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TRANSCRANIAL MAGNETIC MOTOR EVOKED RESPONSE TESTING IN THE HORSE

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ABBREVIATIONS

ALS: amyotrophic lateral sclerosis

CMAP: compound muscle action potential

CMCT: central motor conduction time

CT: computed tomography

CVM: cervical vertebral malformation

EDM: equine degenerative myeloencephalopathy

EEG: electroencephalogram

EMEP: electric motor evoked potential

EMG: electromyography

EMND: equine motor neuron disease

LMN: lower motor neuron

MMEP: magnetic motor evoked potentials

MUAP: motor unit action potential

MRI: magnetic resonance imaging

ms: millisecond

mV: millivolt

PMCT: peripheral motor conduction time

rTMS: repetitive transcranial magnetic stimulation

SSEP: somatosensory evoked potentials

TES: transcranial electrical stimulation

TMS: transcranial magnetic stimulation

UMN: upper motor neuron

PREFACE

The brain is the central computer of our body interpreting outside information and controlling every action. The spinal cord connects the brain with the rest of the body by sending out millions of electrical signals.
The spinal cord contains ascending and descending tracts of nerve fibres to transfer sensory information from the periphery to the brain and motor activities from the brain to the periphery. When there is injury to the spinal cord and this connection breaks neural function below the level of the injury is lost. In most instances, spinal cord disease renders a horse unsuitable for athletic performances. Signs of symmetric ataxia and tetraparesis are described to be typical in horses with cervical spinal cord disease. Depending on the severity of the focal pressure-induced lesion, the clinical neurological signs are varying from obvious to very subtle. Especially in this latter case, a diagnostic test enabling us to evaluate objectively a functional deficit along the spinal cord would be extremely helpful.

In man, the development of transcranial magnetic stimulation (TMS) in 1985 opened new possibilities for studying the descending motor tracts. Barker and co-workers created a new type of cortical magnetic stimulator, based on the principle of electromagnetic induction.

Since in equine medicine, neurological examination is mainly based on clinical evaluation and since TMS is described to be non-invasive, very sensitive and nearly pain-free, it was a challenge for us to test its usefulness in horses as a diagnostic tool and as an index of descending motor pathway dysfunction.

CHAPTER 1

TRANSCRANIAL MAGNETIC MOTOR EVOKED RESPONSE TESTING

REVIEW OF THE TECHNIQUE, BASIC PRINCIPLES AND APPLICATIONS

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SUMMARY

Transcranial magnetic stimulation has developed as a powerful, non-invasive tool for studying the descending motor tracts in humans. The applications of the techniqie in animals are for the moment restricted to small animals. However, this non-invasive, sensitive and painless technique appears promising as a test of motor tract function in horses where the neurological examination is mainly restricted to clinical evaluation and some ancillary tests, such as radiography, cerebrospinal fluid analysis and electromyography. In this review, we discuss the history, basic principles, technique and applications of transcranial magnetic stimulation in humans and small animals and indicate the possibilities for its use in horses. Since the great portion of this review is based on human studies, it is worthwhile to mention that the reports being described are from humans unless otherwise specified.

1. HISTORY

Electromagnetic induction, i.e. the induction of an electrical voltage in a circuit subjected to a changing magnetic field, was first discovered in 1831 by the English physicist Michael Faraday. This was the first experimental observation of magnetic stimulation and the term "magnetic field" was born. A relationship between magnetism and electricity was reported at the beginning of this century by d'Arsonval who found that the brain could be magnetically stimulated by a coil carrying a high current. The induced eddy currents in the retina, when placing one's head in a coil driven from an alternating 110 volt supply at 30 amperes, produces magnetophosphenes or flashes of light (d'Arsonval 1896).

The MEP, or motor evoked potential, comprises a class of tests of conductivity in central nervous system pathways. The technique of stimulating the motor cortex and recording the muscle twitch or surface potential responses in the periphery was established by the work of Merton & Morton, who showed in 1980 that it was possible to stimulate the motor cortex of the human brain through the intact scalp by using very short duration and large amplitude *electrical* pulses, delivered through a pair of surface electrodes (transcranial electrical stimulation, TES). After stimulation a relatively synchronous muscle response, the electric motor evoked potential (EMEP) is produced. It was immediately clear that this would be useful for many purposes. The required intensity, however, was very high (in the order of 1-1.5 kV), uncomfortable and poorly tolerated (Merton *et al.* , 1982). The major problem with this form of stimulation is that only a small fraction of the applied current actually flows into the brain. Much of the electric current flows between the electrodes on the scalp and produces local discomfort and contraction of the scalp muscles. In the mid-1980s, however, Barker and colleagues demonstrated for the first time that stimulation of the human motor cortex and peripheral nerves can be performed using a brief and strong external *magnetic* field. The magnetic stimulator works by charging one or several capacitors and then rapidly transferring the stored energy from the capacitor(s) to a circular wire coil. The subjects reported that the muscle twitches were produced "without causing distress or pain". Transcranial magnetic stimulation (TMS) is now routinely used in humans for a variety of clinical and scientific applications, including testing of motor function, vision, language and studying the pathophysiology of brain disorders (Mills & Murray, 1985; Rossini *et al.*, 1987; Fehlings *et al.*, 1989; Hess *et al.*, 1987a; Boniface *et al.*, 1991; Cantello *et al.*, 1991; de Noordhout *et al.*, 1998; Berardelli, 1999; Kohara *et al.*, 1999; Naka & Mills, 2000). It may even be useful for therapy, particularly in psychiatry (George *et al.*, 1997, Feinsod *et al.*, 1998; Klein *et al.*, 1999; Hasey, 2001) and is especially useful as a monitoring tool for anaesthetised patients undergoing spinal surgery (Shields *et al.*, 1989; Bartley *et al.*, 2002, Aglio *et al.*, 2002).

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While routinely used in humans, application of TMS in other species is rare. The unique features of TMS are particularly useful in evaluating spinal cord injury and recovery (Fehlings *et al.*, 1987, 1988; Magnuson *et al.*, 1999) and anaesthesia (Ebert & Ziemann, 1999, Ghaly *et al.*, 1999) in animal models. The methodology for reproducible assessment of motor potentials evoked by TMS has been characterised in different animal studies on rodents, cats and dogs (Heckmann *et al.*, 1989; Linden *et al.*, 1990; Van Ham *et al.*, 1994; 1995; 1996a; 1996b; Nakatoh *et al.*, 1998, Luft *et al.* 2001). Several studies have demonstrated its safety (Russell *et al.*, 1994; Post *et al.*, 1999; Van Ham *et al.*, 1994, 1995, 1996a, 1996b). In horses, Mayhew and Washbourne (1996) have shown that transcranial magnetic stimulation is able to induce magnetic motor evoked potentials (MMEPs) in unanaesthetised normal ponies. The diagnostic usefulness of the technique was also demonstrated in horses with cervical spinal cord lesions (Nollet *et al.*, 2002).

2. TECHNIQUE AND BASIC PRINCIPLES OF MAGNETIC STIMULATION

2.1. Basic principles

Magnetic stimulation is a technique for stimulating peripheral nerves and cerebral cortex in order to help quantify the integrity of the motor nervous system, especially to measure conduction times. Its purpose is to create a pulsed electric current, induced by the time-varying magnetic field (Barker *et al.*, 1985), that will momentarily depolarise the nervous system. It is important to acknowledge that the actual pathways being investigated are not known; however, they incorporate the fastest conducting fibres which presumably include the pyramidal tracts (Corthout *et al.*, 2001).

A magnetic field is generated by passing an electric current through a coil of wire, called the magnetic coil (Fig. 1), which is placed above the scalp. Faraday's law states that whenever a magnetic field changes there is an induced electric field which impedes the changing magnetic field. The magnetic pulse produced from an electric current pulse will thus induce in turn a current in an electrically conductive region, such as the human or animal body. This induced electric current flows perpendicularly to the magnetic field and circulates up to a few centimetres away from the coil's external edge, and with a direction opposite to the current flowing in the coil and an intensity proportional to the magnetic field.

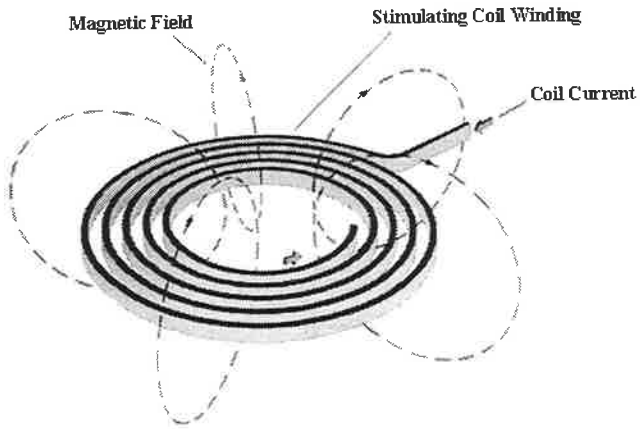

Figure 1. A magnetic field is produced by passing an electric curent through a coil of wire. In an air-core winding the magnetic field intensity is directly proportional to the current flowing through the coil (with permission of R. Jalinous; Magstim Ltd).

The intensity of the magnetic field can be represented by flux lines around the coil (Fig. 2) and is measured in tesla (T). The magnetic field is oriented perpendicular to the coil and, for currently available devices, can reach values of up to 4 Tesla (Barker *et al.*, 1985). The precise stimulating characteristics depend upon the model of stimulator used. For example the Magstim 200 stimulator (used in our experiments) produces a magnetic field which rises to peak within about 150 μs and then decays slowly to zero over the next millisecond. Such a rapidly changing magnetic field induces electric eddy currents in any conductive structures nearby. Because the skull presents a low impedance to magnetic fields of this frequency, eddy currents are produced in the brain, and these currents can stimulate neural tissue. Currents induced on the scalp by magnetic stimulation are much weaker than those produced by transcranial electrical stimulation, because they cross the extracerebral layers (scalp, skull and meninges) with minimal or no activation of the pain receptors and result in a well tolerated procedure (Rossini & Rossi, 1998). Therefore, the sensation produced by magnetic stimulation is very slight (Jalinous, 1991).

In a homogenous medium, the electric field will cause the current to flow in loops parallel to the plane of the coil. The loops with the strongest current will be near the circumference of the coil itself. The current loops become weak near the centre of the coil, and there is no current at the centre itself. The magnetic field decreases rapidly with increasing distance from the coil: with a typical 12 cm diameter round coil the strength falls by half at a distance of 4-5

cm from the coil surface (Hess *et al.*, 1987b). Since the cerebral cortex can be 1-2 cm from the surface of the scalp, and since the central sulcus itself can be 2 cm deep in man, this means that stimulation is severely attenuated at deep sites such as basal ganglia or thalamus.

Magnetic motor evoked potential (MMEP) testing can be regarded as a counterpart of the longer-established procedure of somatosensory evoked potential (SSEP) monitoring, where small "cortical" potentials are recorded over the scalp in response to peripheral nerve stimulation (Machida *et al.*, 1988; Macdonell *et al.*, 1989; Ghaly *et al.*, 1999).

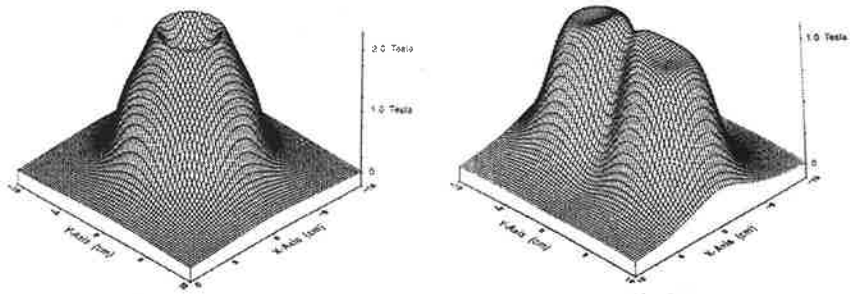

Figure 2. A three dimensional representation of the peak magnetic flux produced on the surface by the 90mm circular coil (left) or 10 mm below the surface of the double 70mm coil (right). For the circular coil (left) the field distribution is symmetrical about the central axis and the maximum magnetic field strength normally occurs next to the innermost turn.
Double, butterfly or figure of eight coils (right) consist of two windings placed side by side producing a maximum electric field under the point where the two windings meet (with permission of R. Jalinous; Magstim Ltd).

2.2. Technical requirements

When magnetic stimulation is performed on the motor cortex, electromyographic responses (MMEPs, magnetic motor evoked potentials) can be recorded in contralateral, particularly distal, appendicular muscles. However, large pulses of magnetic field need to be generated in order to induce electric fields in the body of sufficient amplitude and duration to cause stimulation of the neural tissue in its vicinity. Therefore, magnetic stimulators consist of a coil of wire connected to a large electrical capacitance. *A magnetic stimulator* works by charging one or more energy storage capacitators and then rapidly transferring this stored energy from

the capacitor(s) to the stimulating coil as it discharges. Current (with a peak value of 5000 amperes or more) thus flows from the capacitor through the stimulating coil generating the required magnetic field.

The difficulty in producing magnetic neural stimulators is related to the high discharge currents, voltages and power levels involved in producing the brief magnetic pulse. Typically 500J of energy has to be transferred from the energy storage capacitator into the stimulating coil in around 100μs. Power, measured in watts, is equivalent to joules per second. From this, the power output of a typical magnetic stimulator during the discharge phase is 5MW (5.000.000 watts) – adequate to provide the electricity necessary for 1,000 homes for $1/1000^{th}$ of a second. During the discharge, energy initially stored in the capacitator in the form of electrostatic charge, is converted into magnetic energy in the stimulating coil in approximately 100 μs. This rapid rate of energy transfer produces a time varying magnetic field build-up which induces tissue currents in the vicinity of the coil in the order of 1-20 mA/cm^2 (Jalinous, 1991). However, the amount of thermal energy deposited in tissue due to magnetic stimulation is very small. At maximal output, assuming a maximal stimulus repetition rate of one pulse every 3 seconds, the average power deposited in the brain is calculated to be less than 2mW. This represents less than 0.01% of the heat generated in the adult brain due to the normal basal metabolism. Heating of the brain is of the order of 10^{-6} °C/pulse and unlikely to cause deleterious effects (Barker, 1991). Similarly, international standards suggest that the continuous thermal energy deposited in tissue by electromagnetic radiation should not exceed 0.4 W/kg to avoid thermal stress. For a typical human adult brain mass of 1.5 kg, this limit is 300 times greater than the average thermal energy deposited by the magnetic stimulator at maximal output, even if stimulation is carried out continuously (Barker *et al.*, 1987).

The stimulating coil, normally housed in moulded plastic covers, consists of one or more tightly wound and well insulated copper coils together with other electronic circuitry, such as temperature sensors and safety switches. At the present time, most commercial magnetic stimulators are supplied with a circular coil of 5-10 cm diameter. Different coil types are nowadays available each with their advantages and disadvantages. Large coils cannot produce very focal stimulation of the brain, but have the advantage that a reasonable depth of penetration can be achieved. Although the circular coil is a very useful general purpose coil the site of stimulation is not well defined. For example, with a standard round coil, the induced current in the brain flows in an annulus, underneath the coil, which is usually some 8-12 cm in diameter. Clearly a large volume of neural tissue may be activated by such a device. Improvement in focusing of the stimulus can be achieved by tilting these circular coils so that they lie at an angle to the skull. The greater the angle between the skull and the coil, the more focal the stimulation. Unfortunately, such improved focusing of stimulation is offset by a decrease in the effectiveness of stimulation. Recently, coils wound in a figure-of-eight shape (also termed butterfly or double coil) have been used, and in these, the induced electric field (Fig. 3) under the junction region of the 8 is twice as large as that under the two wings (Rothwell, 1997). These coils have a lower induction than circular coils of the same number of turns, and hence larger currents can flow in them. However, the hypothesis that the double coil only stimulates under its centre should be viewed with caution. There are also smaller peripheral peaks of approximately half the amplitude of the central peak on either side of the winding.

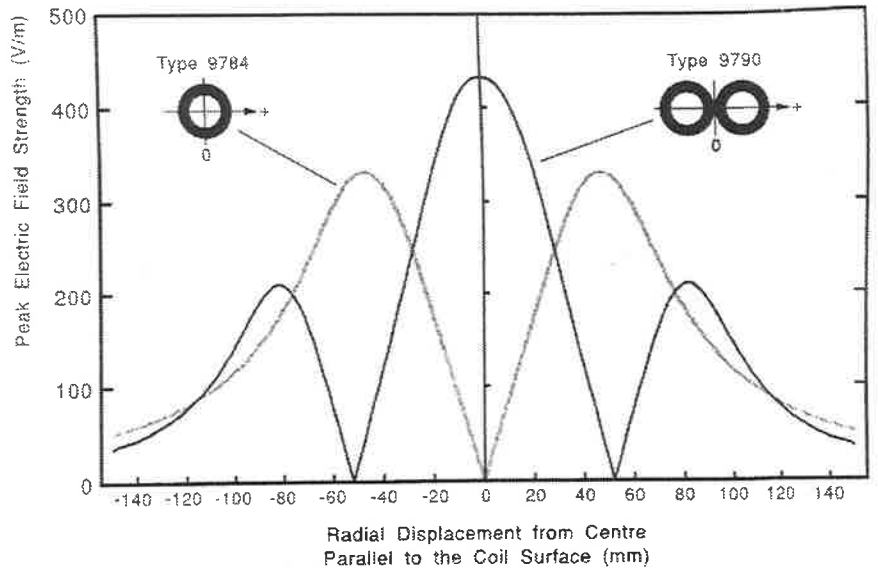

Figure 3. The induced electric field profile of single circular and double circular coils differ widely because of their geometry. The induced electric field of a circular coil is zero directly under its center and rises to a maximum in a ring under the mean coil diameter. In the case of double coils the field is at its maximum directly under the junction of the two coils and has two smaller characteristic peaks on either side. These are less than one half the amplitude of the central peak (with permission of R. Jalinous; Magstim Ltd).

2.3. Comparison with electrical stimulation

Like magnetic stimulation, electric stimulation of the motor cortex evokes electromyographic responses in contralateral, particularly distal, appendicular muscles. Motor evoked potentials resulting from either transcranial electric or magnetic motor cortex stimulation may be used to demonstrate the functional integrity and conduction properties of the descending motor nervous system (Merton & Morton, 1980; Rothwell *et al.*, 1987).

Magnetic stimulation has three main advantages over conventional electrical stimulation. First, the primary benefit of magnetic stimulation is its ability to penetrate all body structures without attenuation. Because this increased field penetration compared to surface electrical stimulation, it allows better stimulation of regions below layers of bone, for instance the brain. The cells are still activated by electric currents, but the magnetic field penetrates the tissue more efficiently and induces current within the brain itself. The mechanism of stimulation at the neural level is thought to be the same for both magnetic and electrical stimulation, namely current passes across a nerve membrane and into the axon, resulting in depolarisation and the initiation of an action potential that then propagates by the normal method of nerve conduction (Barker *et al.*, 1987). Although the magnetic field and hence the induced electric field theoretically should be unaffected by the bone of the spine, there is however a stimulation of the nerve roots at their spinal exit but not the spinal cord when the magnetic coil is placed over the spine. Machida *et al.* (1992) showed that magnetic stimulation could excite the thoracolumbar spinal cord after laminectomy and pediculotomy in dogs. It is suggested that the bony structure surrounding the spinal cord interferes with the spread of magnetically induced eddy currents to the spinal cord. The current induced by the magnetic coil is theoretically maximum in the annulus under the coil. The charge buildup on the bone at the points where the induced current loops enter and leave the spine will reduce the current in the spinal cord. Therefore, the bony vertebrae act as an insulator between the spinal cord and the external tissue and the current probably tends to flow around the spinal cord rather than through it. Hence stronger magnetic fields are needed for spinal stimulation and novel coil geometries may be able to improve the coupling between the induced currents and the anatomy of the spine (Barker *et al.*, 1989).

Second, the electrical field induced with a coil (of 100 mm in diameter) decreases significantly less with increasing distance into the body than a field induced by currents applied via surface electrodes (Barker *et al.* 1987). Electrical stimulation injects current into the body via surface, needle, or implanted electrodes. The charge from an electrical stimulator

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is carried by electrons flowing in the wires to the stimulating electrodes and is transferred to an ion flow at the electrode-tissue interface. A small percentage of these ions will flow into nearby axons, resulting in membrane depolarization. Magnetic stimulation differs from electrical stimulation in that it uses a pulse of magnetic field to cause an electric field (a voltage difference between two points) in the tissue and results in stimulation. Hence the magnetic field functions as the vehicle that causes ion flow (or electric current) in the body and does not itself stimulate the nerve (Barker, 1991). The primary circuit is the stimulating coil, through which the stimulator drives current pulses, but which is not the electrical contact with the tissue. The magnetic field generated by the current flow in the coil is proportional to the rate of change of the magnetic field with respect to time. At the frequencies used in magnetic stimulation, the magnetic field is not affected by the electrical properties of the body and passes through both bone and soft tissue (and even clothing and air) without being affected by them and without causing large electrical fields at the surface.. An additional factor that may contribute to the difference between the two forms of stimulation is that the electric field induced by magnetic stimulation has quite a different distribution to the field produced by transcranial electrical stimulation. In the latter the current flows beneath the electrodes in all directions away from the anode both radially and tangentially to the cortical surface and will tend to stimulate structures close to the surface in this orientation. The electric field resulting from magnetic stimulation is much more homogeneous and is parallel to the surface of the coil at all points and hence will tend to stimulate structures with a different orientation. (Tofts, 1990). These two facts were thought to explain the lack of pain associated with magnetic stimulation and its ability to stimulate, without discomfort, deep structures such as the lumbar roots, the brachial plexus, and the sciatic, radial, and femoral nerves in humans (Krain *et al.*, 1989; Mills *et al.*, 1987). However, the depth of penetration depends on anatomical factors, coil size, coil geometry and the intensity of the applied stimulus. In the simple homogeneous model, the volume within which straight nerves can be stimulated, for both circular and figure-of-eight coils, is shaped roughly like an egg. Its maximum dimensions are at the surface and it decreases in cross-sectional area to zero at the maximum depth at which the stimulation threshold is reached (Barker, 1999).

Third, magnetic stimulation does not require either physical or electrical contact with the body. Hence, no skin preparations are required, and clothing need not be removed at the stimulation site. Although the coil is normally placed in contact with the body for convenience, stimulation can be achieved with the coil held some millimetres away from the

body. This could be valuable in situations such as the stimulation of traumatized regions where physical contact may cause further damage or infection. The stimulating coil can be moved freely over the area of interest, which makes the location of the optimal stimulation site rapid and easy (Barker *et al.*, 1987).

Ever since the introduction of TMS, there has been considerable debate over which structures within the cerebral cortex are activated. The first hypothesis was proposed by Day *et al.* (1989) on the basis of single motor unit studies in the hand. His statement was that direct electrical stimulation through the skull preferentially activates corticospinal fibres directly within a few millimetres of the cell body. This is referred to as direct activation, and results in D-waves conducted down the pyramidal system. Further studies (Di Lazzaro *et al.*, 1998a, b) revealed that pyramidal neurons could be activated trans-synaptically only at higher intensities. In contrast, the lowest threshold form of TMS over the hand area of the motor cortex tends to preferentially activate corticospinal neurons trans-synaptically or indirectly, resulting in I-waves in the pyramidal tract. With higher stimulus intensities both direct (D-wave) and trans-synaptical (I-wave) activation occur (Day *et al.*, 1989, Kaneko *et al.*, 1996, Di Lazzaro *et al.*, 1998a, b). The result is that the EMG responses that are recorded at threshold in response to transcranial magnetic stimulation often occur 1-2 ms later than those recorded following transcranial electric stimulation of the brain (Rothwell *et al.*, 1991). Why there should be this difference between electrical and magnetic forms of stimulation is unclear at the present time. However, it is presumed to be related to the fact that TMS induces electrical current that flows parallel to the surface of the brain. In contrast, electrical stimulation causes current to flow in all directions both parallel and radial to the surface. The result is that radially oriented neurons will have a higher threshold for magnetic than electric stimulation (Rothwell *et al.*, 1999). The response of lower limb muscles has a similar latency with electrical and magnetic stimulation. This suggests that both techniques have the same activation site in the initial segmental or proximal nodes of pyramidal axons as they leave the cortex and readily produce D wave activity (Rothwell, 1997).

2.4. Safety

Since 1985, many thousand subjects have been examined using low-repetition-rate magnetic stimulators to assess motor function of the peripheral and central nervous systems. There is now a considerable volume of data supporting the safety of magnetic stimulation. There have been no ill effects reported with magnetic stimulation of the peripheral nervous system, and,

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in the case of cortical stimulation, the incidence of side effects has been very low and within that expected by available statistics for various patient groups (Kandler, 1990; Hufnagel *et al.*, 1990).

The main area of concern has been the triggering of epileptiform activity in individuals at a high risk for epilepsy. Since TMS has been successfully used in the study of epilepsy and the determination of the site of the epileptic focus, there have only been a few reports of seizures occuring at or shortly after the magnetic stimulation (Homberg & Netz, 1989; Hufnagel *et al.*, 1990; Classen *et al.*, 1995). However, the more recently used repetitive TMS (rTMS) can, depending on the stimulation parameters, evoke seizures in normal subjects and in patients with neurological disease (Wassermann, 1998).

The presence of pacemakers and other electronic implants is also considered as a contraindication because of damage of the internal electronics due to the induced electric fields and currents resulting from the magnetic pulse.

Since implanted metal structures in the brain will have mechanical forces exerted on them due to induced currents, they also should be regarded as a contraindication (Barker *et al.*, 1989).

