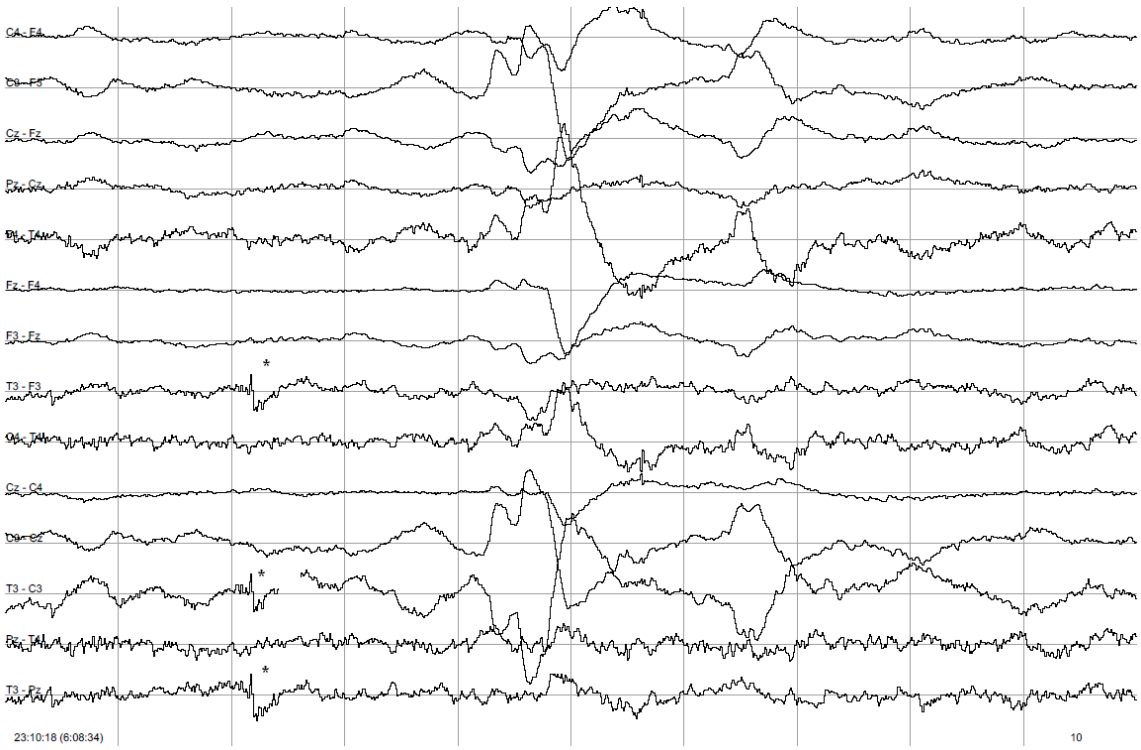


*Fig.14 EEG 50uV/cm. Spikes** are visible as sharp waves *with an amplitude of 20-40uV and a frequency of 70ms. The spikes showed only on the signals of the temporal electrodes. Case 12.*

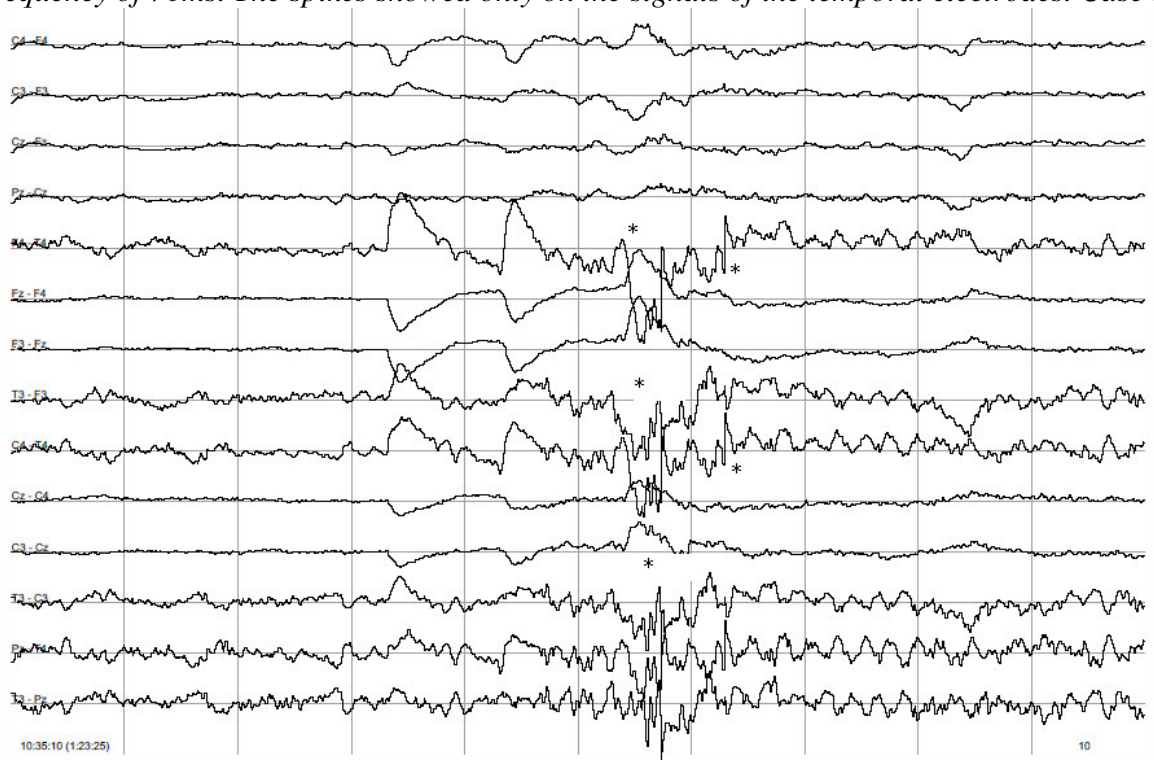


*Fig.15 EEG 50uV/cm. Spikes** are visible as sharp waves *with an amplitude of 50-130uV and a frequency of 150ms. The spikes showed only on the signals of the temporal electrodes. Case 15.*

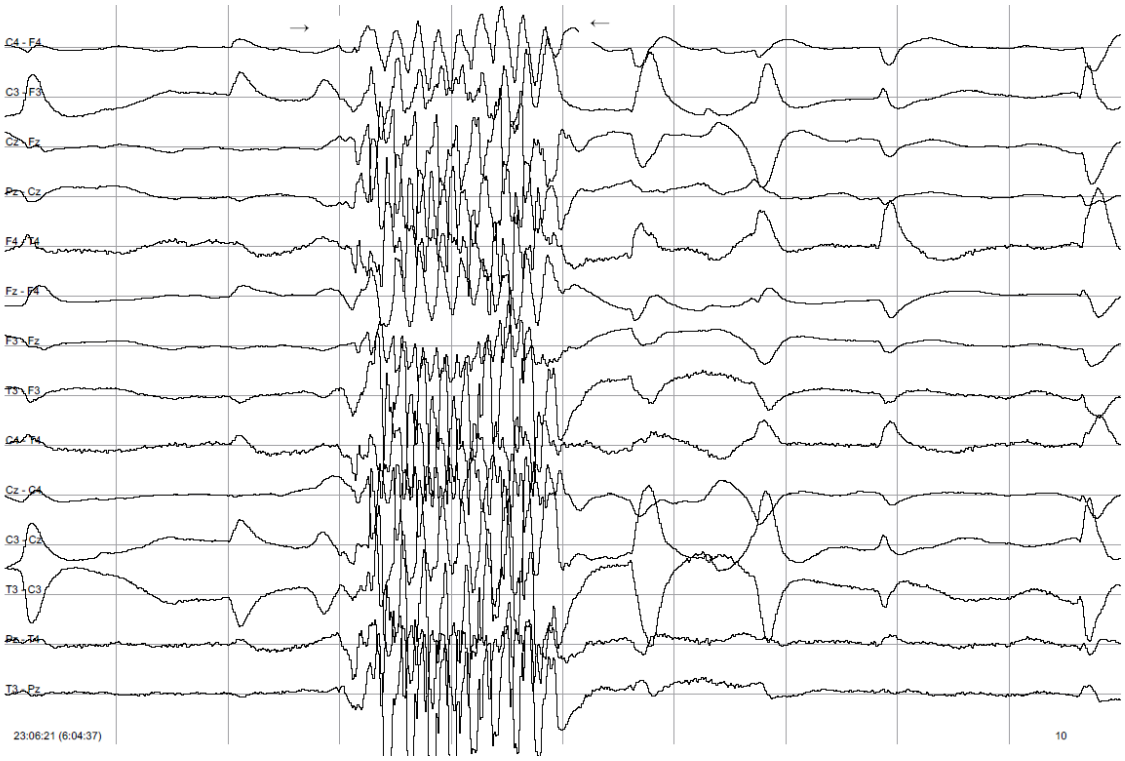


Fig.16 EEG 200uV/cm. Large discharge between the → ← with an amplitude of 600-800uv and a frequency of 4-7Hz over all the electrodes. Case 12.