2.5. Procedure and measured parameters

2.5.1. Procedure

Stimulation of the motor cortex is in most cases achieved via a circular coil hand-held over the scalp. The subject feels only a moderate tapping sensation on the scalp, and the limb twitch. Recordings in humans are made from surface EMG electrodes attached to the skin overlying peripheral muscles using an EMG machine. In some animal studies skin alligator clip electrodes were fastened to the skin (Mayhew & Washbourne, 1996), but in most needle electrodes were inserted in the muscle (Young *et al.*, 1994, Van Ham *et al.*, 1994; 1995; 1996a; 1996b; Nollet *et al.*, 2002). The stimulator triggered the sweep of a standard electromyogram (EMG) machine, enabling the latency between the stimulus and the onset of the response to be measured. Measurements include the threshold, latency, amplitude and configuration.

2.5.2. Measured parameters

Threshold reflects the global excitability of the motor pathway and is often defined as the strength or % of maximal stimulation output that produced an identifiable MMEP of 50-100 μV in 50% of 10 to 20 consecutive occasions (Hufnagel *et al.*, 1991; Ellaway *et al.*, 1998).

Threshold in human adults is independent of age, gender and hemisphere, but varies with different target muscles (Mills & Nithi, 1997; Wassermann *et al.* 1992). A lower threshold is observed in upper (51) than in lower (73% of maximal stimulator output) limbs. Even in upper limbs the threshold is lowest for hand muscles and highest for proximal arm muscles. This may reflect the larger cortical motor areas controlling the hand muscles (Tabaraud *et al.*, 1989; Rothwell *et al.*, 1987; Furby *et al.*, 1992).

Amplitude refers to the recorded voltage of the response. It may be measured from the baseline to the negative peak or from the negative to the positive peak (peak-to-peak amplitude). Mostly amplitude is expressed in absolute terms, as μV or mV. Sometimes it is expressed as a percentage of the maximal response after stimulation of the appropriate peripheral nerve.

The amplitude can have a high degree of inter-trial as well as intra-individual variability (Hess & Ludin, 1988, Amassian *et al.*, 1989), especially when stimulating at slightly suprathreshold level. With increasing stimulus intensity (Kiers *et al.*, 1993) or when a subject makes a voluntary effort in a muscle (Nielsen, 1994) MMEPs are increased and become less variable in their amplitude presumably as a consequence of moving to a flatter region of the stimulus/response curve. The variability appears to be generated spontaneously and may be explained, at least in part, by differences in the state of relaxation of the muscles (Dimitrijevic *et al.*, 1992). As will be discussed below under facilitation, even mild muscle contraction will increase the amplitude and it is difficult to state whether the response was elicited with the muscle relaxed or not, especially in animals. Also, small alterations in the position of the magnetic stimulating coil over the surface of the cranium can result in large changes in variability of MMEP responses to TMS which may reflect fluctuations either in the proportion of available target corticospinal neurons close to threshold (Brasil-Neto *et al.*, 1992) or in the magnitude of the induced current under the coil (Kraus *et al.*, 1993). However, clamping the coil to the head in studies done by Ellaway and co-workers (1998), failed to affect the variability of MMEP-amplitude. The same authors suggested that at least some of the observed variability in amplitude is likely to result from spontaneous changes in the size of the descending volley from the cortex and hence reflect fluctuations in the excitability of the motor cortex.

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Latency is the interval between the delivery of the stimulus and the resulting response and reflects total motor conduction time from cortex to the target muscle. The latency may be measured to the onset of the action potential and is expressed in milliseconds (ms).

MMEP latency is affected by the size of the fibre, the abundance of myelin, and the number of synapses the impulse must cross (Sylvestre *et al.*, 1993). Physiological and clinical studies have focused on the shortest latency responses to provide an estimation of conduction velocities in the fastest descending spinal tracts. Much of the signal is dominated by conduction in a few large fibres. Those fastest descending tracts have been shown to connect monosynaptically to spinal motor cells (Cheney *et al.*, 1985; Porter *et al.*, 1987; Dimitrijevic *et al.*, 1992). However, there have been reports of longer latency responses being recorded from extensor and flexor carpi radialis muscles of the thoracic limbs and from tibialis anterior and triceps surae muscles of the pelvic limbs in healthy human subjects (Holmgren *et al.*, 1990). In addition to the fastest descending corticospinal fibres, there exist other indirect descending corticospinal pathways with fibres that terminate on the spinal interneurons in the intermediate zone of the spinal gray matter (Kuypers, 1981). These descending pathways to spinal motor cells via polysynaptic networks may mediate MMEPs with longer latencies.

One single cortical stimulation is able to produce multiple descending volleys in the pyramidal tract (Hess *et al.*, 1987a). Both spatial and temporal summation of impulses reaching the spinal motoneuron are necessary before it fires; therefore, reduction in the descending volley due to conduction block in some fibres or to loss of the fastest conducting fibres by degeneration and use of slower ones will lead to delay in excitation of the anterior horn cell, resulting in lengthening of latency.

The ***configuration*** of the MMEPs evoked in the muscles of the hand is in most instances bi- or triphasic (Maertens de Noordhout, 1998). A polyphasic configuration (more than five phases) has to be considered as abnormal in those muscles, whereas a polyphasic configuration is more frequently seen with MMEPs evoked in more proximal muscles and muscles of the leg, even in normal subjects.

In children however, MMEPs are generally polyphasic in early childhood and gradually become triphasic, reaching adult levels at the age of 13 years (Nezu *et al.*, 1997).

Researchers also describe an influence of the stimulation intensity on the configuration of MMEPs. At just suprathreshold levels, cortical stimulation produces EMG responses which are generally quite simple and comparable with those following stimulation of peripheral nerves. However, at higher intensities, the cortical responses (whether after electrical or

magnetic stimulation) become polyphasic, due to the multiple descending volleys set up by moderate to high levels of cortical stimulation. Motor units may fire on receipt of any one of several EPSPs (excitatory postsynaptic potentials) which these volleys release. This gives rise to an asynchronous activation of motor units in muscle. The EMG responses from each unit interfere, reducing the maximal amplitude of response and increasing its duration (Rothwell *et al.*, 1991).

The typical waveform of a MMEP recorded in the cranial tibial muscle of a horse, with latency and amplitude parameters identified, is shown in figure 4.

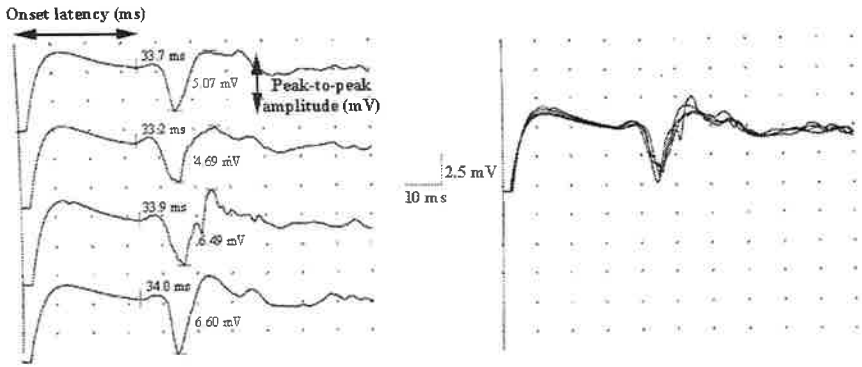

Figure 4. MMEPs recorded in the cranial tibial muscle of a 4-year-old mare (height at withers 151 cm) after transcranial magnetic stimulation. Stimulation started at the beginning of the sweep (vertical line). Onset latency and peak-to-peak amplitude of each recording is marked. On the right, the four superimposed potentials are presented.

2.6. Factors influencing the latency and amplitude of MMEPs

2.6.1. Effect of voluntary contraction: facilitation

Slight voluntary contraction of the target muscles (Fig. 5) shortens the onset latency, lowers the threshold, and increases the amplitude (Barker *et al.*, 1986; 1987; Hess *et al.*, 1987b; Rothwell *et al.*, 1987; Berardelli *et al.*, 1990; Thompson *et al.*, 1991; Di Lazzaro *et al.*, 1998a). The underlying mechanisms for facilitation are not entirely understood. Some researchers suggest that the effect would be caused by changes in cortical excitability: voluntary contraction increased the size and number of descending volleys evoked by a given stimulus. In the voluntary contraction state, the summation of descending voluntary impulses from cortical areas, afferent impulses from muscle spindles and descending potentials

secondary to magnetic brain stimulation can activate the spinal motor neurons earlier than under resting conditions and shorten the MMEP onset latency (Claus *et al.* 1988; Rossini *et al.*, 1987a;b; Kaneko *et al.*, 1996; Di Lazzaro *et al.*, 1998a). Experiments done by Kaneko and co-workers (1996) demonstrated that the shortening of latency can range from 2 to 3.5 ms, and that the amplitude during voluntary contraction can be increased to 150-500% of that recorded during the resting state. They also showed, by recording the evoked responses at the cervical epidural space and at the muscle simultaneously, that the shortened latency and increased amplitude of the MMEPs during voluntary contraction originated more in changes in spinal than supraspinal (cortical) excitability. Presumably the effect of voluntary activation raises the resting potential of spinal motoneurons closer to their discharge threshold and therefore the initial descending volley is capable of discharging at least some motoneurons. This can explain the predominant role of spinal excitability (Di Lazzaro *et al.*, 1998a). Furthermore response latency shortening during voluntary contraction is likely to reflect application of the size principle of Henneman: the first corticomotoneuron cells to fire during a voluntary contraction are those that conduct most slowly and with increasing contraction, larger, faster conducting spinal neurons are recruited (Henneman *et al.*, 1965), thus shortening the onset latency (Weber & Eisen, 2002).

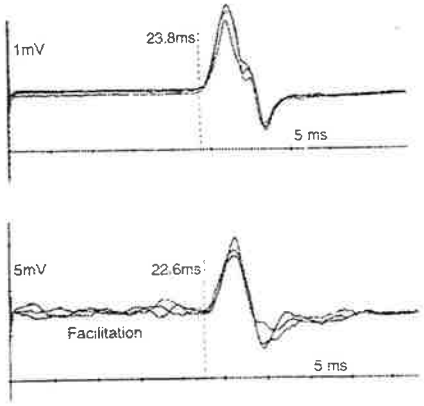

Figure 5. Waveforms recorded over the left first dorsal interosseus (in a human being) after stimulation of the motor cortex with 90mm circular coil placed centrally on the vertex – three superimposed responses each. Responses in the lower tracings are facilitated by slight pre-activation of the target muscle. Note the different scale for the two tracings (with permission of R. Jalinous; Magstim Ltd).

Facilitation can be used when muscle responses are small because of central nervous system pathology (e.g. spinal cord trauma, multiple sclerosis,...). In this situation, the ongoing muscle activity of a facilitatory contraction may make precise measurement of onset latency impossible. Therefore complete relaxation of the muscle, but moderate contraction of the same muscle on the opposite side (contralateral facilitation), will result in the same degree of latency reduction and amplitude increase (Hess *et al.*, 1987c).

In animals, no data are available in the literature concerning the influence of facilitation on MMEPs. Many animal studies are carried out under general anaesthesia where a conscious muscle contraction is impossible to perform. However, some awake and sedated animals do contract their muscles during transcranial magnetic stimulation, but such facilitation is not controllable.

2.6.2. Effect of coil position

In the majority of subjects, the lowest threshold for stimulation occurs when induced current in the brain (which is the opposite direction to that of the coil) flows from posterior to anterior at an angle approximately perpendicular to the line of the central sulcus (Mills *et al.*, 1992).

As a general rule, when the coil was placed over the scalp, maximal and quicker responses could be elicited on the right if the stimulating current in the coil flows counterclockwise. To obtain the largest and quickest response on the left the current flow had to be reversed. The reasons for this difference are poorly understood but presumably reflect differences in the direction and distribution of current flow within the brain produced by the two orientations of the coil. These findings were most obvious at threshold. However, during voluntary contraction, about 20% of the MMEPs were maximal (amplitudes) on the left when the current flowed counterclockwise and vice-versa (Furby *et al.*, 1992).

The most stable responses were obtained from muscles of the upper, or lower limb, with the round coil centered on the vertex, or slightly anterior to the vertex, respectively (Terao *et al.*, 1994, Kaneko *et al.*, 1997). For a given stimulus intensity, responses always were largest in distal, particularly hand muscles (Thompson *et al.* 1989).

In veterinary medicine however, no influence of the coil current could be observed in dogs (Van Ham *et al.*, 1994) and horses (Nollet *et al.*, 2003b).

2.6.3. Effect of age, height and gender

Latency time is strongly correlated with height. No statistical difference was reported between gender for threshold and amplitude. The statistical difference found between gender for latency seems to result from the correlation between latency and height (Barker *et al.*, 1987; Chu, 1989; Furby *et al.*, 1992). It is concluded that height is an important variable in defining the normal MMEP. Most studies however have concentrated on the 20 to 50 year old range and there has been no comprehensive description of the normative results of hand and leg MMEPs over a wide range of ages in a substantial sample of male and female subjects. Some authors described a linear increase in latency with increasing age, but with a very weak correlation (Eisen *et al.*, 1990b). Tobimatsu and colleagues (1998), who examined persons from 19 to 74 years old, observed a significant gender difference in the MMEP latencies of the leg (in males longer onset latency than in females), but not in those of the hand. Both height and age had a significant positive effect on the leg MMEP latencies. An interesting finding is the different effect that was seen regarding age on the hand and leg MMEP latency. In the cortical motor area, 75% or more of Betz cells showed age-related morphological changes, while changes of small pyramidal neurons were less severe than those of Betz cells (Scheibel *et al.*, 1977). The study by Lassek (1940) showed that 75% of Betz cells were in the motor area supplying the leg, 18% in the arm region and only 7% in the hand area, despite the dedication of far more extensive cortical areas to the head and arm than to the leg. In young children, latency of the MMEP, however, does not attain adult values until about age 11 years (Koh & Eyre, 1988). These results thus suggest that physical variables are important in defining normal MMEPs, especially in the lower limbs.

3. INDICATIONS

3.1. Magnetic stimulation in humans

3.1.1. Diagnosis

A diagnostic test should be sufficiently accurate and should have specified clinical indications. Several authors (Eisen & Shtybel, 1990a; Di Lazzaro *et al.*, 1999; Mills, 1999) reported that the overal accuracy of MMEPs is high. Such accuracy and thus the clinical utility makes MMEPs efficacious in evaluating corticospinal tract function. Corticospinal tract function can be assessed with reliability by a rigourous clinical examination, therefore, only if the test can demonstrate abnormalities not revealed by an accurate clinical evaluation will it

assume a definite clinical value. The ability of MMEPs in documenting a subclinical involvement of central motor pathways has been documented by Di Lazzaro *et al.* (1999) who found a high rate of subclinical abnormalities in motor neuron diseases, muscle disorders, multiple sclerosis and spinal cord diseases.

In patients with spinal cord disorders, MMEPs may be useful in demonstrating the site of spinal cord lesions and also in monitoring a disease, as for example in cervical spondylotic myelopathy. In such conditions, serial MMEP recordings might be useful in ascertaining progressive forms and for selection of patients who may benefit from surgical treatment.

Multiple reports (Rossini *et al.*, 1985; Snooks and Swash, 1985, Eisen and Shtybel, 1990a) suggest that the technique is more sensitive than other evoked potentials in increasing the accuracy of diagnosis of multiple sclerosis, but this should not imply there is specificity.

In Parkinson's disease (Eisen & Shtybel, 1990a, Mills, 1999) MMEP latencies have been shown to be normal, although sometimes increased amplitudes have been reported (Eisen & Shtybel, 1990a).

Presently, electrophysiology is the only means of confirming suspected amyotrophic lateral sclerosis (ALS) (Eisen, 2001). In addition to electromyography performed to determine lower motor neuron involvement, the introduction of transcranial magnetic stimulation has allowed the assessment of central motor pathway function (Urban *et al.*, 2001). A common finding is that TMS fails to evoke a muscle response or evokes a response with a reduced amplitude, despite high intensity stimulation (Mills, 1999). The MMEP latency is only modestly prolonged (Mills, 1999). This can be attributed to the degeneration of the corticomotor cells or reduced firing frequency in corticospinal fibres with consequent impaired temporal summation at the motoneuron (Mills 1995). Moreover, it provides a sensitive means for the assessment and monitoring of upper motor neuron involvement in motor neuron disease (Triggs *et al.*, 1999).

3.1.2. Prognosis

Especially in human medicine, it is worthwile to have early indicators of significant motor recovery, especially for the patient's motivation. Furthermore, obtaining early and reliable indications of the final degree of motor function recovery would also be useful for optimizing rehabilitation strategies and evaluating their costs. The quality of motor recovery after stroke is difficult to predict on the basis of only clinical data. Since 1989, studies have been conducted to assess the value of MMEPs in patients with stroke. The application of TMS has yielded contradictory results (MacDonell *et al.*, 1989; Arac *et al.*, 1994; Timmerhuis *et al.*, 1996; Escudero *et al.*, 1998), probably because of the great variability of stroke patients included and differences in the methodologies used. However, most authors agree that the evoked potentials measured in the acute stage had useful predictive value (Timmerhuis *et al.*, 1996; Rapisarda *et al.*, 1996; Pennisi *et al.*, 1999). For instance, Pennisi *et al.* (1999) reported that the absence of responses to TMS in the first 48 h is predictive of absent or very poor functional hand motor recovery.

The use of TMS as a predictive test in patients with traumatic cervical spinal cord injury does not provide more useful information regarding motor recovery than the physical examination, but may be of benefit in uncooperative or incomprehensive patients (McKay *et al.*, 1997; Kirschblum & O'Connor, 1998). Meyer and Zentner (1992) reported that TMS is a valuable diagnostic tool for detection of lesions along the spinal cord, but found no linear correlation between the clinical motor status and the electrophysiological changes.

For facial nerve outcome after acoustic neuroma surgery, Wedekind and colleagues (2000) reported no prognostic significance of preoperative TMS.

3.1.3. Monitoring

There are two clinical indications for monitoring neurological function during surgical procedures: to detect inadvertent damage early when the resulting dysfunction might still be reversible, and to guide the surgeon with regard to the extent of safe operative resection (e.g. in tumor resection) or curve correction (e.g. in scoliosis surgery). For these purposes, the ideal neurophysiological technique should (a) have high sensitivity and specificity, (b) provide real-time feedback, (c) not intrude physically into the operative field, (d) not hinder access for the anesthetist, (e) not prolong the operation unduly, (f) not be subject to artifactual changes that could be misinterpreted as incipient or actual neural dysfunction (i.e. there should be a low incidence of "false-positives") and (g) be equally useful in patients with and without preexisting neurological deficits.

Since the introduction of TMS, MMEPs as well as SSEPs are recorded routinely during major spinal surgery in many centres, for more complete information on both the descending corticomotoneuron tracts and ascending sensory pathways (Shields *et al.*, 1989; Bartley *et al.*, 2002, Aglio *et al.*, 2002). However, in animal experiments, motor pathways have been reported to be more susceptible than sensory pathways to spinal cord trauma and ischaemia (Machida *et al.*, 1988; Fehlings *et al.*, 1989; Kai *et al.*, 1995); therefore, identifiable motor dysfunction might be expected to precede sensory dysfunction and a technique to monitor conduction in the corticospinal pathways (e.g. TMS) is thus to be recommended.

In addition to spinal cord monitoring during spinal operations, other potential roles for the technique may be to monitor sciatic nerve function during hip replacement (Schoenfeldt *et al.*, 1987), peripheral nerve and brachial plexus integrity during hand surgery (Kaplan *et al.*, 1984; Schmid *et al.*, 1990), facial nerve function during surgery (Hatem *et al.*, 2001) and spinal cord status during aortic surgery (Friedman *et al.*, 1987).

3.1.4. Therapy

Transcranial magnetic stimulation is able to modify neuronal activity locally and at distant sites when delivered in a series of trains of pulses: repetitive TMS (rTMS). Data from stimulation of the motor cortex suggests that the type of effect on the excitability of the cortical network depends on the frequency of stimulation (Chen *et al.*, 1997; Berardelli *et al.*, 1998; Pascual-Leone *et al.*, 1998). Recent studies with rTMS for the treatment of psychiatric disorders (depression, schizophrenia, etc.)(Feinsod *et al.*, 1998; Klein *et al.*, 1999; Hasey, 2001) and motor disorders (Parkinson's disease, task-related dystonia (e.g. writer's cramp), tic disorders and epilepsy) appear promising for the future and the authors concluded that the technique may possess tremendous potential as a treatment for these disorders (Hallett, 1998; Tergau *et al.*, 1999; Shimamoto *et al.*, 2001; Wassermann & Lisanby, 2001). However, much research is still needed to optimise the technical considerations, such as stimulus frequency, intensity, and magnetic coil position, and to investigate the neurophysiological changes that result.

3.2. Transcranial magnetic stimulation in animals

While routinely used in humans, application of TMS in other species is rare and few data are available on the characteristics of animal MMEPs. In recent years rodent studies have elucidated TMS mechanisms (Wang *et al.*, 1996), demonstrated its safety (Russell *et al.*, 1994; Post *et al.*, 1999) and have demonstrated the effect that spinal cord injury (Magnuson *et al.*, 1999) and anaesthesia (Ebert & Ziemann, 1999, Van Ham *et al.*, 1995, 1996a, 1996b) have on the MMEPs. Mainly because magnetic transcranial stimulation is not invasive and painless, clinical studies on dogs and cats have increased over the last few years. In 1987 (Konrad *et al.*, 1987) MMEPs were described in dogs and the influence of ischaemia of the spinal cord on the responses was examined. Heckmann and colleagues (1989) described the technique in awake dogs and in dogs awaking from general anaesthesia. In 1993, Sylvestre and co-workers reported TMS for assessing spinal cord integrity in dogs with thoracolumbar

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intervertebral disc disease. They concluded that MMEPs were very sensitive to lesions of the spinal cord in dogs, as indicated by the significant changes in the waves in patients with mild or no neurological deficits and in the loss of response in dogs that still demonstrated purposeful movement. There was a significant attenuation of the amplitudes in all clinical cases, even if the dogs demonstrated back pain alone. Significantly prolonged latencies were associated with neurological deficits. Even when clinical signs indicated one side of the spinal cord was affected by the extruded disc material, significant lateralization was not shown by TMS. This is in contrast to the results published by Owen *et al.* (1988) where lateralization of MMEPs were reported. Recently, Poma and co-workers (2002) noted the potential of TMS to become a useful screening tool for cervical spinal cord disease in large-breed dogs. Impairment of the functional integrity of the spinal cord, regardless of the severity of the neurological signs, was revealed by TMS. Of special interest is the group of 7 dogs with neck pain only and no other neurological abnormalities, wherein all MMEP latencies and peak-to-peak amplitudes were significantly abnormal and in 5 of 7 dogs undergoing myelopgraphy cervical spinal cord lesions were confirmed. Despite the lack of a significant difference among dogs in the three neurological categories (neck pain alone; ataxia all 4 limbs; non-ambulatory), a linear association was observed when the mean latencies and peak-to-peak amplitudes of all affected dogs were correlated with severity of neurological deficits.

The effects of different forms of anaesthesia and sedation on MMEPs elicitated in dogs have been described (Sylvestre *et al.*,1992; Young *et al.*, 1994; Van Ham *et al.*, 1994, 1995, 1996a, 1996b). The sedative combination of droperidol and fentanyl was reported to be the best to record MMEPs with shortest onset latency and highest amplitude (Van Ham *et al.*, 1994). Sylvestre and others (1992) reported that TMS could be accomplished in normal dogs sedated with oxymorphone, midazolam or acepromazine and did not find a significant difference between the three drugs.

At surgical levels of anaesthesia, Van Ham and co-workers (1995, 1996a, 1996b) only could elicit MMEPs when using sufentanil in combination with midazolam or either fentanyl or sufentanil with nitrous oxide. No MMEPs were obtained when propofol, thiopental, diazepam and ketamine, and halothane were used in dogs. Young and colleagues (1994) demonstrated that MMEPs can be reliably recorded under methohexital anesthesia. In horses, the sedative combination of detomidine and buprenorphine (Nollet *et al.*, 2003a) has no significant influence on MMEP recordings.

The effects of coil orientation of a figure-of-eight coil was described in rats and cats in 1998 (Kamida *et al.*, 1998; Nakatoh *et al.*, 1998). In 2001, the rodent motor evoked potentials

resulting from TMS were characterized and a methodology for reproducible assessment of motor excitability was developed in the rat (Luft *et al.*, 2001). In 1996, Mayhew and Washbourne reported that TMS was possible in unanaesthetized or minimally sedated (acetylpromazine) normal ponies. In 2002, the usefulness of MMEPs in horses with cervical cord lesions was described (Nollet *et al.*, 2002).

a b c

Figure 6.
a. Position of the magnetic coil on the forehead of the horse.
b. Position of the needle electrodes in the forelimb.
c. Electromyography machine (left) recording the magnetic motor evoked potentials elicited by the magnetic stimulator (right).

These few reports are already promising concerning the diagnostic value of TMS in veterinary medicine. However, an extensive evaluation of the technique will certainly expand the diagnostic impact of magnetic stimulation in neurological veterinary practice in the near future. TMS may become an additional diagnostic tool, especially for revealing subclinical lesions of the central motor pathways in several neurological disorders in dogs and horses or for investigating complaints of back pain in performing horses. Finally, the technique may open new avenues for assessing central motor pathway function in disorders such as equine motor neuron disease.

Studying the effect of TMS as a therapeutic tool would also be very interesting. Neurostimulation therapy for epilepsy in humans is growing in popularity. Reduced seizure frequency has been reported in human patients treated with repetitive transcranial magnetic stimulation at varying low-frequency stimulus rates. For the moment, no such data are available in veterinary patients. Epilepsy is very rare in horses but in dogs the condition is often seen. To study the effect of rTMS would be indeed interesting, however, safety studies,

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the effect of high and low-frequency stimulus rates and different parameters (stimulation frequency, stimulation period, coil position) have to be tested in each of these animal species.