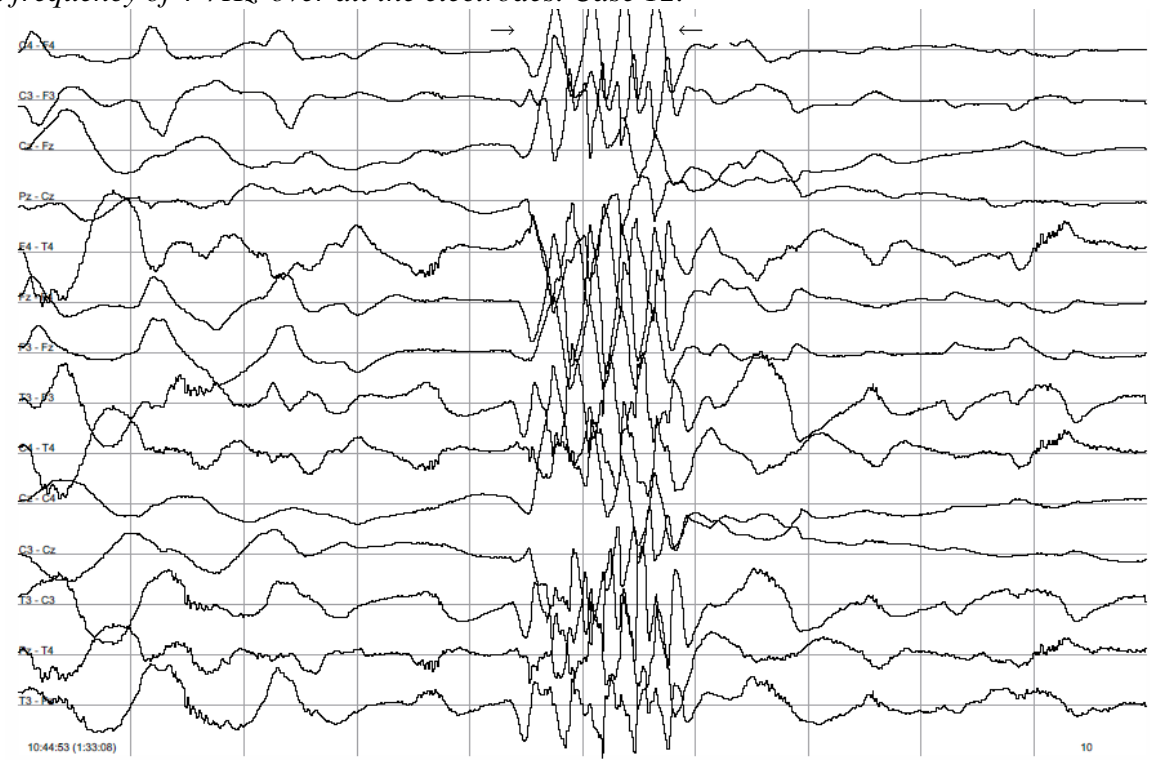


Fig.17 EEG 200uV/cm. Large discharge between the → ← with an amplitude of 600-800uv and a frequency of 4-7Hz over all the electrodes. Case 15.