CONCLUSIONS

In order to use transcranial magnetic stimulation in horses, we have described the basic theory and some practical applications of the technique in human beings and some animal models. We can conclude that the technique will provide reliable information about the functional integrity and conduction properties of the corticospinal tracts and motor control in animals and hope that it will be a complementary diagnostic test in the neurological examination, especially in the horse, where the information from clinical examination, as well as other tests are limited due to the animal's size and often poor cooperation.

An application of the technique in horses with cervical cord lesions is already published (Nollet *et al.*, 2002) and preliminary results in other neurological conditions look promising.

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CHAPTER 2

SCIENTIFIC AIMS

In horses, the examination of the nervous system is mainly restricted to a clinical neurological examination. The results of the neurological examination are not always easy to interpret (e.g. spinal reflexes, pain perception) and some tests are difficult or sometimes dangerous to perform in ataxic horses. In diseases of the spinal cord at the very best an idea about the localisation of the lesion can be obtained. Furthermore, ancillary diagnostic tests only give limited additional information. Cervical radiographs can be contributory to the diagnosis but good visualization of more caudal vertebrae is somewhat difficult in adult, large, well-muscled horses. In that region, scintigraphy can highlight suspected vertebral lesions. Myelography can be useful to define spinal cord compression, but a general anaesthesia is necessary and this makes the procedure more risky. Electromyography tests the function of the lower motor neurons and muscle and is therefore of minor importance in upper motor neuron disorders. Routine laboratory blood work and cerebrospinal fluid analysis, give limited indications as to the nature of any damage to the nervous tissue itself. In conclusion, there are no specific ancillary diagnostic tests that evaluate objectively the integrity and function of the spinal cord in alive horses.

Transcranial magnetic stimulation has been used as a clinical diagnostic tool for several years in man and to a lesser extent in small animals. In these species it is described as a very sensitive and accurate tool in detecting lesions along the descending motor tracts in the spinal cord. One of the major advantages, when compared to electrical stimulation, is that magnetic stimulation is painless and thus causes minor discomfort. Therefore, introduction of this technique would be interesting in horses for two reasons. First, it would be possible to objectively measure the conduction along the descending motor tracts and thus to have an idea about the functional integrity of the spinal cord. Second, this painless technique could be used in standing horses. General anaesthesia is not advisable in ataxic horses because of risks while attempting to stand after the procedure.

The purpose of the present study was to examine the usefulness of the technique of transcranial magnetic stimulation in the horse. The objectives were the following:

1. to determine whether transcranial magnetic stimulation can evoke magnetic motor potentials in normal horses, and thus if the technique can be used in horses
2. if it can be used in horses, to standardize transcranial magnetic stimulation in normal horses in order to use it as a complementary diagnostic tool in clinic. In human

patients the stimulus is described as producing no more than a mild discomfort induced by the evoked muscle contraction and a mild local tapping. When applying this technique to horses however, mild sedation is certainly justified to avoid possible adverse reactions, as fear and excitation. Therefore the influence of a sedative combination on the magnetic motor evoked potentials (MMEPs) will be evaluated. Also the influence of coil position, current direction and stimulation intensity on the MMEPs will be tested.

3. to determine reference values for the measured parameters (onset latency and peak-to-peak amplitude) in order to formulate a 95% prediction interval by which values obtained in clinical patients can be determined as normal or abnormal. Subsequently, any influences that variations in height, weight, age and gender of the horse might have on these values will be determined.
4. to evaluate the usefulness of transcranial magnetic stimulation in clinical patients with spinal cord lesions. Therefore two groups of horses will be examined: a group of horses suspected of having cervical spinal cord lesions and another group suffering from hind limb ataxia.

CHAPTER 3

STANDARDIZATION OF THE TECHNIQUE IN A GROUP OF REFERENCE HORSES

CHAPTER 3

Part 1.

Influence of detomidine and buprenorphine on magnetic motor evoked potentials

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SUMMARY

Horses need to be sedated before they are investigated by transcranial magnetic stimulation because of the mild discomfort induced by the evoked muscle contraction and the noise of stimulation. This paper describes the influence of a combination of detomidine (10 μg/kg of bwt) and a low dose of buprenorphine (2.4 μg/kg of bwt) on the onset latency and peak-to-peak amplitude of magnetic motor evoked potentials in normal horses. There were no significant differences between measurements of these parameters made before sedation and measurements made 10 and 30 minutes after the drugs were administered. We can conclude that the combination of detomidine and a low dose of buprenorphine can be used to facilitate obtaining magnetic motor evoked potentials in horses.

INTRODUCTION

Transcranial magnetic stimulation (TMS) was introduced by Barker and others (1985) as a non-invasive and almost painless method for investigating motor function in healthy people and patients with neurological disorders (Barker *et al.*, 1987; Eisen and Shtybel, 1990; Rossini and Rossi, 1998, Di Lazzaro *et al.*, 1999). In animals, the technique is not used routinely but several clinical studies on dogs and cats have been published (Heckman *et al.*, 1989; Van Ham *et al.*, 1994, 1995, 1996a, 1996b; Young *et al.*, 1994, Nakatoh *et al.*, 1998), and it has been reported that the technique is sensitive for detecting lesions along the descending motor tracts in small animals (Sylvestre *et al.*, 1993). The authors have described the usefulness of the technique in horses with cervical cord lesions (Nollet *et al.*, 2002).

The technique uses a large pulse of magnetic field which induces electric currents within the body. The magnetic field is generated by passing an electric current through a magnetic coil placed above the scalp. This changing magnetic field momentarily depolarise the nervous system, for example, the motor cortex of the brain, by creating an induced current. The ensuing magnetic motor-evoked potentials (MMEPs) can be recorded in different muscles.

The safety of any new procedure, especially if it involves the brain, is an important consideration. Many safety studies have revealed no direct evidence of any increased risk in specific groups, such as patients with heart disease, cardiac pacemakers or seizures, and the absence of side-effects in hundreds of patients and normal subjects provides further evidence of the safety of the technique (Rothwell *et al.*, 1991).

The currents induced on the scalp by magnetic stimulation are much less perceptible than those produced by transcranial electric stimulation. Magnetic stimulation is therefore assumed to be almost painless, and recordings can be made in conscious, awake and relaxed people (Rothwell, 1997). In human patients the stimulus produces no more than a mild discomfort induced by the evoked muscle contraction and a mild local 'tapping' sensation (Barker *et al.*, 1987). In horses, however, mild sedation would certainly be justified to avoid possible adverse reactions of fear or excitation. However, it has been reported that various sedatives and anaesthetics can have a depressive influence on MMEPs (Ghaly *et al.*, 1989; Loughman *et al.*, 1989; Van Ham *et al.*, 1994, 1995, 1996a, 1996b). This paper describes the results of an investigation of the influence of the commonly used sedative combination of detomidine, an alpha-2-adrenergic agonist, and buprenorphine, a long-acting opioid analgesic, on the characteristics of the MMEPs recorded in horses.

MATERIAL AND METHODS

The experimental protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine, University of Ghent, Belgium (reference 18/2000). Six horses (one stallion, one gelding and four mares) with a mean (sd) body weight of 453.7 (36.2) kg (range from 440 to 525 kg), an average height at the withers of 155.8 (4.5) cm (range from 152 to 163 cm) and aged from two-and-a-half to nine years (mean: 4.9 years) were stimulated transcranially with a Magstim 200 (Novametrix, UK), using a circular coil 70 mm in external diameter, which generated a maximal magnetic field of approximately 4 Tesla. The stimulus intensity was 100 per cent of the maximal output. The coil was centred over the forehead of the horse with the examiner standing in front of it. To activate each hemisphere preferentially, a clockwise inducing current flow was used to stimulate the right motor cortex, and an anticlockwise flow to stimulate the left motor cortex (Day *et al.*, 1990). Electromyographic responses (MMEPs) were recorded bilaterally from disposable monopolar needle electrodes (TECA Corporation) in the extensor carpi radialis muscle and the cranial tibial muscle; two small, welldelineated and most distal muscles of the front and hindlimbs. The active electrode was inserted in the middle of the muscle belly and the reference electrode was placed subcutaneously respectively to the lateral side of the radial tuberosity of the forelimb and the lateral malleolus of the tibia of the hind limb. A ground electrode (alligator clip) was attached to the forelimb in the region of the elbow and to the hindlimb in the region of the groin. The stimulator triggered the sweep of a standard electromyogram (EMG) machine (Medelec Sapphire; Medelec Ltd., Old Woking, Surrey, England), so that the latency between the stimulus and the onset of the response could be measured. The time base was 100 ms with a gain ranging from 100 µV to 5 mV per division. Bandpass filter settings were 20 Hz to 3 kHz and only single stimuli were applied. Four potentials were obtained and superimposed from each recording site, to compensate for the within-test variation. Onset latency (ms) was measured as the shortest distance between the trigger point and the take-off of the initial phase (negative or positive). The amplitude of the MMEP (mV) was measured between the two largest peaks of opposite polarity (peak-to-peak amplitude).

The reference values were obtained with the horses unsedated. The animals were sedated with a combination of detomidine (Domosedan®, Pfizer, Belgium) at 10 µg/kg of bodyweight, and buprenorphine (Temgesic®, Schering-Plough, Belgium) at 2.4 µg/kg of bodyweight administered intravenously. The same stimulation procedure was applied 10 and 30 minutes after administration of the drugs.

Statistical analysis

The effects of detomidine and buprenorphine on the MMEP were analysed by comparing their latency and amplitude before the horses were sedated, with the results obtained 10 and 30 minutes after sedation, by a linear mixed-effects models (S-plus, Cambridge, MA, USA) with the horse as a random variable. The recording position (left or right front or hindlimb) was also included in the model.

RESULTS

A motor response was obtained after each magnetic transcranial stimulation in all of the horses, whether they were sedated or not. Four of the unsedated horses reacted by kicking when the needle electrodes were inserted, especially in the cranial tibial muscle and all the unsedated horses showed signs of anxiety after they were stimulated.

There were no statistically significant differences between the right and left responses in terms of latency (P=0.87) or amplitude (P=0.44) and Table 1 summarizes the mean (sd) values of latency and amplitude for the front limbs and the hind limbs found of the six horses before and after they were sedated.

	Before sedation	10 minutes after sedation	30 minutes after sedation	P value*
Onset latency, front limb (ms)	20.9 (0.89)	21.1 (0.89)	21.1 (0.98)	0.66
Onset latency, hind limb (ms)	32.6 (1.97)	32.8 (1.76)	32.6 (2.09)	
Amplitude, front limb (mV)	10.5 (4.28)	10.3 (5.27)	10.4 (4.48)	0.98
Amplitude, hind limb (mV)	7.2 (4.19)	7.5 (3.83)	7.4 (3.65)	

Table 1. Mean (sd) onset latencies and amplitudes of the motor evoked potentials after transcranial magnetic stimulation in six horses before and after sedation with detomidine and buprenorphine.

*significance level of the effect of time

As expected there was a significant difference (P<0.001) between the front limbs and hind limbs, with the front limbs having shorter latencies and larger MMEPs. There were no significant differences between the measurements made before and after the horses were sedated (Table1), indicating that the administration of detomidine and buprenorphine did not influence the latency time and amplitude of the MMEPs.

DISCUSSION

Although transcranial magnetic stimulation is painless, it can cause minor discomfort, mainly as a result of the muscle contractions evoked. This muscular reaction can sometimes be quite intense especially with a large stimulus. The noise generated by the magnetic field equipment also seems to cause some horses to show signs of anxiety. Both of these effects can result in the horses reacting, or even becoming agitated or panicking, and this was observed in the unsedated horses. The aims of the study were to assess the efficacy of a combination of sedatives in reducing the discomfort and fear, and to assess whether it would interfere with the test procedure. Little information is available in either human beings or animals about the depressive effect of sedative drugs on MMEPs. In human patients TMS is usually applied when they are awake and relaxed. For peri-operative monitoring, to detect early inadvertent neurological damage, the potentially depressive effect of anaesthetics or sedatives is avoided by the use of special but expensive magnetic stimulators that produce pulse trains of up to four pulses (Lin and Chen, 1998). Van Ham and others (1994, 1995, 1996a, 1996b) described the negative effect of heavy sedation and anaesthesia in dogs, in which the combination of droperidol and fentanyl was reported to be the best with which to record MMEPs with shortest onset latency and highest amplitude. At surgical levels of anaesthesia MMEPs could be recorded in dogs only when using sufentanil in combination with midazolam, or either fentanyl or sufentanil with nitrous oxide. No MMEPs were observed when propofol, thiopental, diazepam and ketamine, or halothane were used in dogs. Sylvestre and others (1992) reported that TMS could be used in normal dogs sedated with oxymorphone, midazolam or acepromazine; no significant difference was observed between the three drugs, but no comparison was made with pre-sedation values. In ponies, Mayhew and Washbourne (1996) found no significant differences between unsedated ponies and ponies given approximately 5 mg acetylpromazine intravenously. However, in the present study, preliminary tests showed that this tranquiliser at a dose of 0.1 mg/kg intravenously (Marroum *et al.*, 1994) was not sufficient to eliminate anxiety reactions of all of the horses, even when

they were apparently fully sedated. This difference may be because in general, horses react more anxiously and more violently than ponies to TMS when they are awake. In these preliminary trials, offering hay to unsedated ponies prevented them showing defensive reactions during TMS, but the method did not work with horses. However, Nolan and Hall (1984) found that ponies sedated with lower doses of acepromazine (0.05 mg/kg) in combination with buprenorphine (0.006 mg/kg) could be aroused by noise.

The combination of detomidine and buprenorphine was tested because detomidine is commonly used in general equine practice as a safe and efficient sedative, either alone or in combination with opiates, for diagnostic or small surgical procedures. A dose ranging from 8 to 20 μg/kg given intravenously produces adequate sedation in most horses, although some fail to respond even when higher doses are administered (Alitalo, 1986; Ricketts, 1986). These animals appear to be deeply sedated but still respond to external stimuli (Clarke and Taylor, 1986). The addition of an opiate, such as buprenorphine, enhances and prolongs the sedation induced by detomidine and decreases the response of the animal to external stimuli (Clarke and Paton, 1988). Similarly, in the present study, the addition of the analgesic buprenorphine eliminated the defensive reactions of the horses. The administration of buprenorphine alone can induce excitation in horses, and the combination with an alpha-2-adrenergic agonists is therefore recommended. In combination with a sedative agent, buprenorphine does not seem to induce severe respiratory depression or adverse cardiovascular effects in clinically normal horses or those with chronic obstructive pulmonary disease (COPD) (Szoke *et al.*, 1998). Finally, there are other opioids that can be used in combination with detomidine, including methadone, morphine and butorphanol.

Detomidine exerts its effect mainly through the stimulation of alpha-2-adrenoceptors. Stimulation of these receptors in the brain results in a decrease in the activity of ascending neural projections to the cerebral cortex and limbic system (Martin *et al.*, 1984; Stenberg, 1986). In human beings there are high densities of alpha-2-adrenoceptors in the locus coeruleus, dorsal motor nucleus of the vagus nerve and the intermediolateral column and substantia gelatinosa of the spinal cord (Unnerstall *et al.*, 1984; Probst *et al.*, 1985). It is accepted that the locus coeruleus is the major site of action for the typical sedative effects of alpha-2-adrenergic agonists. The alpha-2-adrenergic agonists also have a potent analgesic effect which results from both cerebral and spinal effects, possibly in part mediated by serotonin and the descending endogeneous analgesia system. Their characteristic muscle relaxant effects, inducing weakness and swaying, are most probably caused by a direct impairment of the release of excitatory amino acid from spinal interneurons and a concomitant

Chapter 3. Part 1. Influence detomidine and buprenorphine on MMEPs

inhibition of facilitatory coeruleo-spinal pathways, which are involved in the control of spinal cord activities (Coward, 1994; Abbruzzese, 2002).

Buprenorphine is an opioid that raises the pain threshold or decreases the perception of pain by interfering with nociceptive neural transmission centrally. Opioids produce analgesia by actions at several sites, but a major mechanism involves the activation of bulbospinal inhibitory, primarily serotonergic (Yaksh and Tyce, 1979; Mason, 1999) and noradrenergic (Tyce and Yaksh, 1981) pathways, which exert an inhibitory control over the dorsal horn, where the primary sensory neurons involved in pain sensation release predominantly substance P and glutamate. Buprenorphine is a long-acting opiate, whose analgesic action lasts from eight to 12 hours, and it has a relatively slow onset of action which reaches its full effect after 20 to 30 minutes (Thurmon et al, 1996b).

The MMEPs recorded 30 minutes after the administration of the sedative combination were not significantly different from those recorded either before or 10 minutes after it was administered. The optimal dose of buprenorphine for horses reported in the literature is 0.004 to 0.006 mg/kg administered intravenously (Thurmon et al, 1996b). Although a lower dose was used in this study, it was still adequate for the MMEP procedure to be carried out without severe reactions, even 10 minutes after its administration.

Opioids and alpha-2-adrenergic agonists are synergistic and induce both analgesia and sedation for different reasons. First, the alpha-2- and opioid receptors are situated inside similar regions of the brain and even on the same neurons. Secondly, these receptors are coupled to the same signal transducer, that is membrane-associated G proteins, and the signal transduction mechanism is linked to the same effector mechanism, the potassium channel (Thurmon *et al.*, 1996a, 1996b).

The absence of any measurable influence of this sedative combination on the MMEP recordings may partly be explained by the fact that the sites of action of these drugs are not directly involved in the motor tracts tested with TMS.

Another possible explanation is the effect of detomidine and buprenorphine on the neuroregulators. Detomidine has a mainly negative feedback effect on norepinephrine, and buprenorphine acts via norepinephrine and serotonin. Since the main neurotransmitter in the motor nerves is acetylcholine, it is questionable whether the administration of detomidine and buprenorphine would be expected to affect the function of the descending motor tracts (Thurmon *et al.*, 1996a).

CONCLUSION

The TMS technique can be applied to standing horses by using a sedative combination of detomidine and buprenorphine. This combination minimises any discomfort during recordings in normal horses and has been used successfully in horses with neurological signs (Nollet *et al.*, 2002). Its major advantages are the ease of working, the low cost, and the fact that general anaesthesia, which is generally necessary for TMS in small animals, can be avoided. Most general anaesthesia has a marked dose-dependent suppressive effect on the motor-evoked potentials in all species, and its use in ataxic horses might involve a significant risk when they attempt to stand as they recover.

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CHAPTER 3

Part 2.

Influence of coil current, coil position and stimulation intensity on magnetic motor evoked potentials

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SUMMARY

The influence of coil position on the peak-to-peak amplitude and onset latency of transcranial magnetic motor evoked potentials (MMEPs) in the extensor carpi radialis and cranial tibial muscles of horses was evaluated. Seven different stimulating coil positions were obtained by constructing a frame on the forehead. Two stimulation intensities (80% and 100% of maximal stimulator output) and two different coil currents (clockwise and counter-clockwise) were tested. For both recording sites MMEPs with the shortest onset latency and the largest peak-to-peak amplitude were detected when the coil was placed over the midline of the forehead. There was no significant difference between left and right side recordings. The direction of the current flow in the coil had no influence on the onset latency of the MMEPs.

INTRODUCTION

Fritsch and Hitzig (1870) were the first to show that electrical stimulation of different regions of the human cortex resulted in movement of different parts of the body. A striking correspondence between the topography of the skeletal muscles and the arrangement of the regions in the brain cortex, that activate these muscles, has been observed in subsequent studies in humans and animals (Ferrier, 1875; Gualtierotti & Paterson, 1954; Merton & Morton, 1980; Levy *et al.*, 1984a; 1984b) and is referred to as somatotopic organisation.

In recent years, transcranial magnetic stimulation (TMS) has provided a non-invasive and painless means for investigating the topography of the human corticospinal tracts (Meyer *et al.*, 1991; Thickbroom *et al.*, 1998). Transcranial magnetic stimulation involves the passage of a brief current pulse through an insulated coil which in turn induces a brief electromagnetic pulse (Barker *et al.*, 1985; 1987). The intensity of the pulsed field attenuates only with distance from the coil (Epstein *et al.*, 1990; Jalinous, 1991). When the coil is placed on the scalp, the electromagnetic pulse is capable of inducing an eddy current within the brain that leads to excitation of the descending motor tracts (Amassian *et al.*, 1990; Roth *et al.*, 1991). The evoked responses in the muscles can be used to assess the functional integrity of spinal cord motor pathways. For mapping studies, the magnetic stimulator is systematically moved over the scalp and after stimulation with a standard stimulus intensity the MMEPs are measured. This will produce a map of MMEPs with variable amplitude and usually the amplitude will be highest in the centre of the map and taper off to the edges. The site of maximal amplitude can be called 'the optimal position' (Rothwell *et al.*, 1999). In human medicine optimal responses are elicited in hand muscles with the coil centered on the vertex, while for leg muscle responses the coil is preferentially placed more anteriorly (Ingram *et al.*, 1988; Hess *et al.*, 1990).

In horses, no such information is available. The purpose of the present study was to look for optimal stimulation sites and to assess the importance of factors, such as current direction and stimulation intensity, for transcranial magnetic stimulation in the horse.

MATERIAL AND METHODS

The experimental protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine, University of Ghent, Belgium (reference 18/2000). Seven horses (six mares and one stallion) with an average body weight of 485 ± 54.7 kg, an average age of 6.4 ± 1.5 years and an average height of 152.7 ± 4.8 cm were sedated with detomidine (1 mg/100 kg) and buprenorphine (0.24 mg/100 kg). Administration of this sedative combination does not influence the measured parameters of the MMEPs (Nollet *et al.*, 2003). The horses were then transcranially stimulated (Magstim 200, Novametrix) with a 70mm diameter round coil, capable of generating a maximal magnetic field of approximately 4 Tesla. In order to record the positioning of the stimulating coil, a reference system covering the forehead was constructed for each horse with adhesive tape according to the following procedure. Tapes were placed on the forehead between the dorso-medial aspect of the bony orbit (a) and the rostral aspect of the base of the ear (b) and between the dorsal aspect of the bony orbits (aa) and between the rostral aspect of the base of the ears (bb). A grid was then produced by placing further tapes in the middle of the quadrangle and parallel to the other lines. In this way seven different coil positions were obtained (Figure 1). For each stimulation site, a clockwise inducing current flow as well as a counter-clockwise inducing current flow, as viewed from in front of the horse, were tested. All sites were first stimulated at a stimulus intensity of 80% of maximal output and afterwards at an intensity of 100% of maximal output.

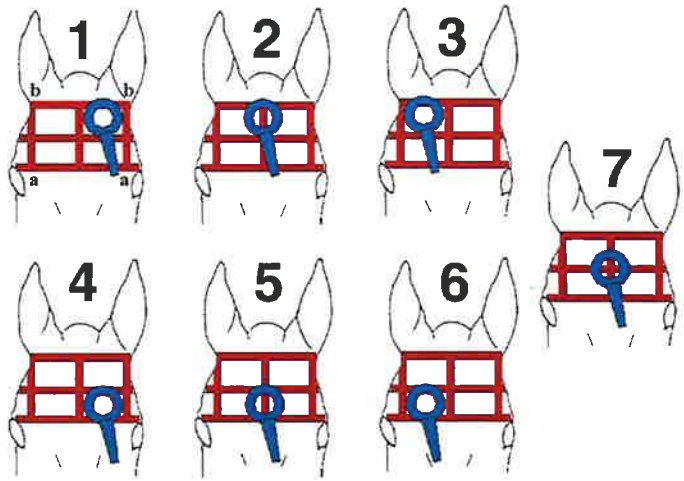

Figure 1. The seven studied coil positions on the equine forehead.

Electromyographic responses (MMEPs) were recorded bilaterally from needle electrodes in the extensor carpi radialis muscle and the cranial tibial muscle, two small, well-delineated and most distal muscles of the equine front and hind limbs. The active electrode was inserted in the middle of the muscle belly and the reference electrode was placed subcutaneously on the lateral side of the radial tuberosity of the forelimb and the lateral malleolus of the tibia of the hind limb. A ground electrode (alligator clip) was attached to the skin of forelimb in the elbow region and of the hind limb in the groin region. The stimulator triggered the sweep of a

standard electromyography machine (Medelec Sapphire; Medelec Ltd.), enabling the latency between the stimulus and the onset of the response to be measured. The time base was 100 ms with a gain ranging from 100 μV to 5 mV per division. The bandpass filter settings were 20 Hz to 3 kHz and only single stimuli were applied. Two MMEPs were recorded for each stimulation site to account for within-test variation. The responses were analyzed for latency and peak-to-peak amplitude. Therefore, 224 stimuli were applied to each horse (2 repetitions x 2 intensities x 2 coil currents x 7 stimulation sites x 4 recording sites).

Statistical analysis

The effect of coil position, direction of the current flow, recording position (front or hind limb, left or right limb) and intensity of the stimulus on MMEP was statistically analysed by comparing the latency time and peak-to-peak amplitude for the different situations using linear mixed effects models (S-plus, Cambridge, MA, USA) with horse as a random variable. The effect of each parameter was first evaluated in a univariate analysis, secondly all parameters with a $p < 0.1$ were included in the multivariate analysis. All two-way interactions were tested and only the significant interactions were included in the model.

Table 1. P-values of the multivariate analysis showing the significance of the effect of the parameters on onset latency and peak-to peak amplitude.

	Onset latency	Peak-to peak amplitude
Coil current	0.357*	0.007
Coil position	<0.001	<0.001
Forelimb versus hind limb	<0.001	<0.001
Left versus right limb	0.524*	0.006
Stimulus intensity	<0.001	<0.001
Coil position x forelimb versus hind limb (interaction)	<0.001	0.308
Coil current x left versus right limb (interaction)	**	0.970

* univariate analysis results that were not significant and therefore not included in the multivariate model

** not tested since the main factors (coil current and left versus right limb) were not included in the multivariate model

RESULTS

The p-values from the multivariate analysis are presented in Table I. With stimulation at positions 2 and 7, MMEPs with the shortest onset latencies and largest peak-to-peak amplitude were obtained in the front limbs as well as in the hind limbs (Figs. 2, 3 and 4). On these positions the onset latencies also had the smallest standard deviations.