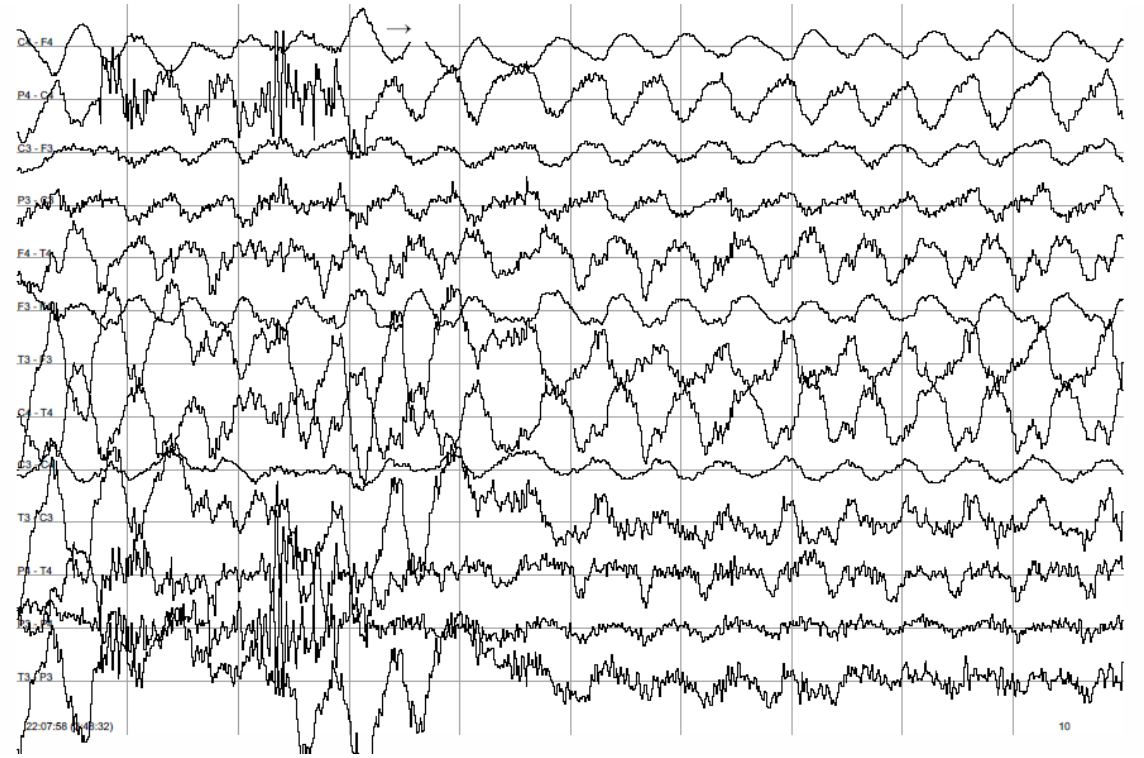


Fig.18 EEG 50uV/cm. Rhythmical activity is seen from the → entirely to the right of the figure on all the electrodes with an amplitude of 30-70uV and a frequency of 1-2Hz. Case 1.

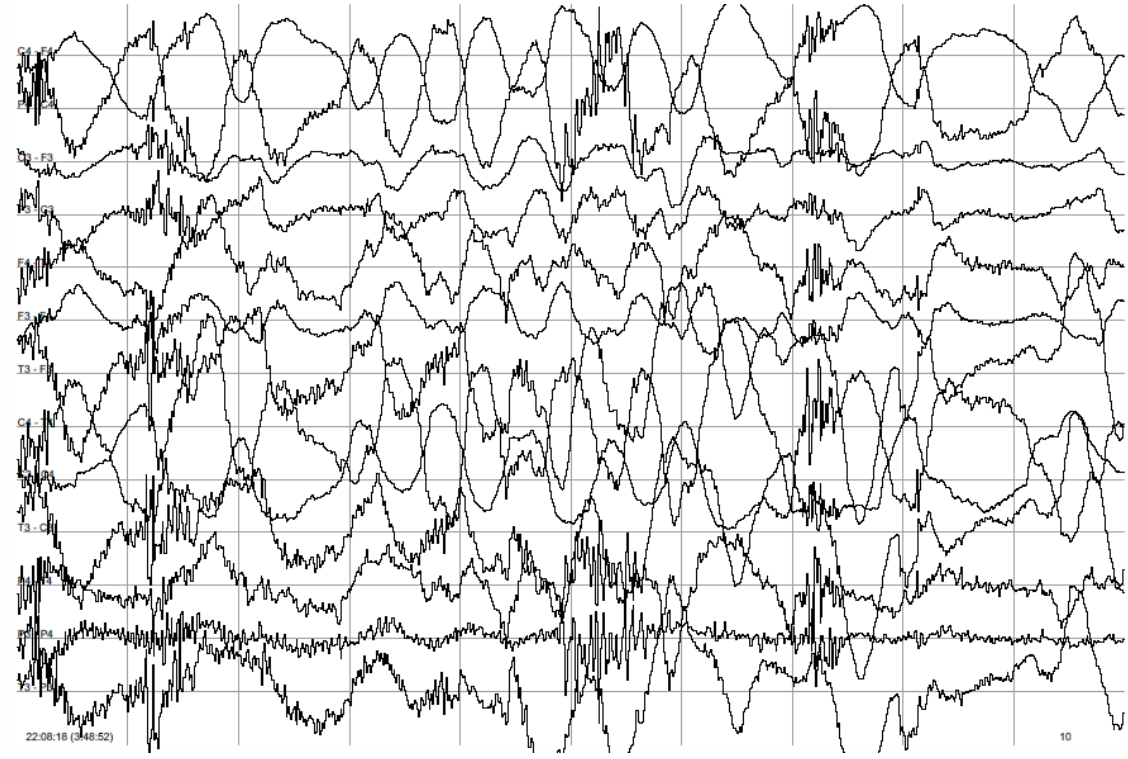


Fig.19 EEG 50uV/cm. After the rhythmical activity(Fig.13) the EEG shows large irregular and uncoordinated loops in the EEG over al the electrodes. Case 1.