No statistical difference in onset latency was found between the right side (39.28 ± 15.05 ms) and the left side (39.75 ± 14.84 ms) and between stimulation when the current in the coil flows in clockwise (39.85 ± 15.28 ms) or in counterclockwise (39.18 ± 14.60 ms) direction. Both the current in the coil and the side of measurement (left or right) had a significant influence on the peak-to-peak amplitude of the MMEPs (Table I). However, no significant interaction between the coil current and the right or left side could be found, indicating that the effect of the coil current is equal for both sides. The average difference between the left (4.08 ± 3.53 mV) and right (3.70 ± 3.74 mV) side is very small. An average peak-to-peak amplitude of 3.70 ± 3.55 mV could be found when the current flows in a clockwise direction in the coil or of 4.08 ± 3.71 mV with the counterclockwise flowing current.

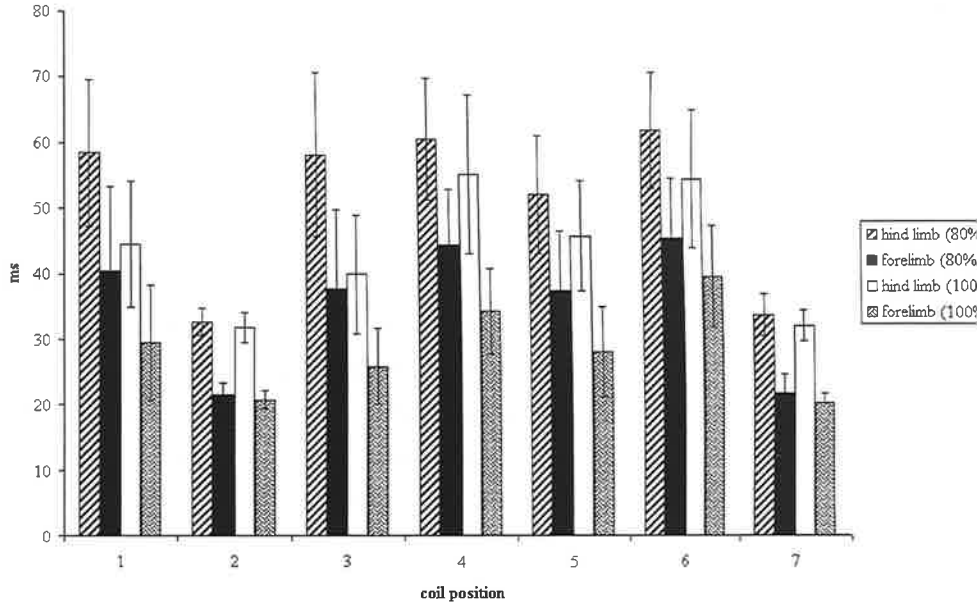

Figure 2. Average (±SD) onset latencies for MMEPs (ms) recorded in front limb and hind limb in seven horses when stimulated at different head positions with 80% or 100% of the maximal stimulator output.

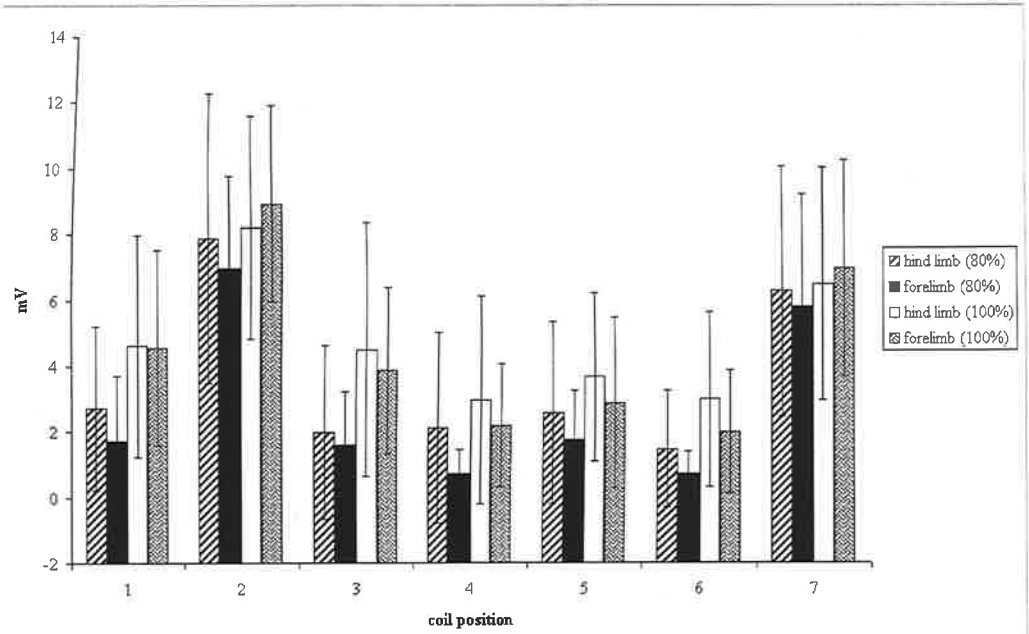

Figure 3. Average (±SD) peak-to-peak amplitudes (mV) for MMEPs recorded in front limb and hind limb in seven horses when stimulated at different head positions with 80% or 100% of the maximal stimulator output.

Coil position, forelimb versus hind limb and stimulus intensity (80% or 100% of maximal output) were significant for onset latency as well as peak-to-peak amplitude. With stimulus intensity at 100 % of maximal output of the stimulator, larger MMEPs (4.62 ± 3.62 mV) and shorter onset latencies (35.80 ± 13.06 ms) were found than with stimulus intensity at 80 % (respectively 3.15 ± 3.50 mV and 43.22 ± 15.77 ms, respectively) of maximal output.

The significant interaction between coil position and front versus hind limb indicates that at the same position of the coil on the head significant shorter onset latencies could be found between MMEPs registered in the front limb compared to those in the hind limb. This effect was not significant for the peak-to-peak amplitude.

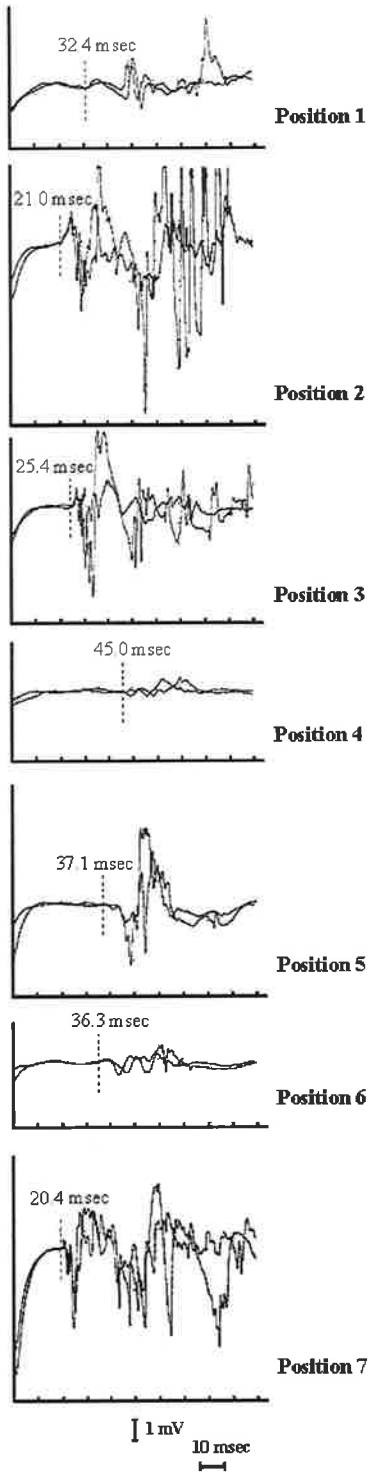

Figure 4. MMEPs (each time two superimposed responses) recorded over the left extensor carpi radialis muscle after transcranial magnetic stimulation of the seven stimulation sites in one horse. The vertical dotted lines with the numeric value (in ms) are the take-off points for onset latency determination. Stimulator output was set at 100%. Coil current was counter-clockwise.

DISCUSSION

An interesting finding in this study was the difference of optimal coil position between humans and horses. In the horses, for both left and right forelimb and hind limb muscles, the best MMEPs were recorded with the coil placed over the midline of the forehead (positions 2 and 7). This central location corresponded well with the findings of Breazile *et al.* (1966) who applied an electrical stimulus to the cortical surface and also found a distinct somatotopic representation, dividing the motor cortex in four areas, namely upper and lower lip muscles, nostril dilator muscles, shoulder and neck muscles and limb muscles. The motor area for the limb musculature was central, reaching the dorsal median fissure. The anatomical representation of the hind limbs was in most instances more caudal than that of the forelimbs, but much overlap was observed, which can explain our results since a large area is stimulated with the circular magnetic coil used.

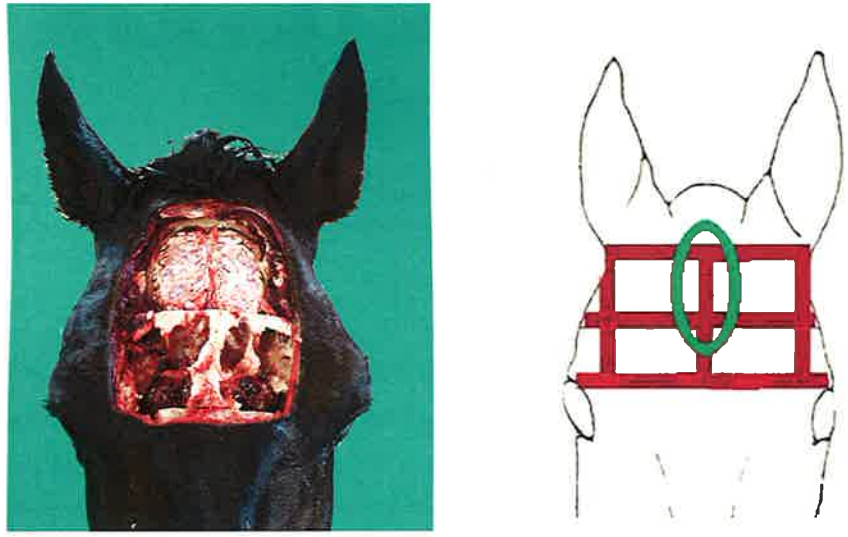

Figure 5. Position of the brain in the equine skull (left) and optimal coil position for recording MMEPs in extensor carpi radialis or cranial tibial muscle (right). The central location (cfr. position 2 and 7) corresponds well with the anatomic representation of the motor cortex. Magnetic stimulation of other areas rostral or more lateral to the seven presented positions on the equine skull surface could not elicit MMEPs and these correspond with the anatomic representation of the brain in the skull (left).

In humans, however, the optimal localisation for obtaining responses in the upper limbs is seen when the coil is centered on the vertex. For the leg muscles the optimal position of the coil on the scalp varies from subject to subject but is mostly more anteriorly (Hess & Ludin, 1988). In dogs, Van Ham *et al.* (1994) reported that the best stimulation site to find MMEPs with the shortest onset latency, the largest amplitude and the best reproducibility was found to be the vertex for both recordings from the forelimb and the hind limb.

As the origin and conducting pathways of TMS in humans have not been completely elucidated, reasons for this interspecies difference can only be proposed. In human medicine, magnetic stimulation appears to stimulate the motor cortex, resulting in activation of output pathways, i.e. the corticospinal tract, to produce electromyographic (EMG) responses in muscles (Barker *et al.*, 1985; 1987). The primary motor cortex of humans starts in the precentral gyrus, the fold of the cortex just anterior to the central sulcus (Woolsey, 1961). In animals, the motor cortex of Carnivora is also located around the central sulcus but the location of the central sulcus is not homologous to the central sulcus of primates. The motor cortex of both the cat and dog extends somewhat caudal to this limit (Sisson & Grossman, 1953). In 1966, Breazile and colleagues (1966) investigated the delineation of the motor cortex and the homologue of the central sulcus in the horse, by application of an electrical stimulus to the cortical surface and observation of muscle movement. They concluded that the region of the motor cortex of the horse occupied nearly the entire rostral one-half of the dorsal surface of the cerebral hemisphere and is homologous to the precentral cortex of primates. Noninvasive mapping of the equine skull surface using magnetic stimulation with different coil positions can confirm this localisation since stimulation of the seven above described positions did evoke MMEPs and stimulation of other areas beneath or more lateral to the presented positions could not elicit MMEPs (unpublished observations).

Another possible explanation could be the widespread magnetic field which is the same beneath all segments of the circumference of the coil and thus capable of stimulating quite extensive cortical areas. This is especially true in small animals, where because of the relatively small size of their brain and motor area on the brain, the whole motor area of the brain will probably be stimulated (Van Ham *et al.*, 1994). In horses a larger brain area can be stimulated, but the motor cortex only occupies the rostral one-half of the dorsal surface of the cerebral hemisphere. MMEPs found after stimulation at different head positions also confirm this local area, since stimulation of the lowest parts (position 4, 5 and 6) did not evoke responses in all instances and only with a smaller peak-to-peak amplitude and very long onset latency.

In humans the latency of EMG responses varies according to the orientation of the induced current in the brain when monophasic stimulus pulses are applied (Day *et al.*, 1989; Werhahn *et al.*, 1994). In the majority of subjects, the lowest threshold for stimulation occurs when the induced current in the brain (which is the opposite direction to that in the coil) flows from posterior to anterior (Mills *et al.*, 1992). Therefore, the lowest threshold for obtaining responses in the right upper limb in humans is seen when the coil is centered on the vertex and current flows in a counterclockwise direction in the coil (when viewed from above), resulting in a better stimulation of the left hemisphere. Reversal of the current flow (by turning the coil over) also produces EMG responses, but with a higher threshold, in the right limb. For the leg muscles the optimal position of the coil on the scalp is mostly more anterior (Hess & Ludin, 1988).

It will be obvious, therefore, that the vertex placement of the coil activates both hemispheres but preferentially activates the left with clockwise current flow in the brain and the right with counter-clockwise current flow in the brain. The reasons for this difference are poorly understood but presumably reflect differences in the direction and distribution of current flow within the brain produced by the two orientations of the coil. In horses no differences between clockwise or counterclockwise current flow for determining onset latencies of muscle responses in right or left muscles were seen. Only for peak-to-peak amplitude significant difference could be found. However, this difference could not be explained by preferable stimulation of the left or right cerebral hemisphere since the interaction between the coil current and peak-to-peak amplitudes of the MMEPs registered in the left or right side of the forelimb or hind limb, indicating that the effect of the coil current is equal for both sides, is not significant. As described in humans (Hess & Ludin, 1988; Amassian *et al.*, 1989; Ellaway *et al.*, 1998), the peak-to-peak amplitude also has a very large standard deviation in the same horse and the same muscle (unpublished observations). The fact that despite this large standard deviation, we still found a significant difference is difficult to explain. A functional asymmetry in the motor system of the horse might be a possible explanation. Yahagi and Kasai (1999) reported a functional hemispheric asymmetry of the human motor cortex between right- and left-handed subjects. While the right-handed subject imagined flexing the right index finger (dominant hand) the peak-to-peak amplitudes of the MMEPs were significantly larger than those induced by mental imagery of flexing the left (non-dominant hand) finger. In left-handed individuals, however, these left-right differences in MMEP peak-to-peak amplitude were not observed. Moreover, in humans, the left hemisphere has been shown to predominate in many aspects of higher level motor planning (Kimura, 1993; Peters, 1996; Rushworth *et al.*, 1997). In most horses a dominance for the left side has been described (Meij & Meij, 1980). Also, the average peak-to-peak amplitude of the MMEPs registered at the left side are slightly larger than those registered on the right side. There is however up till now unsufficient knowledge about the brain function of the horse to explain this difference. Additionally, since the recordings were taken with the horse standing, it may be that the muscles of both sides are unevenly contracted because the horse is leaning toward one side or the other. This might provide a facilitating effect that would produce a larger amplitude on the side supporting most of the horse's weight and maybe also explain some difference in left versus right amplitudes.

In dogs, Van Ham *et al.* (1994) reported no significant differences in onset latencies and peak-to-peak amplitudes between recordings from the left or from the right limb when reversing the coil current from clockwise to counterclockwise.

A second explanation for this interspecies difference could be the difference in significance of the pyramidal versus the extrapyramidal tracts. The major portion of the corticospinal tract in humans crosses at the pyramidal decussation at the level of the foramen magnum, forming the lateral corticospinal tract, with a small proportion of uncrossed fibres forming the ventral corticospinal tract. It is suggested that this latter tract in humans rarely descends below the thoracic part of the spinal cord and that some uncrossed fibres may cross to join the lateral corticospinal tract (Brodal, 1981). Thus, in humans, the major effect of a cortical stimulus may be expected in the contralateral limb, but ipsilateral as well as contralateral responses are also clearly evident in some patients during intraoperative monitoring (Levy *et al.*, 1984b). The observation that each single cortical stimulus evokes muscle twitches and limb movement may suggest a very dense corticomotoneuronal connection in humans or that, because of the magnitude of the stimulus intensity, current spread may have activated other corticofugal fibres. Corticorubral and corticoreticular fibres would be likely candidates for activation of rubrospinal and reticulospinal tracts, respectively, which are more important motor pathways in horses. In our opinion, no anatomic evidence of orientation of the fibres in the motor cortex is available in horses.

A third possible explanation could be the strength of stimulation (suprathreshold) and the non-controlled facilitation in horses. In humans, when determining threshold at rest, the current flow induced by a monophasic pulse will preferentially activate one hemisphere and thus induces a larger EMG response in the opposite limbs. However, when stimulating at 100% of the maximum output of the device and with slight voluntary contraction, Furby *et al.* (1992)

observed about 20% of the maximum MMEPs ipsilaterally. They explained this phenomenon by asymmetrical levels of alpha motoneuron preactivation.

The reason for using a suprathreshold stimulation in our study is two-fold. Firstly, the purpose of TMS is to develop the technique for clinical use in neurological cases in horses. Therefore, we have attempted to standardize the test for 100% of maximal stimulation output in normal horses in order to compare it with the MMEPs registered in ataxic horses, since MMEPs are already difficult to detect in some horses with lesions along the spinal cord when stimulating at the maximal capacity (Nollet *et al.*, 2002). A second and more practical reason is that determination of threshold is very difficult in horses since we can not eliminate the effect of non-controlled facilitation that also affects the threshold determination (unpublished results). Therefore a fixed value of 80% of the maximal stimulation output was chosen for the lower stimulation intensity. In nearly all horses this value is above threshold.

Chapter 3. Part 2. Influence of coil current, coil position and stimulation intensity

CONCLUSION

We can conclude that transcranial brain stimulation using a magnetic pulse, a technique that has been proven to be particularly suitable for exciting the motor cortex in conscious humans, also elicits reproducible MMEPs in horses. The midline of the forehead seems to be the optimal coil position for recording MMEPs in the extensor carpi radialis or cranial tibial muscle. The orientation of the current in the coil seems to have no influence for recording MMEPs in the extensor carpi radialis and cranial tibial muscle in the horse. In this study the same coil positions gave best MMEP responses in both the forelimbs and the hind limbs. Refined coil orientation by using small butterfly or figure-of-eight shaped coil, used for mapping studies in humans, maybe can differentiate more between specific areas. This type of coil, however, elicits a more focal stimulation but produces a relatively weak and less penetrating magnetic field. Since our aim of using TMS in horses is to provide an ancillary test in the neurological examination of horses, obtaining reproducible MMEPs is more important than knowing the exact motor organisation of the cerebral cortex.

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CHAPTER 4

Reference values of magnetic motor evoked potentials in 84 healthy horses and influence of height, weight, age and gender

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SUMMARY

Transcranial magnetic stimulation (TMS) was performed on 84 healthy horses. Reference values for onset latency and peak-to-peak amplitude of magnetic motor evoked potentials (MMEPs) recorded from the extensor carpi radialis and cranial tibial muscle were obtained.

A significant difference was found between recordings made in the front limbs and in the hind limbs, where MMEPs recorded in the front limbs have a shorter onset latency and a higher peak-to-peak amplitude. Mean (±SD) normal values for onset latency of 19.32 ±2.50 ms and 30.54 ±5.28 ms and for peak-to-peak amplitude values of 9.54 ±3.73 mV and 6.62 ±3.62 mV were obtained for extensor carpi radialis and cranial tibial muscle respectively. The left-to-right difference in onset latency and peak-to-peak amplitude was not significant. In the same horse differences up to 0.82 ms and 1.53 ms for the extensor carpi radialis and cranial tibial muscles respectively lie within the 95% confidence limit and are considered normal. In contrast to onset latency, peak-to-peak amplitude showed a very large intra- and inter-individual variability, even in the same muscle. To reduce the variability and to predict normal values of new individual cases, influence of height, weight, age and gender on the magnetic motor evoked potential were determined. No significant effects of gender were observed on onset latency and peak-to-peak amplitude. The age of the horse had only a small but significant effect on peak-to-peak amplitude, with larger responses in older horses. Height at the withers and weight of the horse, parameters that strongly correlate with the size of the horse, had an important significant influence on onset latency but not on peak-to-peak amplitude.

The age of the horse and the height at the withers was used to predict peak-to-peak amplitude and onset latency reference ranges for horses.

INTRODUCTION

A magnetic motor evoked potential (MMEP) represents the electrical activity in the descending motor tracts in response to an external magnetic stimulus to the cerebral motor cortex. Transcranial magnetic stimulation of the motor cortex can produce MMEPs of the extremity muscles more safely and painlessly than high-voltage electrical stimulation of the brain. Since its introduction in 1985 (Barker *et al.*, 1985) numerous studies and clinical applications, including diagnosis of lesions of corticospinal descending pathways, anterior horn cell deterioration, nerve root compression and, recently, intraoperative monitoring of the spinal cord, in humans (Barker *et al.*, 1987; Herdmann *et al.*, 1993; Thompson *et al.*, 1991: Tabauraud *et al.*, 1993; Wehling *et al.*, 1995; Di Lazzaro *et al.*, 1999; Mills, 1999; Triggs *et al.*, 1999; Aglio *et al.*, 2002; Bartley *et al.*, 2002) and small animals (Heckman *et al.*, 1989; Sylvestre *et al.*, 1993; Van Ham *et al.*, 1994, 1995, 1996a, 1996b; Young *et al.*, 1994, Nakatoh, 1998; Poma, 2002), have been published.

Cervical spinal cord dysfunction is also a common problem in equine veterinary medicine. Currently available tests (clinical examination, radiography, myelography, scintigraphy, CT-scan) give no objective information about the functionality of the nervous tracts, and are sometimes difficult to perform and not without risk in ataxic horses. We already reported the possibility of eliciting MMEPs in sedated horses (Nollet *et al.*, 2003) and the usefulness of the technique for the detection and localization of lesions along the descending motor tracts (Nollet *et al.* 2002). As described in humans, conduction times higher than "mean latency + 2 times the standard deviation" may indicate pathologic slowing of the conduction, while an increased latency of MMEPs in cases of "subclinical" paresis can serve as an important diagnostic tool for the early detection of motor deficits in cervical compression radiculopathy and myelopathy (Dvorak *et al.*, 1990; Wehling *et al.*, 1995).

Currently, data acquired from healthy horses using TMS are insufficient. Therefore, in this study the technique was applied to 84 healthy sedated standing horses of different height in order to frame reference values of onset latency and peak-to-peak amplitude of MMEPs and to evaluate the possible effect of height, age and gender on the neurophysiological measurements.

MATERIAL AND METHODS

Horses

Eighty-four healthy horses (24 stallions, 30 geldings and 30 mares) aged from 8 months to 20 years (mean ±SD, 7.91 ± 4.43 years) were used for this study. The horses had an average body weight of 382.55 ± 153.11 kg (range from 106 to 650) and an average height at the withers of 137.80 ± 27.07 cm (range from 85 to 175). In order to determine the possible effect of the size of horses on MMEP parameters, different measures, such as height at the withers, distance between occiput and withers, distance between withers and lumbosacral space, distance between withers and base of the tail, distance between occiput and lumbosacral space and distance between wing of the atlas and front of the shoulder, were determined.

The study was approved by the faculty ethics committee (reference 18/2000). Although the stimulation is painless, the mild discomfort induced by evoked muscle contraction and the noise of stimulation can agitate some horses. Therefore the horses were sedated with a combination of detomidine (Domosedan®, 10 µg/kg of bwt; Pfizer, Belgium) and buprenorphine (Temgesic®, 2.4 µg/kg of bwt; Schering-Plough, Belgium) intravenously. Administration of this combination does not influence the measured parameters of the MMEPs (Nollet *et al.*, 2003).

Magnetic stimulation

All horses were stimulated transcranially (Magstim 200; Novametrix, U.K) using a round 70 mm coil, generating a maximal magnetic field of approximately 4 Tesla at the coil surface.

The stimulus intensity was 100% of the maximal output. The coil was centered over the forehead. In horses, the current flow within the coil had no influence on the recorded responses (unpublished observation).

The stimulator triggered the sweep of the electromyogram (EMG) machine, recording the evoked potentials, allowing the latency between the stimulus and the onset of the response to be measured. For each recording site four individual stimulations were delivered and superimposed, to compensate for the within test variation (Figure 1). Consequently, a minimum of 16 stimuli were applied to each horse (4 repetitions × 4 recording sites).