Coincidental findings

Case 13 showed hypo activity. This showed by slow delta waves with an amplitude of 20-100uV with a frequency of 1 Hz(Fig.20). This continued for about twenty seconds and occurred 2 times.

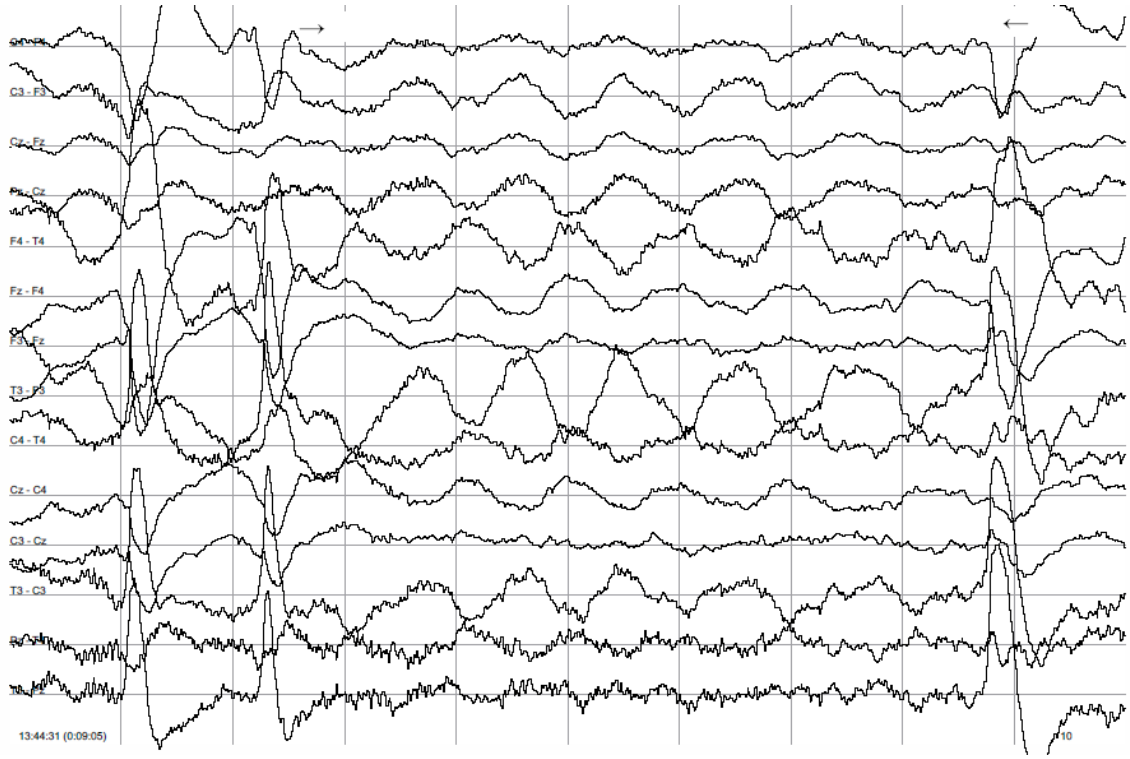


Fig.20 EEG 50uV. Hypo-activity shown as slow delta waves with an amplitude of 20-100uV and a frequency of 1 Hz. The delta waves are seen between the → ← over all the electrodes. Case 13.

MRI of case 13 after euthanasia

The MRI made of the head of case 13 showed a large abscess in the left hemisphere of the brain(Fig.21 a,b,c).

MRI scan of the abscess in the brain of case 13.