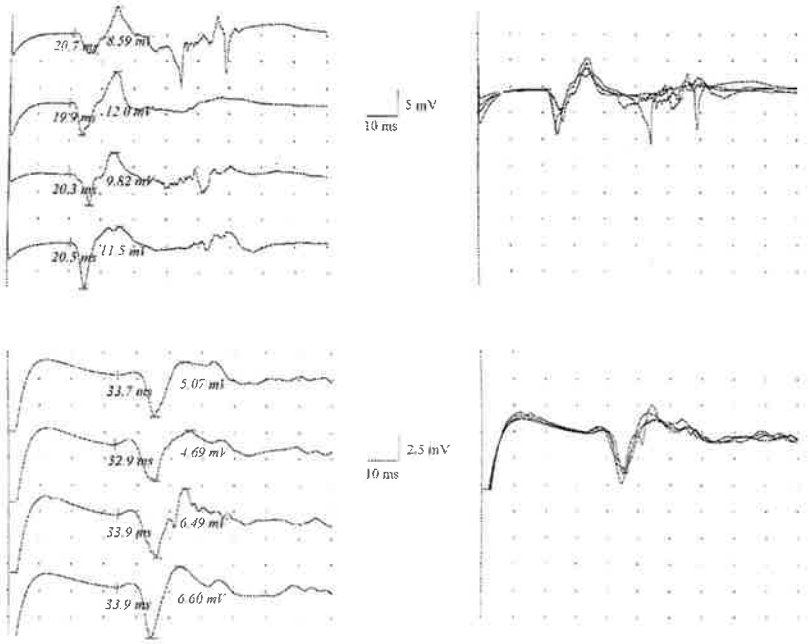

Figure 1. Magnetic motor evoked potentials recorded from the extensor carpi radialis muscle (upper tracings) and the cranial tibial muscle (lower tracings) of one horse (gelding, 6 years old, height 155 cm). Four potentials were obtained to evaluate reproducibility of the evoked potentials. The onset latency (ms) and peak-to-peak amplitude are marked on the figure. The potentials shown on the right are the superimposed individual responses of the left tracings.

Recording of MMEPs

Electromyographic responses (MMEPs) were recorded bilaterally from needle electrodes in the extensor carpi radialis muscle and the cranial tibial muscle, using a standard EMG machine (Medelec Ltd., Old Woking, Surrey, England). The active electrode (25 mm monopolar, disposable, insulated, stainless steel needle; TECA Corporation, Pleasantville, NY) was inserted in the middle of the muscle belly and the reference electrode was placed subcutaneously, to the lateral side of the radial tuberosity of the forelimb and the lateral malleolus of the tibia of the hind limb. A ground electrode (alligator clip) was attached to the forelimb in the elbow region and to the hind limb in the groin region. The time base was 100 ms with a gain ranging from 100 µV to 5 mV per division. The bandpass filter settings were 20 Hz to 3 kHz.

Onset latency (in ms) was measured as the shortest distance between the trigger point and the take-off of the initial phase (negative or positive). Amplitude (in mV) was measured between the two largest peaks of opposite polarity (peak-to-peak amplitude).

Statistical analysis

For onset latency as well as for peak-to-peak amplitude, the significant effect of different parameters (including gender, weight, age, left-to-right difference and the different linear measures) was determined using linear mixed models with horse as the random variable. The means were considered to be significantly different if $p \leq 0.01$ (S-plus; Cambridge, MA, USA).

First all parameters were analysed univariatly. Predicted values and 95% prediction interval of onset latency and peak-to-peak amplitude, based on the parameters that had a significant effect, were calculated using simple linear regression analysis. When several highly correlated factors were significant in the univariate analysis, the parameter resulting in the smallest deviance was selected. Each model was run for fore and hind limbs separately (S-plus; Cambridge, MA, USA).

The 95% predicition interval was calculated using the following formula:

$$95\%\ \text{prediction interval} = y_0 \pm t_{n-2,\alpha/2} \cdot s \cdot \sqrt{1 + \frac{1}{n} + \frac{\left(x_0 - \bar{x}\right)^2}{\sum_{i=1}^{n}\left(x_i - \bar{x}\right)^2}}$$

with:

y_0 = predicted value

s = standard error of the residuals

x_0 = height at the withers for onset latency; age for peak-to-peak amplitude

$t_{n-2,\,\alpha/2}$ = value from the students t-distribution for (n-2) degrees of freedom and an accepted error of $\alpha/2$

n = number of observations

$\bar{x}$ = average value of x (height at withers; age)

The 95% upper limit confidence interval for left-to-right difference was calculated as the mean difference of the absolute values + 1.64*SD.

RESULTS

Motor responses to magnetic cortical stimulation were obtained in all horses in all four limbs. Representative examples of the MMEPs in the fore and hind limbs are shown in figure 1. In most horses MMEPs recorded from the extensor carpi radialis muscle have a bi- or triphasic shape. A more polyphasic configuration is encountered when recording is done from the cranial tibial muscle, especially when the horse contracts the muscle.

A significant difference was found between recordings made from the front limbs and from the hind limbs, where MMEPs recorded from the front limbs had a significantly shorter onset latency and a significantly larger peak-to-peak amplitude than those recorded in the hind limbs.

No significant difference in the onset latency and peak-to-peak amplitude was found between the right and left responses. The average left-to-right difference in onset latency in the same horse and the same muscle was 0.37 ± 0.27 ms for the extensor carpi radialis muscle and 0.49 ± 0.63 ms for the cranial tibial muscle. The upper limit for the 95% confidence interval of the differences between left and right of the extensor carpi radialis muscle and the cranial tibial muscle are upto 0.82 ms and 1.53 ms respectively. In contrast to onset latency, peak-to-peak amplitude showed a very large intra- and inter-individual variability, even in the same muscle (see figure 1). Consequently the upper limit of the 95% confidence interval of the left-to-right difference is 7.16 mV (mean ± SD; 2.91 ± 2.59) for the extensor carpi radialis muscle and of 8.32 mV (mean ± SD; 3.29 ± 3.06) for the cranial tibial muscle.

In table 1, p-values showing the significance of the effect of the parameters on onset latency and peak-to peak amplitude are given.

No significant effects of gender were observed on onset latency and peak-to-peak amplitude.

The age of the horse had only a significant effect on peak-to-peak amplitude.

In contrast, the different parameters describing the size of the horse all had a significant effect ($p < 0.0001$) on onset latency but not on peak-to-peak amplitude. Of these measures, height at the withers explained the largest variation (= smallest deviance). The weight of the horses also had a significant effect on the onset latency, but this parameter strongly correlated with the size of the horse.

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Table 1. P-values of the univariate linear mixed models analysis showing the significancy of the effect of the parameters on onset latency and peak-to peak amplitude.

	Onset latency	Peak-to peak amplitude
Forelimb versus hind limb	<0.0001	<0.0001
Weight	<0.0001	0.7900
Left-to-right difference	0.3159	0.0305
Gender	0.3431	0.0199
Age	0.1819	0.0012
Height at withers	<0.0001	0.4539
Wing of atlas - front of shoulder	<0.0001	0.5400
Occiput - withers	<0.0001	0.4600
Withers - lumbosacral space	<0.0001	0.6900
Withers - base of tail	<0.0001	0.6317
Occiput - lumbosacral space	<0.0001	0.5359

To predict normal values of onset latencies and peak-to-peak amplitudes in the extensor carpi radialis and cranial tibial muscle in normal horses, height at the withers (figures 2 and 3) and age are used. The calculated regression equations are given in table 2. On these figures the 95% prediction interval is also marked.

Table 2. Regression equations of predicted onset latency and peak-to-peak amplitude of MMEPs recorded from extensor carpi radialis and cranial tibial muscle after cortical magnetic stimulation in healthy horses. *(ECR: extensor carpi radialis; CT: cranial tibial; height = height at withers; amplitude = peak-to-peak amplitude)*

	ECR muscle	CT muscle
Predicted onset latency (ms) Y=	8.55 + 0.078 × height (cm) (R^2= 0.71)	6.72 + 0.17 × height (cm) (R^2= 0.79)
Predicted amplitude (mV) Y=	8.02 + 0.19 × age (years) (R^2= 0.05)	5.36 + 0.16 × age (years) (R^2= 0.04)

In table 3 the calculated equations for the 95% prediction intervals for onset latency and peak-to-peak amplitude are given.

The coefficient of determination (R^2) (table 2) indicates the proportion of variation of y explained by the regression function. Based on the different R^2 it is obvious that the regression model for onset latency has a much better predictive value and as a consequence smaller prediction intervals than the peak-to-peak amplitude. This is a result of the very large variation of the peak-to-peak amplitude within and between horses.

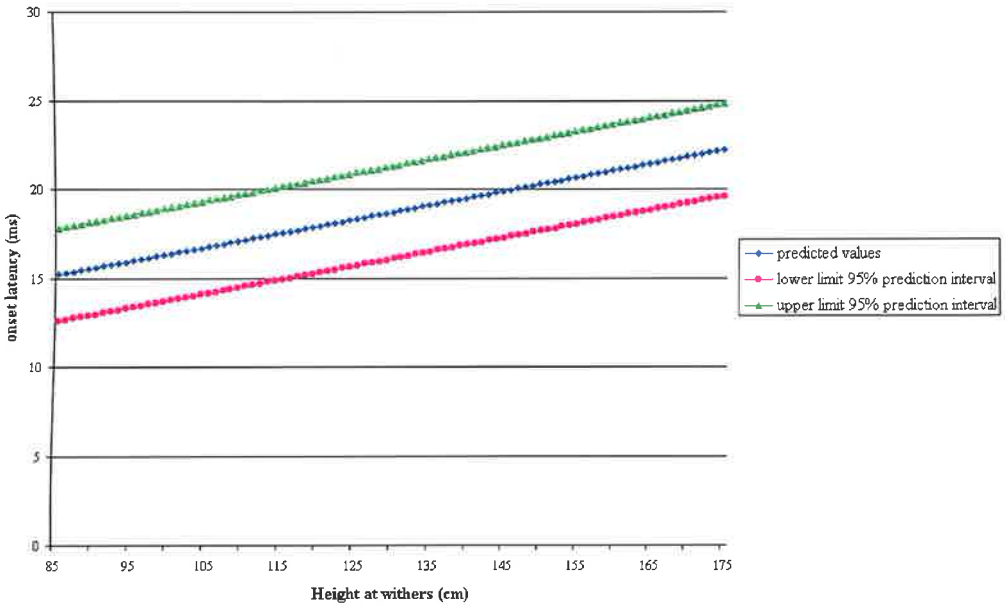

Figure 2. Graph of predicted values and 95% prediction interval of onset latency of MMEPs recorded from the extensor carpi radialis muscle of normal horses, based on the height at the withers.

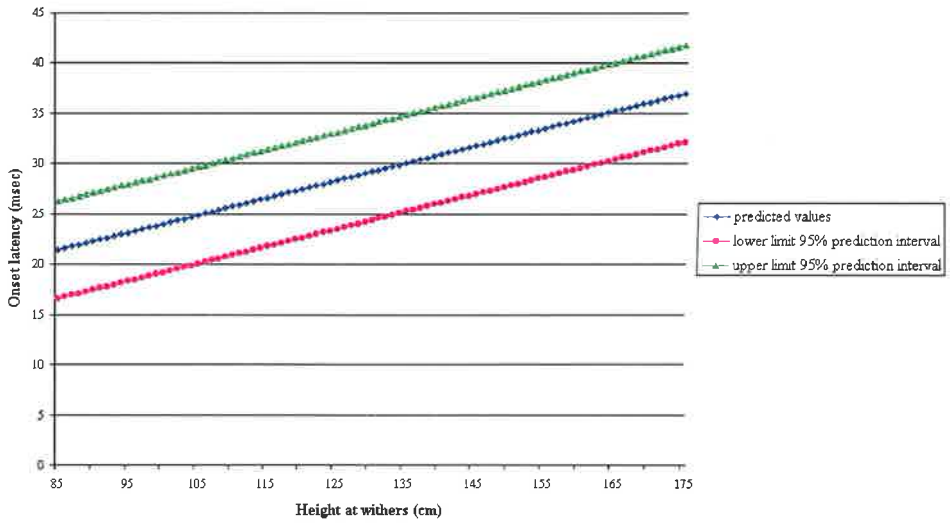

Figure 3. Graph of predicted values and 95% prediction interval of onset latency of MMEPs recorded from the cranial tibial muscle of normal horses, based on the height at the withers.

Table 3. 95% prediction interval of onset latency and peak-to-peak amplitude of MMEPs recorded in extensor carpi radialis and cranial tibial muscle after cortical magnetic stimulation in healthy horses.

	M. extensor carpi radialis
Onset latency (msec)	$y_0 \pm 1.9635 \times 1.3293 \times \sqrt{1 + \frac{1}{672} + \frac{(x_0 - 137.8036)^2}{492116.0714}}$
Peak-to-peak amplitude (mV)	$y_0 \pm 1.9635 \times 3.6366 \times \sqrt{1 + \frac{1}{672} + \frac{(x_0 - 7.9143)^2}{13173.78}}$

M. tibialis cranialis	
Onset latency (msec)	$y_0 \pm 1.9635 \times 2.4533 \times \sqrt{1+\frac{1}{672}+\frac{(x_0-137.8036)^2}{492116.0714}}$
Peak-to-peak amplitude (mV)	$y_0 \pm 1.9635 \times 3.5529 \times \sqrt{1+\frac{1}{672}+\frac{(x_0-7.9143)^2}{13173.78}}$

DISCUSSION

In human medicine several authors described that magnetic stimulation of the motor cortex evokes descending volleys in the corticospinal tract (Barker *et al.*, 1985, 1987). The present data show that in all horses MMEPs were obtained from all examined muscles without any exception. An unanswered but nevertheless important question is the role of the corticospinal tract in ungulates, especially horses. The primary motor cortex in humans has both direct and indirect projections motor system regions of the medulla oblongata and the spinal cord: it projects indirectly (extrapyramidal tract) to the medulla and spinal cord via the red nucleus, reticular formation, and other brainstem nuclei and directly to the spinal cord via the corticospinal (pyramidal) tract. The corticospinal tract, however, is a fibre system unique to mammals but the functional significance of this tract in ungulates has been questioned on the basis of data derived from both physiological and anatomical experiments (Bagley, 1922; Barone, 1959; King, 1911). By investigating the motor cortex, these authors indicated that the corticospinal tract exists in ungulates, but terminates within the caudal medullary and cervical spinal cord levels. Therefore the question could arise as to whether or not magnetic stimulation of the equine motor cortex could evoke MMEPs in the limb musculature; from this and other studies (Mayhew and Washbourne, 1996; Nollet *et al.*, 2002, 2003) it is obvious that it is. In 1942, Lassek compared the fibre composition of the corticospinal tract of several ungulates with that of man and found that, although the largest neural fibres which form this tract in ungulates are smaller than the largest fibres found in man, the number of fibres present was large enough to indicate that they serve important functions in ungulates. Moreover, physiological investigations of the motor cortex of several ungulates (Breazile *et al.*, 1966b; Simpson and King, 1911), including the horse (Breazile *et al.*, 1966a), indicated that these species do possess a corticospinal fibre system which functions in somatic motor control of both thoracic and pelvic limb musculature. By electrical stimulation of the pyramids of horses, Breazile and colleagues (1967) were also able to elicit action potentials in peripheral nerves of the forelimb and hind limb, indicating that the corticospinal tract is of functional significance in this species. They suggested that findings of previous anatomical studies (Bagley, 1922; Barone, 1959; King, 1911), namely that this tract terminates in cervical levels of the spinal cord of ungulates, were likely due to limitations of the techniques used in tracing these neural fibres. Another possible explanation for the evoked EMG responses after TMS in horses could be the stimulation of the more important extrapyramidal tracts in horses. Kamida *et al.* (1998) recently reported that TMS in rats had been attributed to the stimulation of structures other than the motor cortex, because transecting the dorsal corticomotoneuronal tract, which contains the corticospinal tractus, the main pyramidal tract (Brown, 1971), did not abolish magnetic MMEPs. Other studies in rodents and in cats showed that the rubrospinal tract is involved in transmission of the motor evoked potential by transcranial stimulation (Kawai *et al.*, 1992; Yu *et al.*, 2001a, 2001b). In these studies 'spinal cord recorded transcranial motor evoked potentials' were examined. They consisted typically of three to four early negative peaks followed by several small multiphasic late waves. Measurements of the mean conduction velocities of each peak and studies of the influence of brainstem electrical stimulation, sequential transection of the spinal cord or ablation of the sensorimotor cortex and pyramidotomy revealed that TMS in cats and in rats evokes complex responses in both pyramidal and extrapyramidal tracts. A third explanation could be the influence of the strength of stimulation. Previous studies in cats and primates have demonstrated that the MMEP evoked by near-threshold stimulation of the motor cortex consists of a series of waves that are generated in the motor cortex and conducted along the corticospinal tract (Haghighi *et al.*, 1995; Hiraizumi *et al.*, 1996; Ghaly *et al.*, 1999). However, Konrad *et al.* (1990) found that suprathreshold stimulation of the motor cortex in cats produced large MMEPs which travelled in the ventral funiculus and therefore were most likely associated with extrapyramidal tracts, such as the reticulospinal and the vestibulospinal tracts. Consequently, it is reasonable to presume that conduction occured along the extrapyramidal tracts with the suprathreshold stimulation used in our horses.

In most human MMEP studies threshold stimulus intensity is recorded. This cortical threshold reflects the global excitability of the motor pathway, including large pyramidal cells, cortical excitatory and inhibitory interneurons, and spinal motoneurons. According to the size

principle of Henneman (1957) it is currently held that, at threshold intensities, the smaller fibres are stimulated first since neurons of a small size reach threshold before neurons having a larger cell body. As the intensity is increased, the larger, faster conducting fibres are activated, resulting in shorter latencies, and a larger number of neurons are recruited, resulting in larger peak-to-peak amplitudes (Sylvestre *et al.*, 1992). The reason why in this study we chose to stimulate all horses at 100% is that in some horses with neurological problems, MMEPs could only be recorded at a stimulation output of 100% (Nollet *et al.*, 2002). Therefore, in order to compare MMEPs recorded in these horses, reference values were recorded at maximal stimulation. A second reason why threshold determination was not done in our horses is the uncontrollable effect of facilitation in horses. Slight voluntary contraction of the target muscle (less than 5% of maximum) results in an enhanced compound muscle action potential (CMAP) with a shorter onset latency and reduced cortical threshold (Helmers *et al.*, 1989; Weber and Eisen, 2002). In horses, evaluation of muscle relaxation is subjective and anticipation of imminent stimulation is clearly perceived in many horses.

In all horses MMEP responses were obtained without any difficulty, even in the hind limb muscles. Therefore we can assume that a missing or insufficiently reproducible potential must be regarded as pathologic in horses. In humans, however, it has been shown that the MMEPs recorded from the limb are more difficult to elicit and of a smaller peak-to-peak amplitude than those recorded from the hand (Barker *et al.*, 1987). In addition, one publication mentioned that they cannot be recorded in 8% of normal subjects (Eisen and Shtybel, 1990). In dogs, several authors (Heckmann *et al.*, 1989; Van Ham *et al.*, 1994) reported good recordings of MMEPs from the extensor carpi radialis and cranial tibial muscle.

MMEPs recorded from the cranial tibial muscle in our horses had a lower peak-to-peak amplitude than those recorded from the extensor carpi radialis muscle, and the parameter peak-to-peak amplitude had a very large variation in comparison to onset latency in the same horse. This intra- and inter-individuals variability is also seen in humans and in dogs. Many factors account for the variations (Levy *et al.*, 1987; Simpson *et al.*, 1987; Chu, 1989; Xing *et al.*, 1990; Strain *et al.*, 1990; Dull *et al.*, 1990; Sylvestre *et al.*, 1993) and most of these are difficult or impossible to control in the clinical setting: coil position over the cortex, minimal angulation of the coil, changes in the placement of the recording electrodes for each trial, variation in the current fluxes induced by magnetic stimulation, and variations intrinsic to the horses themselves, such as level of relaxation at which modest muscle contraction facilitates the response. Therefore, peak-to-peak amplitude can be assumed to be of limited clinical value. However, Weber and Eisen experienced that a side-to-side difference of 50% or greater

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can be regarded as abnormal in human patients without lower motor neuron disease (Weber and Eisen, 2002). In some of our normal horses a larger left-to-right difference was found, but notwithstanding this variability, all responses had a larger mean peak-to-peak amplitude when compared to responses found in horses with cervical cord lesions (Nollet *et al.*, 2002b), where most MMEPs had a mean peak-to-peak amplitude of ca. 1 mV or smaller.

The onset latency of MMEPs recorded from the extensor carpi radialis and cranial tibial muscles highly correlated with all quoted linear measures. Also in humans latencies are highly correlated with height, especially the leg MMEP latencies (Chu, 1989; Tobimatsu *et al.* 1998). Because in our horses the best predictive value was found for height at the withers and in clinical circumstances it also seems easier and more practical to consider this measure, height at the withers is used to predict onset latency in other normal horses. The left-to-right difference for onset latency is very small, and therefore differences exceeding 0.82 ms and 1.53 ms for the extensor carpi radialis and cranial tibial muscle, respectively, in the same horse can be considered as pathologic.

The MMEPs mainly had a biphasic or triphasic wave, especially in the extensor carpi radialis. In the cranial tibial muscle a more polyphasic configuration can be seen. In humans in most instances a bi- or triphasic configuration of the MMEPs has been described in the muscles of the hand (Maertens de Noordhout, 1998). Consequently, a polyphasic configuration (more than 5 phases) has to be considered as abnormal in those muscles. However, a polyphasic configuration is more frequently seen with MMEPs evoked in more proximal muscles and muscles of the leg, even in normal subjects. In children, however, MMEPs are generally polyphasic in early childhood and gradually become triphasic, reaching adult levels at the age of 13 years (Nezu *et al.,* 1997).

Also in the contracting muscle, an increase in the complexity of the EMG waveform was seen in our horses. Nevertheless, the level of relaxation or contraction is uncontrollable even in sedated horses. The high stimulus intensity used in this protocol can also cause complex and polyphasic muscle responses (Maertens de Noordhout, 1998). Therefore in humans a threshold stimulus intensity is determined and patients are then studied at an intensity of 30% above this threshold. For the above mentioned reasons we have opted for this maximal stimulus intensity. However, the increase of complexity of waveform is not comparable with the small polyphasic waves seen in horses with cervical spinal cord lesions (Nollet *et al.*, 2002b). Sylvestre *et al.*, 1993 also described polyphasic shapes of MMEPs in dogs with

extruded disc material. Due to demyelination or disruption of the axons the propagating impulse can be delayed and this will result in staggered impulses causing asynchronous activation of the peripheral neurons and, hence, the motor units.

Gender did not influence the onset latency and peak-to-peak amplitude of the MMEPs recorded in our horses. In humans, significant longer latencies were reported in man compared to women, when recording MMEPs in leg muscles. This was not the fact in hand muscles. They suggested that this finding probably reflects the difference in height (Tobimatsu *et al.*, 1998).

Age of the horses in this study had a very small significant influence on the peak-to-peak amplitude, but not on the onset latency. This is in contrast to humans, where the developmental profile of central conduction times to upper and lower extremity muscles showed an age-dependent acceleration with adult values not being reached before the age of about 10 years. Clear responses in the upper extremity could be obtained after the first year of life, whereas in the lower extremity this was not until the fourth year of life (Müller *et al.*, 1991; Nezu *et al.*, 1997). Eyre and co-workers (1991) even reported that MMEPs could not be evoked under the age of 6 years. They suggested that electrophysiological maturation of the corticospinal motor pathways (changes in the extent of myelination, changes in fibre diameter of the descending efferents and/or maturation of intrinsic intracortical microcircuitry), when all parameters of MMEPs reach adult characteristics, is complete at the age of 13 years (Koh and Eyre, 1988; Eyre *et al.*, 1991; Müller *et al.*, 1991; Nezu *et al.*, 1997). Brody *et al.* (1987) even found that within the spinal cord the degree of myelination of corticospinal efferents was more advanced in the cervical than in the lumbar region. At older ages, latencies were found to increase in humans, especially in the leg and to a lesser degree in the hand (Kloten *et al.*, 1992). This could be explained by a loss of nerve cells in the central nervous system (Wright and Spinx, 1959; Tobimatsu *et al.*, 1998)

Concerning the timing of myelination, there are, however, wide interspecies variations. In the foal, the acts of standing up and ambulating almost immediately after birth make it obvious that this species is born with a fairly developed motor system. From morphological studies only limited information has been gained about the precise maturational profile of descending fibres in the equine motor system. Recently, findings of Szalay (2001) relevant to cellular maturation and the development of myelinated areas of the central motor system in horses support this assumption: the main motor centres and pathways are present prenatally.

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Nevertheless, this investigator observed a spectacular maturation of the motor areas regarding both cytology and fibre quality up to postnatal day 45, after which growth rather than maturation became the leading phenomenon.

In our study, no foals were included. The youngest animal was 8 months old and of the 84 horses only 3 were younger than 2 years and 5 were 2 years old. However, in foals with ataxia, we experienced more difficulties in reproducing MMEPs than in adults with ataxia (personal experience). Whether these difficulties are due to the cervical cord lesions in these particular cases or to the immaturity of the descending motor tracts is still an open question. However, demonstrating the ability of transcranial magnetic stimulation to show this maturation between birth and postnatal day 45 in the same foal can be an interesting subject for further investigations.

CONCLUSION

In this study we have presented normal values for MMEP parameters of horses of different height. Since general anaesthesia in horses is not without risk (Johnston *et al.*, 1995) and expense and the fact that most horses which need to undergo TMS have some neurological abnormality, it is clear that TMS is an excellent addition to the few tools we have for noninvasive imaging of the function of the equine descending motor tract. Magnetically evoked motor potentials are highly reproducible and recent advances suggest that the applications of TMS in horses will continue to grow rapidly.

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CHAPTER 5

CLINICAL APPLICATIONS OF THE TECHNIQUE IN HORSES WITH SPINAL CORD DISEASE

CHAPTER 5

Part 1.