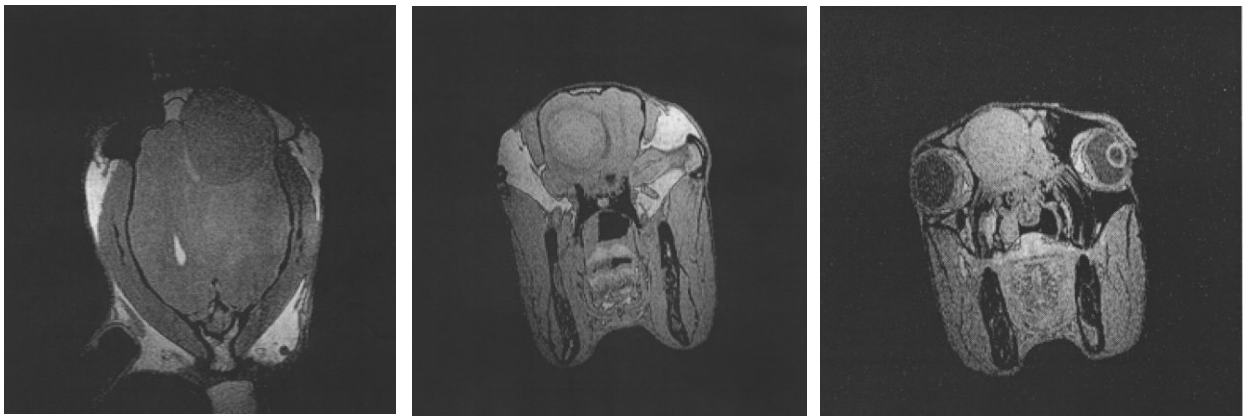


Fig. 21 a,b,c An abscess in the left hemisphere of the brain. a. A dorsal view. The abscess is visible as a grey circle on the upper right part of the figure. b. A caudal view. The abscess is visible as a circle in the upper right part of the figure. c. A caudal view. The abscess appears between the eyes as a circle. Case 13.

Discussion

Summery of the results
Using AEEG, based on the anatomical structures of the horses head, it is possible to make long-term records of good quality of the horses brain waves. All the cases showed normal brain signals. 57% of the patient group showed brain signals indicating pathology. 43% of the patient group and 12%(n=1) of the control group showed epileptic activity.

Material & Methods: position and application of the electrodes
In the horse, EEG is practised for 10 years now(Aleman et al. 2006; Lacombe et al. 2001; Williams et al. 2008). Despite that, there hasn't been developed a system for electrode placement based on the anatomical structure of the horses head. When you consult the literature different placement systems are used(Aleman et al. 2006; Lacombe et al. 2001; Lacombe et al. 2001; Williams et al. 2008), all based on the human system. The 10/20 international system for electrode placement for humans is developed as followed. By using EEG, the right spots to place the electrodes on the scalp were determined. Then these places were marked. Afterwards holes were drilled in the scalp, so the location on the brain was revealed. With Indian ink the cortex of the brain was marked also. After brain removal from the head the location of the electrodes 'on' the brain were analysed (Klem et al. 1999). The electrodes were place symmetrical over the whole head. Today we have enough possibilities for using scanning methods to locate situations inside the body. The goal of this experiment was to develop a standard basic system for electrode placement for EEG in the horse. To find out the best positions for the electrodes a MRI scan of the horse head was made. The MRI showed the right positions of the electrodes. This was based on the anatomical structures of the horses head and the human and equine literature. The system of electrode placement used for this research is rightly developed to measure horses brain signals. In horses however the location of the brain in the scalp is different than in humans. Instead of filling "half the head", as in humans, the brain in horses fills only the part between the ears and the eyes seen from a lateral and frontal view(Budras et al. 1994a; Budras et al. 1994b; Nickel et al. 1975a; Nickel et al. 1975b). Only there the brain lies close enough to the scalp so an EEG recording will be relevant.
The researchers who practiced EEG in the horse placed the electrodes they used only on the forehead of the horse.(Aleman et al. 2006; Lacombe et al. 2001; Williams et al. 2008) This is understandable since the location of the horses brain is roughly between the ears and eyes seen from a frontal aspect and between the eyes and the ears seen from lateral (Fig.4). Just placing electrodes on the forehead, means missing more than half of the signals of the brain. Since the parietal and the frontal lobe lie beneath the forehead, only the signals from those lobes will be registered. Seen from lateral view the brain also lies between the ears and eyes of the horse. In humans it is known that signals for instance from the temporal lobe are just as or even more important when diagnosing epilepsy. In humans TLE(Temporal Lobe Epilepsy) is diagnosed with (A)EEG especially from the electrodes placed 'above' the temporal lobe of the brain(Beleza et al. 2009; Noachtar and Rémi 2009; Tassi et al. 2009). In horses this kind of epilepsy obviously isn't known. By placing electrodes on the temporal lobe of the horse more epileptic signals should be obtained. Maybe even interictal deviations. Of course horses are not humans and there are differences that should be considered. First the horse's brain lies 90 degrees turned insides the head. The parietal and frontal lobes lie beneath the fore head, while in humans they are positioned underneath the scalp on the top of the head. Second the brain lies further inside the head from a lateral point of view. Between the brain and the skin lie two big chewing muscles.

The musculus temporalis covers almost the whole brain from a lateral view and the musculus masseter is positioned from the jaw till just beneath the ear and thus covers half the brain. These differences probably could have made the results from the temporal electrodes more sensitive for Artefacts. The results shows that most of the abnormal signals as spikes were seen in the temporal lobe, as in humans. Despite some Artefact seen on all the electrodes at a time, the temporal lobe electrode seemed useful in this study to capture different brain signals than normal. It recorded spikes that were not visible on other electrodes and it didn't record more EMG or artifact then the other electrodes.
Also the other normal and abnormal findings in the EEG records were comparable with human EEG signals. Since the large temporal bone lies in the middle of these anatomical landmarks it was possible to place one electrode on each side of the head on the temporal bone. On the MRI scans was visible that the position of the 9 electrodes, based on the literature and some preparations of a horse head, corresponds with the location of the horses brain(Fig. 4-6).
At first as much electrodes as possible(eleven electrodes) were used so the most signals were recorded. 3 electrodes were placed on the P line; P4, Pz and P3. This resulted in record with a high percentage of EMG Artefact up till >95% of the recordings. Also the P electrodes easily got loose during the experiment, because of the position on top of the head. When the horse would rub its head these electrodes would come of first. There was to little space between the both parts of the m.temporalis to place 3 electrodes. So in case 4, 10 electrodes were used, P4 and P3. This still gave a lot of EMG activity, so the rest of the horses were recorded with 9 electrodes, only using the Pz. Every horse has different structures and muscles, so the results between them were also different. In some horses more EMG was recorded than in others but since the use of 9 electrodes al the horses had legible results. Also the change of electrodes placed with collodium and the sticker electrodes gave better results. The stickers stacked much better to the head, except in one case, no electrodes got loose and al the signals were legible. The independence decreased to 1 or 2 kΩ using the sticker electrodes, while <10 kΩ is still acceptable(Leidse Onderwijs Instelling; Stigsby 1981).

Material & Methods: filter settings
In humans a 50Hz notch filter, a 0,5 Hz high pass and a 35 Hz low pass filter are used(Leidse Onderwijs Instelling). Since in this research the 50Hz interference was minimal, because of the use of shielded cables the notch filter wasn't used. The other filters were also set on 0,5Hz high pass and a 35 Hz low pass to exclude the worst interferences from outside.

Results: EEG, EMG and Artefact
Recording brain signals using AEEG it was possible to make records for 6.2 till 48.4 hours. 24 hour recording is usual in AEEG in humans. In case 13 the 'porti system' did not record anything for a whole day. At the end of this day the horse was euthanized, so no more records could be made. Only the 6.2 hours from the first they could be saved. In case 10 the records showed the first day of recording >95% EMG activity. Since the EEG was illegible for >95% it was decided to record an extra 24 hours, which made a total of 48.4 hours. Also the difference in recording time in the other horses was mainly caused by the same technical difficulties.
When scoring the percentages EEG, EMG and Artefact the first half hour of the recordings was scored. This means the horses were just put in their stables. 49.6% was the mean EMG scored. When horses were put in their stable they often started eating. So most of the EMG scores were caused by chewing Artefact. In case 10 only EMG was scored for the first half hour. This was a horse in which P3 and P4 were used. Also in the rest of her results almost everything was EMG.

This could mean that the electrodes were placed more on muscle than in the other horses. Or maybe this horse was more active with her facial muscles (m. masseter, m.temporalis) during the taping.
Also a lot of Artefact was scored in the first half hour of all the horses. The mean was 21.6% Artefact. This also could be explained by the fact that the horses were just put in their stable. They were not used to the equipment and often had trouble accepting it. All the horses stood in a stable that was new for them, so they started to explore the environment. This could have resulted in the Artefact on the records. For instance when the horse moves around a lot you will get EMG and Artefact, this in combination with the big loops from eye movements, will make an illegible records. To get a complete image of the percentages EEG, EMG and artifact it would have been be better and more reliable to score al the whole records of all the horse.
Also the fifth half hour of Case 1 and 3 were scored, because the first 2 hours they were still clinical sedated. Medication, especially sedatives influence the brain signals(Mysinger et al. 1985; Purohit et al. 1981) and also makes the horse behavior very calm. This means the EEG record is also calm without showing normal brain signal as chewing, and just a few eye movements. Because of this it is necessary to start interpreting the records after the horse is not clinically sedated any more.