The use of magnetic motor evoked potentials in horses with cervical spinal cord disease

Section 1.

Evaluation of transcranial magnetic stimulation in 12 horses suspected of having cervical spinal cord disease

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SUMMARY

The aim of this study was to investigate the use of magnetic motor evoked potentials as an ancillary diagnostic test in horses with cervical spinal cord lesions.

Transcranial magnetic stimulation was performed in twelve ataxic horses and the results of the evoked responses were compared to those found in reference horses. The latency and peak-to-peak amplitude of the potentials in the twelve ataxic horses were significantly different from those measured in reference horses. The configuration of the abnormal potentials was also polyphasic.

Normalisation of the evoked potentials occurred in none of the horses when recorded after a period of clinical improvement. These findings demonstrate that the technique is also able to detect lesions in horses with subtle clinical signs of incoordination.

The magnetic transcranial stimulation is a valuable ancillary test to assess the integrity of the motor tracts. The technique is painless and safe and shows a good sensitivity to detect lesions along the descending motor pathways.

INTRODUCTION

Spinal cord disease with ataxia due to lesions of the descending motor tracts is common in horses (Mayhew 1989, Matthews, 1998, Mayhew, 1999). Neurological examination in the horse is based mainly on a thorough physical examination. When it is difficult to make an accurate diagnosis, ancillary diagnostic tests are needed. Radiography, cerebrospinal fluid analysis and electrodiagnostic tests such as electromyography, nerve conduction velocity tests (Blythe *et al.*, 1988; Wheeler, 1989; Whalen *et al.*, 1994) and somatosensory evoked potentials (Strain *et al.*, 1988) have been described in the horse. Each technique has its own restrictions. Radiography of the vertebral column is limited mainly to the cervical region and shows only bone abnormalities. Contrast myelography requires general anaesthesia. Needle electromyography only gives information on lower motor neuron lesions. Sensory nerve conduction velocity tests and somatosensory evoked potentials only measure the patency of somatosensory pathways. Transcranial stimulation of the motor cortex and subsequent recording of the electromyographic responses is useful in assessing the functional integrity of the motor pathways of the spinal cord. With transcranial electrical stimulation of the motor cortex (Merton and Morton, 1980; Mills and Murray, 1985, Mills *et al.*, 1987), high amplitude electrical stimulation over the scalp is necessary to elicit a muscular response, and the procedure is painful. The introduction of the magnetic coil stimulator by Barker and colleagues in 1985 provided a method for transcranial cortical stimulation with minimal discomfort. Magnetic motor cortex stimulation evokes synchronized descending excitatory volleys in corticospinal pathways (magnetic motor evoked potentials, MMEPs) (Day *et al.*, 1987, Di Lazzaro *et al.*, 1999). Since then magnetic stimulation has largely replaced electrical stimulation in the neurological examination of human subjects. Magnetic stimulation does not induce high densities of current within the intervening tissues as does electrical stimulation; therefore, magnetic stimulation has little excitatory effect on skin receptors and pain fibres resulting in significantly less discomfort for the tested subjects (Barker *et al.*, 1991). It can also painlessly stimulate the peripheral nerves and no serious side effects have been reported either to the cortex or to the peripheral nerve in man (Barker *et al.*, 1986; Krain *et al.*, 1989; Levy *et al.*, 1989; Mills *et al.*, 1987). Other authors described successful excitable tissue activation in dogs (Heckmann *et al.*, 1989; Linden *et al.*, 1990; Van Ham *et al.*, 1994, 1995). Mayhew and Washbourne (1996) showed that transcranial magnetic stimulation is able to induce magnetic motor evoked potentials (MMEPs) in unanaesthetised ponies. Both in man and in animals with spinal cord trauma and ischemia, magnetic stimulation has proven to be a valuable diagnostic tool for detection of lesions along the spinal cord (Merton and Morton, 1980; Levy 1983; Levy and York 1983; Fehlings *et al.*, 1987, 1988; Hess *et al.*, 1987b; Rossini *et al.*, 1987).

The purpose of this study was to establish reference values for horses and to assess the technique of MMEP to test the damage of the motor tracts in horses with traumatic cervical spinal cord injury or suffering from cervical vertebral malformation or malarticulation.

MATERIAL AND METHODS

Horses

The experimental protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine, University of Ghent, Belgium (reference 18/2000). Transcranial magnetic stimulation was performed on twelve control horses, in order to obtain reference values.

Twelve horses, aged from one to four years, were referred to the Department of Large Animal Internal Medicine with clinical evidence of wobbler syndrome or cervical trauma: 6 males, 1 gelding and 5 mares. They were of different breeds: 7 Belgian warmblood horses, 2 trotters, 1 Oldenburger, 1 French saddle horse and 1 Haflinger. All horses first passed a complete clinical and neurological examination. A mild to severe ataxia (incoordination) of the pelvic limbs or of all four limbs was observed. Horses were easily pulled to one side when standing or walking and circumduction of the limbs was also a typical clinical finding. Some horses with mild symptoms were exercised to evaluate their coordination at a trot. In a second stage, all horses underwent transcranial magnetic stimulation. Finally, a radiographic examination of the cervical vertebral column was performed in 11 horses.

Technique of transcranial magnetic stimulation and parameters

To prevent agitation, all horses were sedated with a combination of detomidine (Domosedan®, Pfizer, Belgium; 1 mg/100 kg) and buprenorphine (Temgesic®, Schering-Plough, Belgium; 0.12 mg/100 kg). No significant differences were found between measurements of latency and amplitude with and without the combination of detomidine and buprenorphine (Nollet et al., 2003).

Transcranial magnetic stimulation of the motor cortex was achieved using a Magstim 200 (Novametrix, U.K.). The magnetic pulses were delivered through a 70 mm external diameter circular coil, generating a maximal magnetic field of approximately 4 Tesla at the coil

surface. The stimulus intensity was 100% of maximal output for cortical stimulation. The coil was centered over the forehead. To obtain preferential activation of each hemisphere, a clockwise inducing current flow, as viewed from in front of the horse, was used for the right motor cortex and a counter-clockwise flow for stimulation of the left motor cortex (Day *et al.*, 1990). A marker of the current direction is provided on the coil's frame to ensure that the magnetic field has always the same orientation.

Electromyographic responses (MMEPs) were recorded bilaterally from needle electrodes in the extensor carpi radialis muscle and the tibialis cranialis muscle. The active electrode was inserted in the middle of the muscle belly and the reference electrode subcutaneously on the lateral side of the radial tuberosity of the forelimb and on the lateral malleolus of the hindlimb. A ground electrode (alligator clip) was attached to the forelimb in the elbow region and to the hindlimb in the groin region. The time base was 100 ms with a gain ranging from 100 µV to 5 mV per division. When no responses were detected the time base was changed to 200 or 500 ms. Bandpass filter settings were 20 Hz to 3 kHz and only single stimuli were applied. Four potentials were obtained and superimposed from each recording site, to evaluate reproducibility of the recordings.

Onset latency (in ms) was measured as the shortest distance between the trigger point and the take-off of the initial phase (negative or positive).

MMEP amplitude (in mV) was measured between the two largest peaks of opposite polarity (peak-to-peak amplitude).

MMEP results were considered abnormal if the values exceeded more than 2 SD from the mean reference value.

RESULTS

With sedation, all horses tolerated the procedure very well.

In all control horses MMEPs were easily recorded after each stimulation in all four limbs. Figure 1 illustrates the EMG responses to cortical magnetic stimulation in one control horse.

The reference values for the parameters found after cortical magnetic stimulation are outlined in Table 1. The onset latency shows a small variability within each muscle. Side-to-side latency difference is very small and does not exceed 1 ms in both the extensor carpi radialis and the tibialis cranialis muscles. Values of peak-to-peak amplitude have a larger variability.

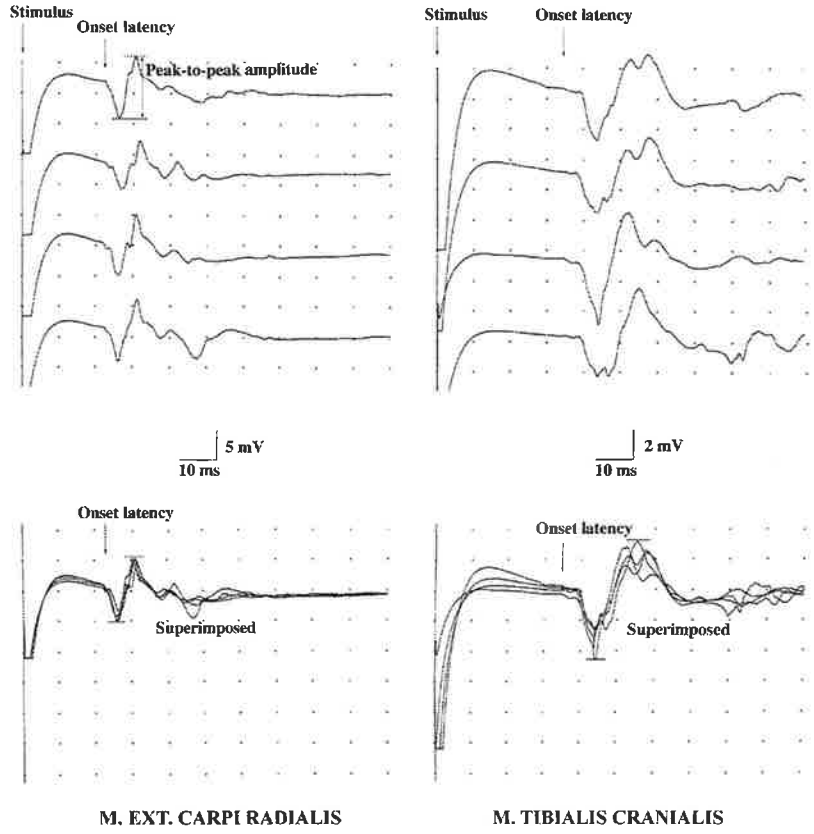


Figure 1. Magnetic motor evoked potentials recorded from the extensor carpi radialis muscle (left) and the tibialis cranialis muscle (right) of a control horse. Four potentials were obtained to evaluate reproducibility of the evoked potentials. The potentials shown in the lower traces are the superimposed individual responses of the top traces.

Table 1. Mean values (±SD) of onset latency and peak-to-peak amplitude of MMEPs after cortical stimulation in 12 control horses.

	M. extensor carpi radialis		M. tibialis cranialis	
	Left	Right	Left	Right
Onset latency (msec)	20.81 ±1.85	20.59 ±1.83	35.94 ±3.43	36.33 ±3.53
Amplitude (mV)	7.37 ±2.69	7.62 ±2.68	5.02 ±3.87	4.26 ±2.55

In figure 2 and 3, MMEPs found in the extensor carpi radialis and the tibialis cranialis muscles of horses suffering from cervical spinal cord lesions can be compared to the configuration in a control horse (figure 1). The MMEPs evoked in these horses demonstrate several typical abnormal features. They have prolonged or variable latency, are of low amplitude, exhibit quite frequently polyphasic waveforms and are labile in appearance. Moreover, more variable delays in onset latencies were seen within one horse and one muscle compared to normal horses.

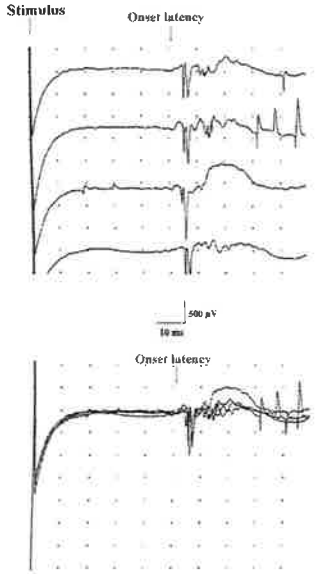


Figure 2. Magnetic motor evoked potentials of the extensor carpi radialis muscle, recorded in a horse suspected of having a cervical spinal cord lesion. Compared to the normal recordings in figure 1, significant latency and amplitude changes are shown.

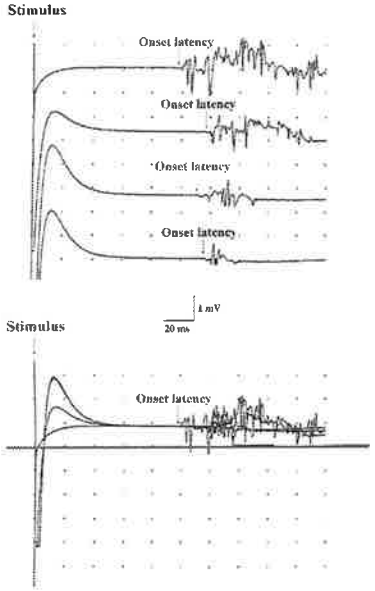


Figure 3. Magnetic motor evoked potentials of the tibialis cranialis muscle, recorded in a horse suspected of having a cervical spinal cord lesion. Compared to the normal recordings in figure 1, significant latency and amplitude changes are shown. The waveforms are very polyphasic.

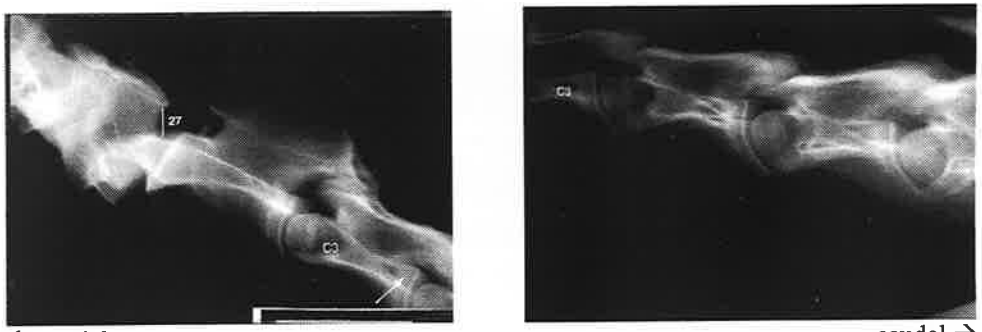
The case histories, radiographic findings on the cervical vertebrae and data of MMEPs for the twelve affected horses are given in Table 2. In some severely ataxic horses (horses 1, 2, 5, 6, 7, 11), MMEPs were not recordable in the hind limbs or in all four limbs. In horse 5 only, a lateralization (large difference in onset latency between the right and left side both in the forelimbs and the hindlimbs) of the MMEP responses was evident.

Table 2. Clinical signs, radiographic findings, mean values (±sd) of onset latency and mean values (+ range) of peak-to-peak amplitude of MMEPs recorded after transcranial stimulation in 12 horses with cervical spinal cord lesions (nf = not found).

Horse	Age (years)	Time between onset of symptoms and MMEP	Clinical signs	Radiographic findings on the cervical vertebrae	Onset latency (msec)				Amplitude (mV)			
					M. ext. carpi radialis		M. tibialis cranialis		M. ext. carpi radialis		M. tibialis cranialis	
					Left	Right	Left	Right	Left	Right	Left	Right
1	3.5	2 weeks	difficulties to keep upright unsteady gait of all four limbs swaying	not done	54.8 ±3.2	56.8 ±2.5	239 ±7.3	nf	1.31 0.42-1.76	1.17 0.75-1.67	0.14 0.13-0.16	nf
1		2.5 months	very mild ataxia shuffling gait when exercised		45.6 ±0.1	43.8 ±0.2	70.2 ±30.	110 ±3.2	0.89 0.27-1.42	0.53 0.43-0.70	0.77 0.33-1.10	1.56 1.00-2.11
2	1.5	5 days	lateral recumbency in pasture severe ataxia	no abnormalities	nf	nf	nf	nf	nf	nf	nf	nf
2		2 months	mild ataxia		40.1 ±3.8	40.3 ±2.8	100.6 ±5.8	102.8 ±7.5	0.11 0.02-0.31	0.18 0.08-0.24	0.33 0.24-0.48	0.18 0.08-0.26
3	4	2 years	hypermetria and stiff gait in pelvic limbs	no abnormalities	52.3 ±2.5	52.3 ±2.5	81.9 ±0.8	69.5 ±0.6	0.18 0.18	0.34 0.34	0.74 0.58-0.90	1.38 0.83-1.99
4	1	1 month	circumduction of thoracic limbs weakness in pelvic limbs	no abnormalities	60.6 ±0.6	61 ±2.5	96.4 ±4.6	100.1 ±3.3	1.07 0.84-1.22	0.81 0.41-1.23	0.55 0.27-0.95	0.32 0.13-0.47
4		6.5 months	only mild improvement		44.3 ±1.6	49.7 ±1.0	108.9 ±8.1	117.3 ±5.5	0.41 0.28-0.71	0.43 0.27-1.20	1.12 0.80-1.58	0.42 0.40-0.44
5	1	2 days	severe ataxia in all 4 limbs	no abnormalities	284.6 ±22.5	106 ±5.6	nf	nf	0.11 0.06-0.16	0.14 0.10-0.17	nf	nf
5		7 months	much improvement slight ataxia slipping at higher speed		45.4 ±1.1	29.2 ±1.8	83.3 ±1.2	54 ±1.23	0.26 0.15-0.51	0.24 0.17-0.34	1.98 1.05-2.82	0.18 0.13-0.24
6	1	3 days	severe ataxia in all limbs	Malformation of the caudal part of C2-C5	103 ±1.0	103.1 ±6.8	nf	nf	0.11 0.07-0.13	0.23 0.16-0.30	nf	nf
7	2	1 month	ataxia and weakness in all 4 limbs	osteochondrosis at the level C5-C7	48.9 ±1.3	48.9 ±1.6	nf	nf	0.14 0.11-0.14	0.20 0.15-0.25	nf	nf

8	1.5	4 months	mild ataxia in the pelvic limbs after a period of severe ataxia	no abnormalities	43.6 ±0.3	42.5 ±0.3	113.5 ±3.4	108.3 ±9.0	0.11 0.08-0.19	0.23 0.15-0.40	0.21 0.05-0.36	0.06 0.05-0.06
8		7 months	little improvement		44.8 ±0.7	43.7 ±0.4	111.6 ±3.6	109.3 ±5.6	0.29 0.24-0.34	0.21 0.04-0.42	0.23 0.15-0.31	0.16 0.14-0.20
9	1.5	2 months	mild signs of hypermetria in pelvic limbs after a period of severe ataxia and weakness	no abnormalities	41.6 ±0.7	42 ±0.2	66 ±2.0	74.9 ±4.5	0.39 0.19-0.45	0.60 0.39-0.82	1.05 0.97-1.14	1.71 1.25-2.12
10	2	several months	straddle-legged gait and hypermetria of the pelvic limbs	subluxation between C1-C2	54.6 ±0.9	53.8 ±0.5	164.2 ±5.7	150 ±14.3	0.22 0.17-0.28	1.46 0.91-1.83	0.64 0.10-0.88	0.59 0.31-0.94
11	1	1 week	severe ataxia and difficulties to keep upright	Malformation of the caudal part of C2-C5	134.5 ±8.8	140.7 ±6.7	nf	nf	0.20 0.13-0.22	0.18 0.17-0.20	nf	nf
12	1.5	1 month	obvious ataxia, weakness and circumduction in all limbs	fracture of a transverse process of C6	37.9 ±0.7	37.9 ±1.1	109 ±9.9	112.7 ±6.1	0.46 0.28-0.79	0.71 0.30-1.02	1.25 1.00-1.46	1.30 0.92-1.62

All horses improved neurologically after several months and some horses were re-examined. However, normalization of the MMEPs did not occur in any of these horses.



← cranial caudal →

Figure 4a and 4b. A 2-year old warmblood horse (horse 10) was slightly ataxic for several months. Subluxation between C1 and C2 is probably present; the vertebral canal diameter at C1-C2 measured 27 mm, the caudodorsal part of the third and fourth vertebral bodies are enlarged (arrow), "C3 more than C4"; narrowing of the vertebral canal is evident at the caudal part of C3 (21mm compared to 27 mm at the cranial part of C3), (C = cervical vertebra).

Radiographic examination did not reveal any abnormalities in 6 out of 11 horses. In one horse (horse 10) a subluxation between C1 and C2 (Fig. 4a) was evident and narrowing of the caudal orifice of the vertebral canal was present at several vertebrae (Fig. 4a,b), the latter due to a caudodorsal enlargement of the vertebral bodies (cervical vertebral malformation, CVM).

In a second horse (horse 7), one small osteochondral fragment was found cranial and dorsal to the cranial epiphysis of C6 and two other fragments in a similar location at C7 (Fig. 5); the caudodorsal vertebral body outlines of C5 and C6 were enlarged as well (CVM). In a third horse (horse 12) a fracture of a transverse process of the 6th cervical vertebra was present.

In two other horses (horse 6 and horse 11) a moderate malformation of the caudal part of the vertebral bodies from C2 to C5 was observed (CVM), with abnormal flexion between C2 and C3. In addition, narrowing of the vertebral canal between C3/C4 and C5/C6 was present in horse 11 as well.

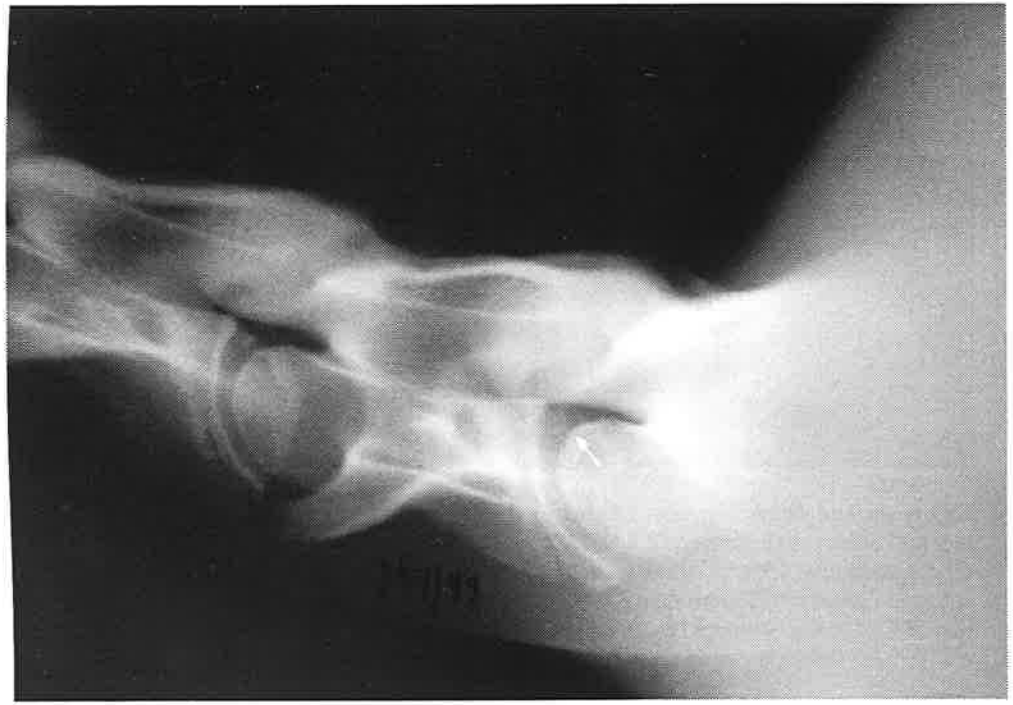


Figure 5. Cervical radiographies of horse 7. There is slight malformation at C5 and C6; one small osteochondral fragment is seen craniodorsal to and probably detached from the cranial epiphysis of C5 and 2 such fragments (white arrow) cranial to and likely detached from the cranial epiphysis of C6. The fragments might be an expression of trauma or osteochondrosis dissecans.

DISCUSSION

Lesions of the spinal cord often produce important abnormalities in movement.

The wobbler syndrome primarily affects the hind limbs. A focal, cervical, pressure-induced necrosis of the gray and especially white matter and some focal loss of neurons are observed. With time secondary Wallerian-like, neuronal fibre degeneration occurs in ascending pathways cranial to and descending pathways caudal to the lesion. The pathogenesis of the syndrome is multifactorial. The damage can result from malformation of cervical intervertebral articular processes, resulting in vertebral subluxation, with subsequent pressure on the spinal cord. Another cause of wobbler disease is dynamic stenosis induced by instability of the vertebrae (Nixon and Stashak, 1982, Wagner *et al.*, 1985, Ekman, 1990). Most affected horses are young, which may indicate that a growth disturbance of the vertebrae is an important factor in the pathogenesis.

Chapter 5. Part1. The use of MMEPs in horses with cervical spinal cord disease

The anatomical evidence of cervical spinal cord lesions should rely on radiographic findings. However, clinical neurological signs often do not correspond to the radiographic location of the lesions. Incidental radiographic abnormalities without clinical signs are common, and electrophysiological studies are important in determining whether anatomic abnormalities are functionally and clinically relevant.

The radiographic changes are unquestionable, but their significance might be questionable in the affected horses in this series. The effect of flexion and extension was not assessed and it is possible that a positive effect on the narrowing of the vertebral canal might or might not have been present in some cases. Small osteochondral fragments detached traumatically at the edge of an epiphysis are always a clear indication of severe trauma. Neurological signs of spinal ataxia can easily be explained if the fragments are traumatic. However, if such fragments are part of an osteochondrosis dissecans, their effect on spinal ataxia is doubtful and difficult to explain. Malformation of the vertebrae (CVM) without definite subluxation and definite narrowing of the vertebral canal can hardly explain the effect on the spinal cord.

Transcranial magnetic stimulation is an electrophysiological technique used to elicit motor evoked potentials (MMEPs) that makes it possible to evaluate the functional integrity of the fastest conducting descending motor fibres in the brain and spinal cord (Hess *et al.*, 1987a; Barker *et al.*, 1985). In contrast to electric shocks, magnetic cortical stimuli are painless and can be administered easily to awake patients. A potentially useful application of this technology is to assess the degree of acute spinal cord injury. Meyer and Zentner (1992) showed the high sensitivity of MMEP for detection of lesions along the descending motor pathways in man. Pathological MMEP findings were observed in all patients with clinical signs of motor deficits. False negative findings were not encountered. They even suggested that MMEP is able to detect lesions in patients with subtle or no clinical signs. Fehlings *et al.* (1989) induced an extradural compression injury of the cord at C7 in rats with a modified aneurysm clip. These authors concluded that a significant linear relationship exists between the severity of spinal cord injury and MMEP findings. In dogs, subtle lesions of the spinal cord or subclinical deteriorations in monitored patients were again detected (Sylvestre *et al.*, 1993). The MMEPs registered in the affected horses of this study indicate the potential of cortical magnetic stimulation to detect abnormal conduction in the descending motor pathways in compressive and traumatic diseases of the cervical spinal cord in horses.

The latencies of the MMEPs in the twelve normal horses were slightly longer than those described by Mayhew and Washbourne (1996). However, these authors described MMEPs elicited in Welsh crossbred ponies that are much smaller than the horses used in this study.

The latency reflects total motor conduction time from cortex to the target muscle. MMEP latency is affected by the size of the fibre, the abundance of myelin, and the number of synapses the impulse must cross (Sylvestre *et al.*, 1993). One single cortical stimulation is able to produce multiple descending volleys in the pyramidal tract (Hess *et al.*, 1987b). Both spatial and temporal summation of impulses reaching the spinal motoneuron are necessary before it fires. Therefore, reduction in the descending volley due to conduction block in some fibres or to loss of the fastest conducting fibres by degeneration and use of slower ones, leads to delay in excitation of the ventral horn cell, resulting in latency lengthening. Changes in the latencies were seen in all affected horses in this study.

The more variable delays seen in onset latencies of the affected horses compared to normal horses probably reflected an insufficient synchronization of the descending volley impinging upon the "facilitated" alpha-motoneuronal pool due to an increased temporal dispersion of the propagated impulse along the central motor tracts. Repetitive discharges of the spinal motoneurons due to defective inhibitory processes might partly contribute to the highly dispersed and polyphasic MMEPs.

In man, MMEPs in hand muscles are mono-, bi- or triphasic. Polyphasic morphology is considered abnormal. In more proximal muscles of the arm and muscles of the leg, polyphasic conformation is more frequent (Maertens de Noordhout, 1998). MMEPs recorded in the forelimb of normal horses have a bi- or triphasic configuration, whereas those found in the hindlimb can be more polyphasic. Nearly all MMEPs recorded in the affected horses were severely polyphasic.

The amplitude is influenced by the number of fibres recruited by the stimulus, the number of motor neurons excited by the descending impulses, and the characteristics of the target muscle (Sylvestre *et al.*, 1993). In normal human subjects and in horses, as evidenced by the large standard deviation found in the normal horses, MMEP amplitudes are very variable. The variability appears to be spontaneous and its cause is unknown. It could be explained by the physical changes in the parameters of stimulation (the output of the stimulator, the type and position of the coil) or by neurophysiological changes (the degree of pre-innervation) in the

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excitability of the descending motor pathway (Hess *et al.*, 1986, Ellaway *et al.*, 1998). Therefore, evaluation of amplitudes is difficult even in the same individual and may not always be possible when different patients are compared. In this study, however, a significant attenuation of the amplitudes in all clinical cases was present. Rapid and long-lasting attenuations of MMEP amplitudes were observed in horses with cervical spinal cord lesions even if clinically evident neurological deficits had disappeared. Reduction in response amplitude or absent responses may be due to block, section, or degeneration of the descending motor tract. Depression of spinal motoneuron excitability and increased presynaptic inhibition of descending motor terminals in the spinal cord are other possible mechanisms for amplitude reduction. The smaller the response, the more difficult it is to evaluate MMEP and the onset of the potential may not be easily distinguished from baseline noise associated with facilitatory voluntary pre-innervation of the muscle.

The loss of response in the hind limbs or in all four limbs, seen in some severely ataxic horses, does not necessarily indicate complete paralysis, since this was also observed in human patients showing residual motor function (Meyer and Zentner, 1992). Sylvestre *et al.* (1993) found recordable MMEPs only in dogs with mild or no neurological deficits and in 50% of ambulatory dogs that were severely ataxic. MMEPs could not be elicited from non-ambulatory dogs. In man, this phenomenon is explained by the fact that, in acute traumatic spinal cord injuries, a reduction or absence of voluntary movement and reflex activity below the level of the lesion is apparent for several weeks ("spinal shock") (Adams and Victor, 1989). In horses and dogs, spinal shock is rare and very short, probably lasting for a maximum of 6 hours (Mayhew, 1999). Another explanation for the phenomenon could be found in studies of spinal cord injury in man and animals where MMEPs were recorded simultaneously from the epidural space and the peripheral nerves. In these studies, the waveforms were invariably decreased, delayed, or lost at the peripheral nerve level before changes were noticed in the epidural space (Konrad *et al.*, 1987; Owen *et al.*, 1988, 1989; Kraus *et al.*, 1990). The propagating impulse, although present in the spinal cord caudal to the lesion, may not be strong enough to increase the postsynaptic membrane potential of the motor neuron to its threshold. Therefore, the impulse will not be present in the peripheral nerve. At each synapse with a ventral horn cell, an excitatory postsynaptic potential is generated in response to each wave. Temporal and spatial summation of excitatory postsynaptic potentials occur and, if sufficient to depolarise the anterior horn cell to threshold, it will fire and trigger contraction of its motor unit (Amassian *et al.*, 1987). With these

observations in mind, it is also interesting that, in human medicine, leg (tibialis anterior) MMEPs could not be evoked in 8% of normal human subjects and, therefore, their absence cannot be regarded as abnormal (Eisen and Shtybel, 1990, Maertens de Noordhout, 1998). Compared to the transcranial stimulation in control horses, MMEPs could be elicited in the tibialis cranialis muscle in all horses.

In only one affected horse lateralization of the MMEP responses was evident. In normal human subjects, the side-to-side latency difference recorded from homologous tibialis anterior muscles is described as small (Tobimatsu *et al.*, 1998) and a value exceeding 6 ms was regarded as abnormal (Eisen *et al.*, 1990). Tsai *et al.* (1994) described latency asymmetry of 0.5 ms, detected in arm muscles, as normal in control patients. In our normal horses, there was also only a slight side-to-side latency difference.

All horses improved neurologically after several months and some horses improved sufficiently well to achieve athletic function. The fact that there was no normalization of the MMEPs in any of these horses that were re-tested indicates permanent damage to the cervical spinal cord. These findings also demonstrated the sensitivity of MMEPs in spinal cord lesions. Since all horses were affected before their maximal performance capacity was achieved, it will always be an open question as to whether they ever reached their normal performance capacity. In horses followed-up for several months, the improvement seemed to stabilise at some point. The difference between the minimal clinical signs and the values found with the transcranial stimulation are striking and motivate the use of the technique for an objective evaluation of the integrity of the motor tracts.
In man, the changes in the MMEPs also remain after successful surgical decompression of the spinal cord, independent of the clinical syndrome. This is caused by changes in the vascularisation pattern and by atrophy of the spinal cord, which is often irreversible after a prolonged compression (Sandler *et al.*, 1976a, 1976b; Tator *et al.*, 1991; Maertens de Noordhout, 1998).

Clinical examination is the most essential part of diagnosis and prognosis in a neurological evaluation. However, we believe that MMEP examination by cortical stimulation is a very valuable diagnostic tool for objective assessment of motor function. The method seems to be safe in horses and, as in human subjects, no untoward effects have been experienced. Pathological MMEP indicates damage along the descending pathways, even in cases when

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clinical examination shows only subtle signs. MMEP may indicate the clinically most affected side but fails to provide quantification of the clinical motor deficit, because no linear correlation is found between the clinical motor status and the electrophysiological changes. Further studies are necessary to define more clearly the essential factors influencing latencies and amplitudes of motor responses. They should provide evidence as to whether additional evaluation of other variables, e.g. configuration and/or duration of the potentials, could attribute to a more precise correlation between clinical and electrophysiological findings.

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Section 2.

Evaluation of transcranial magnetic stimulation in 53 ataxic horses suspected of having cervical spinal cord lesions

SUMMARY

Magnetic motor evoked potentials (MMEPs) were recorded in 53 horses suspected of having cervical spinal cord disease. The onset latency and peak-to-peak amplitude of MMEPs recorded in the extensor carpi radialis and cranial tibial muscles were measured. In only 2 horses normal MMEPs were found. In 42 horses, MMEPs had prolonged onset latencies and, in most horses, a small peak-to-peak amplitude. Polyphasic pattern MMEPs were also observed in many of the horses. No MMEPs could be recorded in the front and hind limbs of one horse that was destroyed "after one week at home" because of acute worsening of the signs leading to recumbency. No MMEPs could be recorded in the hind limbs only in 8 horses. Only one of these horses could be re-evaluated after 8 months; MMEPs had returned but had prolonged onset latencies and small peak-to-peak amplitudes.

In only one of 8 re-evaluated horses, MMEPs were normalised 6 months after the first examination. This horse had had abnormal MMEPs only on the left side and showed no radiographic abnormalities of the cervical vertebrae.

Four horses were referred in lateral recumbency. MMEPs recorded in these horses were all prolonged. They had a very small peak-to-peak amplitude and in one of these animals MMEPs could not be found in the hind limbs. In three of these horses a cervical vertebral fracture was diagnosed by radiography and/or postmortem examination.

INTRODUCTION

An initial evaluation of transcranial magnetic stimulation (TMS) in 12 ataxic horses (Nollet *et al.*, 2002; chapter 5, part 1, section 1) revealed a good sensitivity of the technique to detect lesions involving the descending motor pathways in horses. When comparing the MMEPs recorded in the extensor carpi radialis and cranial tibial muscles of these horses to the MMEPs recorded in the same muscles of 12 normal horses of similar height, a significant difference in onset latency and/or peak-to-peak amplitude could be demonstrated, indicating a clinically significant lesion along the cervical spinal cord. Recently, the technique has been standardised in horses (Nollet *et al.*, 2003a; 2003b) and reference values for horses of different heights were defined (Nollet *et al.*, 2003c). In the present work, we report TMS findings in a larger number of ataxic horses.

MATERIAL AND METHODS

All 53 horses had acute or chronic clinical signs of ataxia in all four limbs. Four were referred in lateral recumbency. The age ranged from 14 days to 17 years (4.06 ± 4.19 years). Five horses (4 to 6 years old) were presented with a history of "irregular gait" since purchase. Only in one of these cases (2 years old) very mild ataxia was diagnosed by the local veterinarian when performing a clinical pre-purchase examination. At the time of purchase one owner was told that the horse was fallen not long ago and was therefore a bit lame. Two other horses were thin at time of purchase and the salesman declared they were weak because of leanness. After a few months of feeding, however, the new owners still noticed the "irregularity or weakness" and referred the horses for further examination.

A clinical neurological examination was performed on all horses. Thereafter they were sedated with a combination of detomidine (Domosedan®, Pfizer, Belgium; 10 µg/kg of bwt) and buprenorphine (Temgesic®, Schering-Plough, Belgium; 2.4 µg/kg of bwt) intravenously to perform transcranial magnetic stimulation. Stimulation was done as described in chapter 4. The onset latency and peak-to-peak amplitude of the obtained MMEPs were compared to the reference values described in chapter 4.

In all cases, except three, the cervical vertebrae were radiographed.

In 8 of the 53 cases postmortem findings were available.

RESULTS

Clinical findings and TMS

Fortynine horses showed ataxia in all four limbs and four were tetraplegic.

After TMS, mean (±SD) onset latency in these 53 cases was 49.60 ± 56.98 ms for the extensor carpi radialis muscle and 76.61 ± 38.13 ms for the cranial tibial muscle. The mean (±SD) peak-to-peak amplitude was 3.06 ± 4.46 mV for the extensor carpi radialis muscle and 3.29 ± 10.76 mV for the cranial tibial muscle. A polyphasic pattern was observed in many cases.

In some chronic cases, MMEPs had sometimes only a slightly prolonged mean onset latency, but with a too large intra-measurement difference or they had a small amplitude, a polyphasic configuration or a long duration when compared to normal MMEPs.

In only 2 horses with clear ataxia in all four limbs no MMEP abnormalities could be found.

In 8 cases no MMEPs could be recorded at the first evaluation in the hind limbs only and in one case in all four limbs. However, these horses were not para- or tetraplegic. The latter horse was destroyed at home after one week because of acute worsening of the signs leading to recumbency suddenly could not stand up. Cervical radiographs revealed malformation of multiple vertebrae. Of the 8 horses where MMEPs were found only in the forelimbs, only 1 horse was re-evaluated and after 8 months from the initial examination small and prolonged MMEPs were found in the hind limbs. The remaining 7 horses were not referred for re-evaluation for the following reasons: 4 horses were slaughtered or euthanized because of recumbency or because their gait remained ataxic, 1 horse was sold to a horse-dealer and lost for follow-up, 1 horse is still slightly ataxic according to the owner and is used as a companion animal (not trained) and one horse is still too young to perform and will be re-evaluated at a later stage.

Four horses were referred in lateral recumbency and in all MMEPs were prolonged (54.3 ± 34.66 ms in the extensor carpi radialis muscle and 92.25 ± 62.11 ms in the cranial tibial muscle) and very small (0.67 ± 0.74 mV in the extensor carpi radialis muscle and 0.44 ± 0.28 mV in the cranial tibial muscle). In only one of these paralysed horses MMEPs could not be recorded in the hind limbs. All four horses were euthanized.

In all horses except 1 that were re-evaluated after a certain time (n=7) there was an improvement only of the MMEPs (shorter onset latency or larger peak-to-peak amplitude) but no normalisation, even when the owner stated that the horse walked normally again. In some cases the shape of the MMEPs also changed from very small polyphasic potentials to larger but still serrated waves. One horse in which only the MMEPs on the left side were abnormal

at the initial evaluation (mean (±SD) onset latency of the MMEPs recorded in the right extensor carpi radialis muscle: 21.82 ± 0.05 ms; in the left extensor carpi radialis muscle: 23.02 ± 0.13 ms; in the right cranial tibial muscle: 35.32 ± 0.93 ms and in the left cranial tibial muscle: 41.07 ± 0.97 ms), showed an improvement after 3 months (onset latency of left extensor carpi radialis muscle: 22.85 ± 0.24 ms; left cranial tibial muscle: 39.85 ± 0.13 ms) and a normalisation of the MMEPs after 6 months (onset latency of left extensor carpi radialis muscle: 22.43 ± 0.06 ms; left cranial tibial muscle: 36.92 ± 0.45 ms). In this horse no radiographic bony abnormalities were observed.

In the 5 horses presented with an irregular gait since purchase MMEPs were recordable in all muscles (onset latency: 34.47 ± 10.08 ms in the extensor carpi radialis and 77.75 ± 15.85 ms in the cranial tibial muscle), had already a larger amplitude (3.11 ± 3.31 mV in the extensor carpi radialis and 1.66 ± 2.01 mV in the cranial tibial muscle) and, in most cases, a more organized configuration when compared to more acute cases (see figure 1).

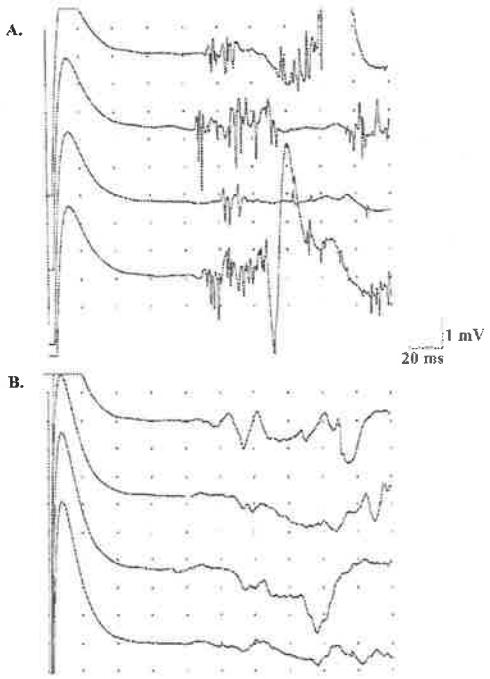

Figure 1. Four MMEPs recorded in the left cranial tibial muscle of **(A.)** a yearling mare with signs of severe ataxia of 2 days duration and of **(B.)** a four-years old gelding who was bought 3 months previously and showing signs of "weakness" since purchase.

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Cervical radiography

One of the three cases where no radiographs of the cervical vertebrae were taken was totally paralysed and euthanized after TMS. A fracture of C3 was found on necropsy examination. The two other cases were not radiographed because they were too ataxic but had normal MMEPs; they are discussed below.

In 15 cases no bony abnormalities were detected on the cervical vertebrae. However, in some of these cases severe ataxia or very abnormal MMEPs were observed.

In the 35 examined horses where radiographic abnormalities were found in the cervical vertebrae, malformations and sub-luxation (n=18), fractures (n=7), osteochondrosis and osteoarthrosis (n=10) were diagnosed.

Necropsy examinations

Of the 8 horses that were euthanized, four were referred in lateral recumbency. In three of these 4 horses a fracture of a cervical vertebra (C3 in two cases, C5 in one case) was diagnosed. In the fourth case, malformation with narrowing of the vertebral canal at multiple sites was diagnosed.

In two of the four remaining horses, the radiographic findings (osteoarthrosis C5-C6 and sub-luxation) could be confirmed. The remaining two horses were those in which, despite severe ataxia, normal MMEPs were recorded. The oldest horse had progressive signs during the last 14 days and had a normal serum vitamin E level (4.7 mg/l; normal > 1.5 mg/l). This horse was euthanized three days after evaluation. Histopathological examination revealed irregularly localised myelopathy and neuroaxonal swelling and dystrophy in the dorsal column of the cervical spinal cord, the ventral part of the lateral funiculus of the thoracic spinal cord and the dorsal part of the lateral funiculus of the lumbar spinal cord. The youngest horse showed acute, severe ataxia during one day and had a low serum vitamin E concentration (1.2 mg/l). Oral supplementation of vitamin E (6.000 IU/day) did not improve the ataxia sufficiently after 9 days and the owner decided to have the horse euthanized. On histopathological examination, severe myelomalacia, neuroaxonal swelling and many gitter cells were found bilaterally symmetrical in the ventral and lateral funiculi of the cervical spinal cord and to a lesser degree in the thoracic spinal cord.

DISCUSSION

The utility of MMEP recordings has been shown in a limited number (12) of horses with cervical spinal cord lesions (see chapter 5, part 1, section 1). Recently TMS has been standardized for application in horses (see chapter 3) and reference values for MMEPs were defined (see chapter 4). Based on these reference values MMEPs were examined in 53 additional horses suspected of having cervical spinal cord disease (see chapter 5, part 1, section 2). Since the results found in this larger group of horses confirm the results of the initial limited study, all 65 cases will be discussed together.

The most severe cases of cervical spinal cord disease can be diagnosed by neurological examination. However, clinical diagnosis is not always confirmed by the currently available diagnostic techniques, including cervical radiography. Also, as described in man (Teresi *et al.*, 1987; Yone *et al.*, 1992), the severity of abnormal radiological findings poorly correlates with the clinical picture. Moreover, it is sometimes difficult to define subtle gait irregularities as being neurological in origin. Therefore, TMS is very helpful since it gives an objective assessment of the functional integrity of the descending motor tracts.

In only two of the 65 cases with severe ataxia in both thoracic and pelvic limbs, normal MMEPs were found. These two cases were warmbloods and could have had a purely sensory ataxia. They also did not have clear weakness. To demonstrate this, somatosensory evoked potentials (SSEPs) should be monitored simultaneously. This technique tests the integrity of the ascending sensory pathways, by giving multiple electrical stimuli to peripheral nerves and recording the small evoked potentials over the brain. Due to the multiple stimuli and the small amplitude of the SSEPs, they are very difficult to record in awake horses. The two warmblood horses were 6 and 17 years old and in neither was there a known familial incidence of ataxia. The severe ataxia together with the normal descending motor tracts and the histopathological findings were suggestive of atypical cases of equine degenerative myeloencephalopathy (EDM). However, EDM is mainly described in very young horses (4 to 24 months) (Mayhew *et al.*, 1977; 1987; Gruys *et al.*, 1994) and seems to be caused by vitamin E deficiency. In other species, e.g. German Shepherds, chronic degenerative myelopathy is mostly seen in aging dogs and is suggested not to be due to vitamin E deficiency (Averill, 1973; Johnston *et al.*, 2001).

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Only 13 of the 65 referred horses could be re-evaluated after several months or years. The major reason for this low follow-up was the fact that most horses still showed residual ataxia and the owners decided to slaughter or to sell them. Among the re-evaluated cases, one horse was only re-evaluated after three years and the initial findings were described in section 1 (horse 11). According to the owners it had been ataxic for one year after the first visit but was now said to be completely sound. In the mean time, the owners had trained the horse and used it on rare occasions as a leisure horse (walking and small jumps). On clinical examination there was still ataxia (grade I) in all four limbs. The MMEPs had improved considerably, but were still abnormal. In all other re-evaluated cases, MMEPs had improved also but had normalised in one horse only. At the initial evaluation in that horse, MMEPs were unilaterally abnormal and radiography showed no bony abnormalities. Therefore it is possible that there was only a relatively mild reversible traumatic insult. Comparable results are reported in humans. Maertens de Noordhout and co-workers (1998) described evoked potentials (MMEPs and SSEPs) as a useful paraclinical diagnostic test in 55 patients with myelography-documented cervical spinal cord compression or intervertebral disk herniation. The studies were repeated after 1 year follow-up of 43 patients that had undergone surgery and in 12 patients treated by conservative approach. These follow-up studies showed that in the majority of patients with long-standing illness, the abnormalities also persisted in spite of clinical improvement in most patients. Although there was a significant trend toward shortening of mean central motor conduction time, they remained abnormal in most cases. This probably reflects permanent vascular or necrotic lesions induced in the cord by spondylotic changes and the same authors also concluded that serial MMEP studies might be useful to monitor disease progression in unoperated patients. In most unoperated patients MMEP studies after 1 year showed little change, which confirms that the natural evolution of cervical spondylotic myelopathy is very slow in many cases (Nurick, 1972). This is further illustrated by the fact that MMEP abnormalities are sometimes observed in patients with few clinical signs of motor dysfunction (Travlos *et al.*, 1992).

In some horses the shape of the potentials changed from very small polyphasic waves to a more organized but still serrated configuration with an apparent longer duration than normal potentials. This was also seen in some of the older horses where signs were present since purchase. Maybe this less complex or more organized shape together with the still prolonged onset latency can indicate the chronicity and the incomplete recovery of the lesion.

Only one of the horses in which no MMEPs were present in the hind limbs or in any limb could not stand up. Additionally, one of the two animals in which no MMEPs could be found in any muscle became totally paralysed after one week and was slaughtered. Thus, in these few cases the term "complete motor blockade" was not an accurate reflection of the neurologic status of the horses. This conduction block can be explained by demyelinization or by desynchronization of the descending volleys generated by cortical shocks and was already discussed in a previous publication (Nollet *et al.* 2002). Briefly, one single cortical stimulation is able to produce multiple descending volleys in the pyramidal and extrapyramidal tracts and both spatial and temporal summation of impulses reaching the spinal motoneuron are necessary before it depolarises. Therefore, conduction block in some fibres or loss of the fastest conducting fibres by degeneration can lead to a propagating impulse which may not be strong enough to increase the postsynaptic membrane potential of the motor neuron to its threshold and an impulse will not be present in the peripheral nerve.

Some horses where initially no MMEPs could be recorded in the hind limbs are now used as leisure horses. On the other hand no MMEPs could be recorded only in one of the four horses referred in lateral recumbency. These findings indicate the poor prognostic capacity of the technique. In humans with spinal cord disease or stroke also contradictory findings concerning prognosis are reported (Clarke *et al.*, 1994;Macdonell and Donnan, 1995). Furthermore, only early MMEP recordings made within 24 hours from the beginning of ischaemic stroke provides useful information on the clinical prognosis and the different mechanisms of motor recovery in man (Trompetto *et al.*, 2000; Hendricks *et al.*, 2002).

One of the major drawbacks of this study is that postmortem results were only available in 8 horses. However, in these few cases, there was a good correlation between the MMEP-results and the findings at necropsy examination.

We can conclude that transcranial magnetic stimulation is very useful as a diagnostic tool in horses with ataxia. In addition to the clinical and radiographic examination, it gives an objective measure of conduction along the descending motor tracts. Especially in more chronic cases, where the more subtle signs of ataxia could not always be differentiated from lameness or weakness by an unexperienced eye, the technique is very promising.

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Nothwithstanding the fact that in most cases of cervical spinal cord lesions both the motor and sensory tracts will be damaged, SSEPs would be interesting to determine whether pure sensory deficits could cause ataxia in cases where normal MMEPs were found.

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CHAPTER 5

Part 2.

THE USE OF MAGNETIC MOTOR EVOKED POTENTIALS IN HORSES WITH BILATERAL PELVIC LIMB ATAXIA

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SUMMARY

The motor cortices of eight horses and one donkey with signs of only bilateral pelvic limb ataxia and weakness of varying degree were stimulated magnetically and the magnetic motor evoked potentials (MMEPs) were recorded bilaterally in the extensor carpi radialis and cranial tibial muscle.

In six animals, MMEPs with normal onset latencies and peak-to-peak amplitude were recorded in the extensor carpi radialis muscle and abnormal onset latencies and peak-to-peak amplitude were recorded in the cranial tibial muscle. In these animals a spinal cord lesion at the thoracolumbar segments was suspected. In three animals, onset latencies and peak-to-peak amplitude of MMEPs recorded in both extensor carpi radialis and cranial tibial muscle were abnormal. In these animals a cervical spinal cord lesion was suspected. We can conclude that TMS can be considered as a valuable diagnostic tool for assessing the thoracolumbar spinal cord and can be used for differentiating thoracolumbar spinal cord disease from mild cervical spinal cord lesions, causing signs in the hind limbs only.