Results: brain signals
With the AEEG setup we were able to differentiate not only between EEG, EMG and Artefact, but also between some of the EEG results. All the cases showed normal and abnormal brain signals. Some theta and delta waves were recorded and also drowsiness, sleep, deep sleep and sleep spindles were recognizable. 57% of the patient group showed brain signals indicating pathology. 43% of the patient group and 12%(n=1) of the control group showed epileptic activity as in spikes, rhythmical activity and fast rhythmical discharges.
Brain signals of horses and humans are have a lot of resembles, this is seen in the comparable frequencies of normal as well as abnormal brain signals. For instance theta and delta waves and the spikes of 70-200 ms (Tab.1-4) (Aleman et al. 2006; Barlow 1993a; Barlow 1993b; Barlow 1993c; Visser et al.; Visser et al.; Visser et al.; Williams et al. 2008). Also the results in this study were comparable with the horses as well as the results in human literature.
Since the 3 patients, showing epileptic activity in their EEG, were suspicious of having epilepsy due to abnormal behaviour of unknown origins the abnormal results were suggestive for epilepsy. Also the similarity between the human as well as the equine literature and the results of this study, made it possible to diagnose the abnormal EEG activity of the patients as epileptic activity. In the other 3 patient the records didn't show any different signal. This does not rule out the diagnosis of epilepsy(Sheridan and Sato 1985). It could be possible that they had abnormal brain signals during or just before and after an attack. Since these horses didn't have an attack during the experiment or during the seventy-two hours before the experiment, the diagnose epilepsy cannot be excluded. The more time the EEG is made after a seizure the less chance there is to record epileptic brain signals(Bridgers and Ebersole 1985; Marsan and Zivin 1970; Sheridan and Sato 1985; Visser et al.). Case 9 had just had one seizure in its whole life. Case 10 had had partial seizures that had stopped after treatment with Rapidexon[2]. Case 14 was suspected of having seizures cause by Cushings disease. So these cases were more uncertain to be caused by epilepsy, in comparison to the other cases, from the start.
Also in dogs is known that EEG recorded in seventy-two hours after an attack the EEG is still positive in 67% of the epileptic cases (Berendt et al. 1999). This might be the case in horses to. Case 1, of the control group, also had abnormal brain signals. In this horse there has never been

noticed abnormal behaviour that could be suggestive for epilepsy. Still this might be a subclinical form of epilepsy also seen in humans (Capovilla et al.; Liasis et al. 2006).
A coincidental finding was Case 13. This horse showed hypo activity in the brain shown by slow delta waves(Fig.20). The MRI of this horse showed a large abscess in the brain(Fig.21a,b,c). Since an abscess took up a lot of space where normally the brain lied, it is very plausible that this is the reason the brain showed less activity. In humans with a brain abscess the EEG results show slow delta activity(Visser et al.). And because a lot of the EEG signals in horses and humans is similar this makes it even more likely that the abscess caused the abnormal EEG recordings.

Conclusion

The aim of the study is test the hypothesis that AEEG can be used for longterm recordings in unsedated horses for identification of abnormalities, for instance epilepsy.

When recording AEEG in horses using 9 electrodes as placed in fig.4 it is possible to record normal as well as abnormal brain signals. Because the AEEG can tape at least 48 hours at a time, the chance of recording abnormal brain signals is much higher then recording one hour or less(Berendt et al. 1999; Ebersole 1987; Sheridan and Sato 1985). AEEG gives also the advantages to record brain signals of a animal during its normal day activities. The longer recording time and the normal day activities makes it easier to divert normal from abnormal brain signals. The first half hour of the recordings showed a lot of Artefact and EMG activity next to the EEG. Also the logbook showed that the horses started to explore their environment and started eating when first arrived in the for them new stable. Since the horses cannot understand when to stand still, with AEEG it is possible to record all sorts of activity, normal and abnormal. There is enough recording time to get used to the signals of the individual.
All the horses showed normal brain signals as well as abnormal signals on the EEG. 57% of the patient group showed brain signals indicating pathology. 43% of the patient group and 12%(n=1) of the control group showed epileptic activity as in spikes, rhythmical activity and fast rhythmical discharges. All the advantages as mentioned above, make it possible to differentiate epileptic activity from the recorded brain signals using AEEG. Intermittent abnormal behavior, indicative for epilepsy, can be normal by the time the veterinarian arrives. Still, horse owners will ask for a diagnosis and therapy. Often for insurance issues or just for their own comfort. It is thereby important that a system to do identify epileptic brain activity has been developed. The results of this research showed that with AEEG it is possible to make long-term records of good quality of the horses brain waves, normal as well as abnormal, for instance epilepsy.

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