INTRODUCTION

A subtle gait irregularity of the hind limbs can be a diagnostic challenge. Lameness, back pain or spinal cord disorders have to be differentiated between. However, it is difficult to evaluate spinal cord lesions as a possible cause of such syndromes. Furthermore, it is clinically difficult to distinguish horses with very mild cervical spinal cord lesions, causing clinical signs in the hind limbs only, from those with spinal cord lesions at the thoracolumbar segments. Radiographic examination of the thoracic and lumbar vertebrae is difficult even by the currently available powerful equipment. Also, scintigraphy does not give any information about the functional integrity of the spinal cord. Therefore, techniques to evaluate the function of the neural motor pathways to the hind limb muscles would be useful. Transcranial magnetic stimulation (TMS) and the recording of the MMEPs allow functional evaluation of these descending motor tracts.

Painless transcranial magnetic stimulation of the cerebral cortex was introduced in 1985 by Barker and co-workers in man, using short magnetic pulses produced by a device designed to stimulate peripheral nerves (Polson *et al.*, 1982). Stimulating currents within the nervous tissue are induced by a time-varying magnetic field, generated by a brief high-voltage current passed through a copper coil (Barker *et al.*, 1987). The major advantage of magnetic stimulation over electrical transcranial brain stimulation, introduced by Merton and Morton in 1980, is the absence of pain when stimuli are applied to the scalp. The technique is a valuable diagnostic tool in the early objective assessment of motor function in spinal cord compression (Barker *et al.*, 1987; Gianutsos *et al.*, 1987; Maertens de Noorthout *et al.*, 1991; Travlos *et al.*, 1992; Linden and Berlit, 1994; Kirshblum and O'Connor, 1998; McKay *et al.*, 1997). In veterinary medicine, TMS has been performed on healthy animals to standardize the method of stimulation (Heckmann *et al.*, 1989; Cook *et al.*, 1990; Mayhew and Washbourne, 1996) or to assess anaesthetic impact on the recorded MMEPs (Sylvestre *et al.*, 1992; Young and Sylvestre, 1992; Sloan, 1994; Young *et al.*, 1994; Van Ham *et al.*, 1994, 1995a, 1995b 1996a, 1996b, Nollet *et al.*, 2003a). In dogs suspected of having spinal cord lesions, TMS could demonstrate a cervical (Poma *et al.*, 2002) or thoracolumbar (Sylvestre *et al.*, 1993) spinal cord lesion. The successful application of the technique was reported in horses with cervical spinal cord compression (Nollet *et al.*, 2002a) and with some other neurological disorders (Nollet *et al.*, 2002b).

The objective of the present study was to determine whether TMS would be a reliable technique to assess the functional integrity of the thoracolumbar spinal cord in horses and to differentiate objectively between mild or severe thoracolumbar spinal cord lesions and mild cervical spinal cord disease.

MATERIAL AND METHODS

A. Magnetic stimulation

The experimental protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine, University of Ghent, Belgium (reference 18/2000). All patients (9 horses and one donkey) were stimulated transcranially (Magstim 200, Novametrix, U.K.) using a round 70 mm coil (generating a maximal magnetic field of approximately 4 Tesla). The stimulus intensity was 100% of the maximal output. The coil was centered over the forehead. In accordance to human studies, side A or side B of the coil was up, when recording was done from the right or left side of the body respectively. In preliminary studies (Nollet et al., 2003b) the direction of the current flow within the coil had no influence on the registered responses in horses.

The stimulator triggered the sweep of the electromyography (EMG) machine, recording the evoked potentials, enabling the onset latency of the responses to be recorded (see later). Only single stimuli were applied.

B. Recording of MMEPs

Recordings were obtained bilaterally from the extensor carpi radialis and cranial tibial muscles using a standard EMG machine (Medelec Sapphire; Medelec Ltd., Old Woking, Surrey, England). Active needle electrodes were inserted in the middle of the respective muscle bellies and the reference electrode was placed subcutaneously at the lateral side of the radial tuberosity and at the lateral malleolus of the tibia. A ground electrode (alligator clip) was attached in the elbow region and in the groin region.

Bandpass filter settings were 20 Hz to 3 kHz. Time base (ranging from 100 to 200 ms) and sensitivity (ranging from 100 μV to 5 mV per division) were adjusted in order to allow the most precise analysis on the screen.

Four potentials were obtained from each recording site and the recordings superimposed, to compensate for the within test variation. Onset latency (in ms) was measured as the shortest distance between the trigger point and the take-off of the initial negative or positive phase. Peak-to-peak amplitude (in mV) was measured between the two largest peaks of opposite polarity. The study was approved by the faculty ethics committee. Although the stimulation is

painless, the mild discomfort induced by evoked muscle contraction and the noise of stimulation can agitate some horses. Therefore the horses were sedated with a combination of detomidine (Domosedan®, 10 µg/kg of bwt) and buprenorphine (Temgesic®, 2.4 µg/kg of bwt) intravenously. Administration of this combination does not influence the measured parameters of the MMEPs (Nollet *et al.*, 2003a).

C. Additional examinations

In horses with a suspicion of a cervical spinal cord lesion from the MMEPs, a radiography of the cervical vertebrae was performed. In 2 horses (lighter horses), the back was radiographed. In horses in which TMS had raised a suspicion of a lesion caudal to Th2, needle electromyography of segmentally innervated epaxial muscles was performed. Any spontaneous activity (positive sharp waves and/or fibrillation potentials) was recorded, indicating an active local lower motor neuron lesion. A rectal palpation was performed to evaluate the ventral aspect of the lumbar and sacral vertebrae.

RESULTS

The patients included one young donkey, one Spanish horse, one trotter, 3 warmbloods, two thoroughbreds, 1 Belgian draught horse and one Romanian horse, from 9 months to 20 years of age (8.3 ± 5.3 years). The donkey (patient 2) and the draught horse (patient 10) were stallions, 4 horses (patient 1, 3, 6 and 9) were geldings and 4 horses were female. Weight ranged from 70 to 900 kg with a mean of 505.0 ± 207.7 kg.

The history and clinical signs are summarized in table 1. The horses were referred for hind limb lameness, or were suspected of having back pain, or weakness or ataxia in the hind limbs. Duration of the clinical signs ranged from 1 day to minimally 1.5 years or more (day of purchase) before referral. None of the patients had weak muscle tone of the tail or an abnormal perineal reflex (ie. signs of cauda equina involvement). On the basis of the neurological examination, ataxia in the hind limbs was the main clinical finding and a spinal cord lesion caudal to the second thoracic (Th2) segment was suspected.

Age, height, radiographic, electromyographic and TMS findings are summarized in table 2. Significant differences from reference values (Nollet *et al.*, 2003b) were detected in mean

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MMEP latencies (increase) and amplitudes (decrease) of both hind limbs or of all four limbs, indicating a thoracic/thoraco-lumbar spinal cord lesion (n=6) or a cervical cord lesion (n=4), respectively.

Table 1. History and clinical signs of patients.
The grading system from 0 to 4 described by Mayhew *et al.* (1978) has been used: Mayhew IG, de Lahunta A, Whitlock RH *et al.* Spinal cord disease in the horse. *Cornell Vet* 1978;68 (Suppl 6):24-29.

Patient N°	History	Clinical signs
1	The horse showed a gait disturbance since purchase (5 months ago), initially thought to result from a kick on its hind limbs. Because there was no improvement, the local vet referred the horse.	Ataxia and swaying (grade III) in the hind limbs.
1'	Horse 1 was referred for reevaluation after having been put on pasture for one year. According to the owner, the horse stumbled less, could stand up better and had a normal gallop.	Same clinical signs (grade III ataxia in hind limbs).
2	Fourteen days previously, the donkey was paraplegic. With help it could stand up and severe ataxia in the hind limbs was observed. Treatment (NSAIDs, acupuncture and homeopathy) was unsuccessful.	Ataxia (grade IV) in the hind limbs. When backing, the donkey would dogsit. By palpating the back, a large bony mass was felt at the left side of the withers.
3	Ataxia and weakness in the hind limbs was present for 6 months. In the acute stage, the horse reacted painfully to palpation of the thoracolumbar region and treatment for back pain (corticosteroids) gave little improvement.	Very mild ataxia (grade I) in the hind limbs, especially seen when circling. Mild toe dragging, especially with the right hind limb.
4	When feeding the horse, the owners observed acute weakness in the hind limbs.	Ataxia (grade III) in the hind limbs. When circling, clear circumduction and crossing of the hind limbs was observed.
5	The horse was in shock and showed symptoms of ataxia after a trailer crash. TMS was not performed until 4 months after the accident.	Ataxia (grade II) in the hind limbs.
6	Hind limb weakness and ataxia, together with hematuria for 1.5 months.	Ataxia (grade III) and swaying in the hind limbs. Hematuria (blood thrombus coming from the right ureter)
7	This trotter (at that time 3 years old) was given to the owner as a present after a victory in a "cycling classic". From the beginning the trainer observed an irregularity in the left hind limb and referred the horse for a lameness examination.	Ataxia (grade II) in the hind limbs. Especially when circling, circumduction and crossing of the hind limbs became obvious. When turning the horse showed stiffness of the neck.
8	The rider had been riding this horse for 2 years and always encountered difficulties on galloping: easily falling in cross gallop and difficulties to collect the gallop. Jumping performance was very irregular and the horse had difficulties in combination obstacles. There was occasional swaying in the hind limbs.	Ataxia (grade II) and swaying in the hind limbs
9	Two years previously,this horse was bought as coach horse. When the horse pulled the coach, the owner observed regular over extension of the pasterns of the hind limbs and referred the horse for lameness.	Ataxia (grade II) with circumduction of the hind limbs.
10	During a show, this four-year-old stallion was judged to be too weak in the hind limbs. The owner never observed anything.	Very mild ataxia (grade I) with mild circumduction of the hind limbs when turning.

Table 2. Data and individual results of MMEPs in the clinical patients.

Patient N°	Height (cm)	Age (years)	RX	EMG	Onset latency MMEP (ms; mean (±SD)) ECR Right	ECR Left	CT Right	CT Left	Peak-to-peak amplitude MMEP (mV; mean (±SD)) ECR Right	ECR Left	CT Right	CT Left	Diagnosis
1	154	9	np	NAD	20.7 (±0.13)	21.0 (±0.11)	114.5 (±4.93)	93.3 (±9.78)	7.1 (±1.11)	6.7 (±1.69)	1.4 (±0.48)	0.88 (±0.22)	Th/Th-L
1'	154	10	np	NAD	21.2 (±0.13)	21.3 (±0.06)	118.0 (±12.33)	95.2 (±11.23)	11.5 (±0.34)	6.9 (±0.42)	1.4 (±0.56)	4.8 (±1.13)	Th/Th-L
2	110	<1 (9m)	Kyphosis, scoliosis, hemivertebra Th6-Th11	Th6-Th11: PSW and FP	18.6 (±0.08)	18.3 (±0.09)	53.3 (±2.13)	53.8 (±5.23)	6.1 (±1.61)	5.2 (±2.17)	1.4 (±0.24)	1.2 (±0.53)	Th/Th-L
3	164	10	np	Th-L + L1: PSW and FP	22.4 (±0.06)	22.8 (±0.06)	74.1 (±3.73)	84.1 (±11.51)	2.9 (±0.49)	3.0 (±0.47)	2.4 (±0.65)	1.2 (±0.45)	Th/Th-L
4	165	11	np	NAD	21.9 (±0.49)	22.5 (±0.10)	64.3 (±1.82)	66.1 (±4.83)	4.2 (±0.54)	2.6 (±0.38)	1.1 (±0.17)	1.2 (±0.17)	Th/Th-L
5	169	8	np	NAD	20.5 (±0.10)	20.7 (±0.0)	39.7 (±2.01)	40.7 (±1.19)	13.5 (±2.23)	14.0 (±3.31)	2.5 (±0.36)	4.7 (±2.39)	Th/Th-L
6	170	20	NAD	NAD	22.2 (±0.08)	21.9 (±0.15)	42.0 (±0.80)	40.2 (±1.83)	6.6 (±2.09)	11.9 (±0.80)	0.9 (±0.38)	0.9 (±0.16)	Th/Th-L
7	168	5	Osteo-arthrosis C6-C7	np	26.1 (±0.39)	26.8 (±0.53)	58.1 (±12.10)	74.2 (±13.89)	0.9 (±0.1)	1.7 (±1.24)	1.2 (±0.27)	1.1 (±0.36)	C
8	167	8	Osteo-arthrosis C5-C6 and C6-C7	np	25.8 (±0.24)	27.3 (±0.53)	47.1 (±1.23)	45.2 (±3.18)	8.5 (±3.79)	5.6 (±1.72)	2.1 (±0.80)	0.2 (±0.06)	C
9	162	6	CVM at multiple levels	np	31.4 (±3.80)	31.0 (±2.61)	53.5 (±3.89)	57.7 (±5.27)	9.6 (±1.90)	2.7 (±0.59)	2.9 (±0.84)	7.8 (±5.25)	C
10	170	5	NAD	np	25.8 (±0.74)	26.7 (±0.41)	43.3 (±1.26)	45.1 (±1.37)	2.4 (±2.55)	0.5 (±0.24)	1.2 (±1.11)	2.1 (±2.15)	C

np = not performed; NAD = no abnormality detected; Th = thoracic; L = lumbar; C = cervical;
CVM = cervical vertebral malformation; PSW = positive sharp waves; FP = fibrillation potentials

In none of the horses lateralization of the lesion could be demonstrated by TMS. MMEPs recorded from the extensor carpi radialis and the cranial tibial muscle, of a normal horse, a horse suspected of having a thoracolumbar lesion (patient 6) and a horse suspected of having a cervical spinal cord lesion (patient 9) are outlined in figures 1 and 2.

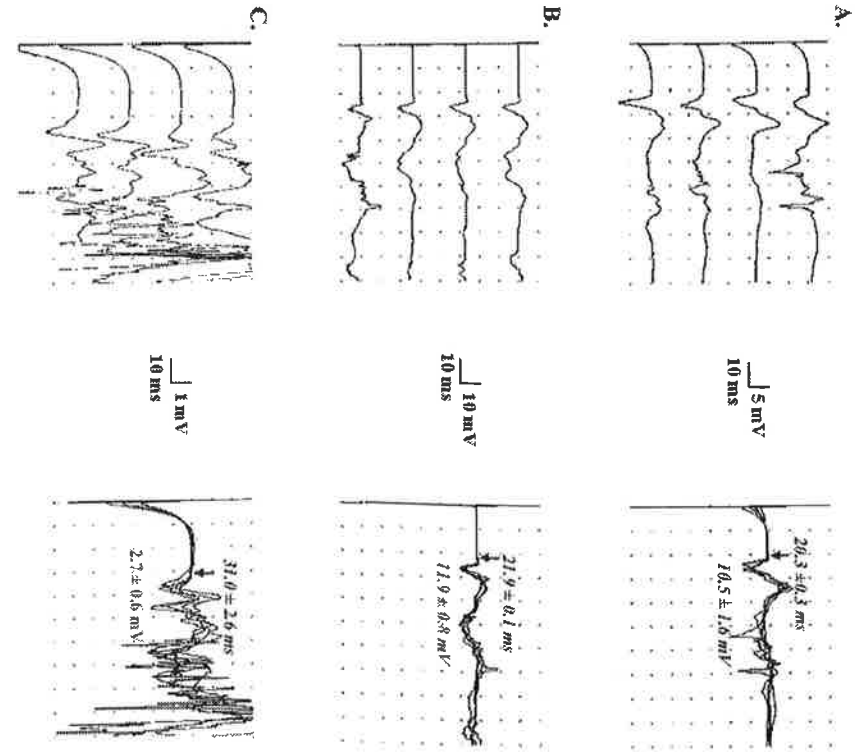

Figure 1. Motor potentials evoked by transcranial magnetic stimulation and recorded from the left extensor carpi radialis muscle in one normal horse (A. gelding, 6 years old, 155 cm height at withers) and two patients with hind limb ataxia (B. patient n° 6, gelding, 20 years old, 170 cm height at withers, thoracolumbar spinal cord lesion; C. patient n° 9, gelding, 6 years old, 162 cm height at withers, cervical spinal cord lesion). Note the different scaling for peak-to-peak amplitude (mV) between the different horses. On the right the superimposed tracings of the left individual tracings are represented. The mean (±SD) values for onset latency (see arrow) and peak-to-peak amplitude are marked on the superimposed tracings.

In horses 5 and 6 the onset latency was only slightly prolonged compared to the 95% prediction interval of the normal latency curve (Nollet *et al.*, 2003b; see page 95 and 96). In both horses the evoked responses from the cranial tibial muscle had a very polyphasic configuration (fig. 2). In horse 6, the peak-to-peak amplitude of the evoked responses from the cranial tibial muscle was very small and the left-to-right difference was larger than in the control population (> 1.53 ms).

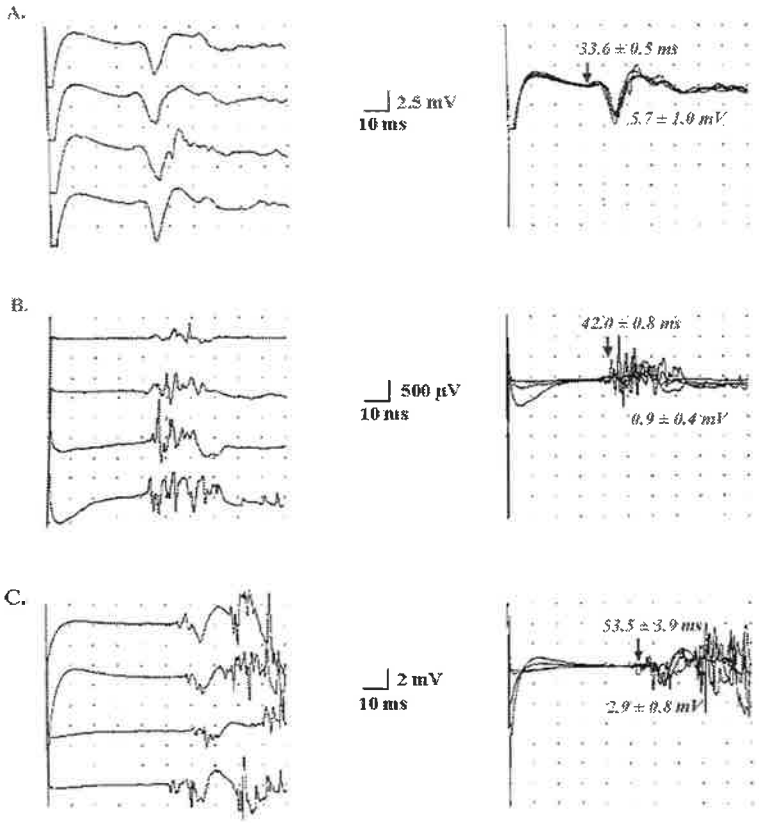

Figure 2. Motor potentials evoked by transcranial magnetic stimulation and recorded from the right cranial tibial muscle in the same 3 horses as in figure 1.
Note the different scaling for peak-to-peak amplitude (mV) between the different horses. On the right the superimposed tracings of the individual tracings (presented on the left) are represented. The mean (±SD) values for onset latency (see arrow) and peak-to-peak amplitude are marked on the superimposed tracings.

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In one of the two patients with a thoracolumbar spinal cord lesion (donkey) scoliosis, fusion and malformation of the eight to the eleventh thoracic vertebral bodies were radiographically observed (figures 3 and 4). In three out of the four cases with cervical spinal cord abnormalities on TMS, osteo-arthrosis (C5-C6 and C6-C7) of the cervical vertebral articulations or cervical vertebral malformation at multiple levels (patient 9) was demonstrated. In the fourth remaining patient no bony abnormalities were evident.
The electromyographic examination (see table 2) was abnormal in cases 2 and 3 with abnormal potentials corresponding to the level of their spinal cord lesion.
Rectal palpation was not rewarding in any patient.

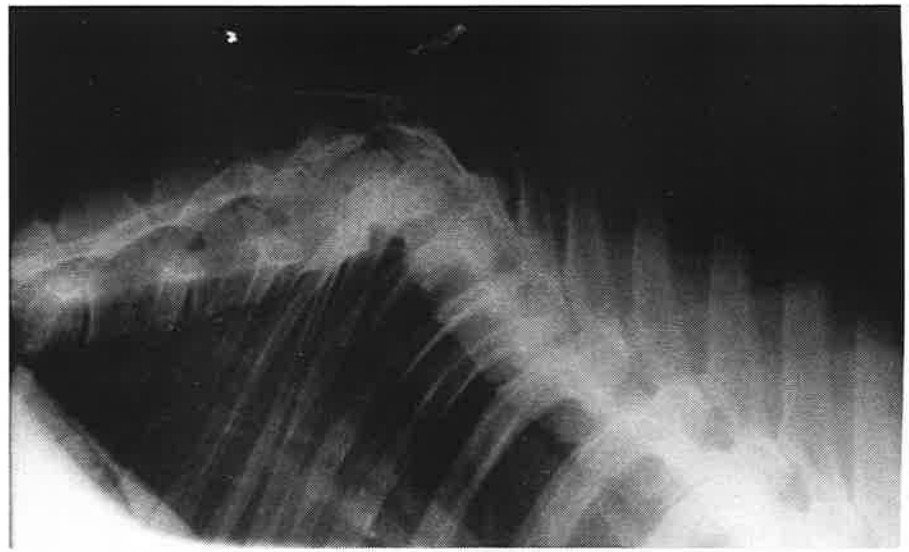

Figure 3. On a laterolateral radiograph of patient 2 a severe kyphosis (118°) is observed with a deformation of the vertebral bodies making individual differentiation of the vertebral outlines from Th8 to Th10 impossible. The detailed information on the specimen, described in fig.4, contrasts sharply with the information on the native radiograph.

Patient 2 (donkey) and horse 4 were euthanized. Necropsy and histopathologic examinations revealed a recent bleeding at the Th18-L1 level in patient 4 with subsequent degeneration of alpha-motoneurons in the ventral horn of the grey matter and mild infiltration of glial cells at that level. Macroscopically a small hemorrhage was also seen at the cranial part of the lumbar spinal cord. In the donkey foal a left sided scoliosis was observed, due to fusion and malformation of especially the eight to the tenth thoracic vertebrae. The sixth and seventh vertebral bodies and spinous processes were asymmetric and deviate to the left. The eighth, ninth and tenth vertebral bodies were partially fused and the spinous processes of the eighth

and ninth thoracic vertebra were completely fused. The ninth thoracic vertebra was abnormally shaped and looked like a wedge or a triangle (hemivertebra), with the right side of the vertebral body being totally hypoplastic and responsible for the scoliosis. The diameter of the fused vertebrae was slightly smaller than the more cranial and caudal vertebrae and smallest at the level of the hemivertebra (Th9). The 11th thoracic vertebral body was also slightly asymmetric (see figure 4).

Only the first horse could be reevaluated after one year. A clinical examination and TMS stated an unchanged but stabilised chronic lesion.

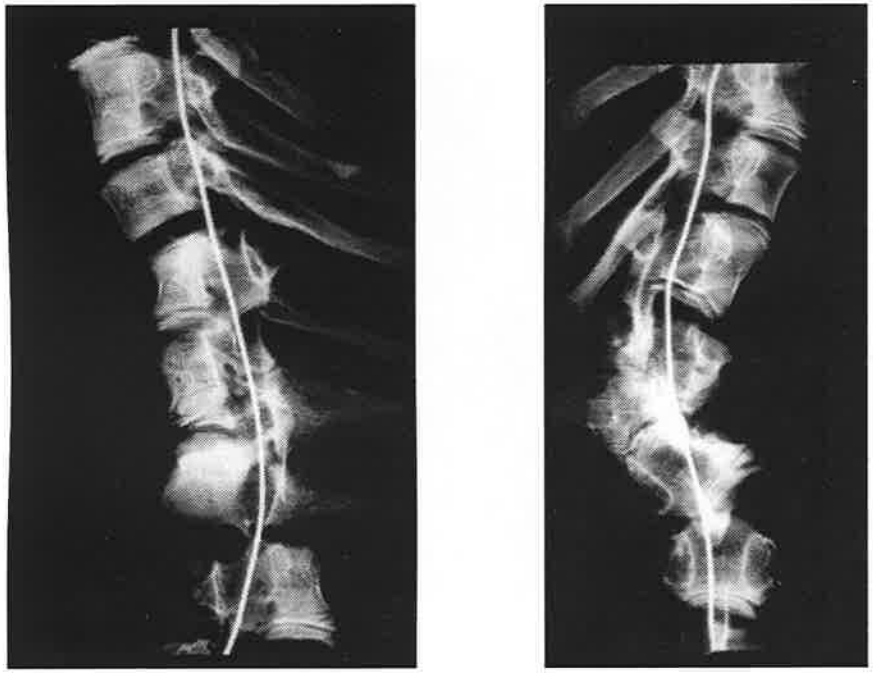

Figure 4. A dorsoventral (a) and laterolateral (b) radiograph of the dissected vertebral column of the donkey (patient 2).
Note: The white line observable in the vertebral canal is an iron wire to hold the vertebrae together.

DISCUSSION

A number of neurological disorders can be responsible for pelvic gait abnormalities in horses. Primary neurological diseases to consider are early, mild cases of cervical vertebral malformation (Mayhew, 1996), equine degenerative myeloencephalopathy (Miller and Collatos, 1997), equine protozoal myeloencephalitis (Furr *et al.*, 2002), the neurological form

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of equine herpes virus-1 infection (van Maanen *et al.*, 2001; Stierstorfer *et al.*, 2002) and parasite migration in the vertebral canal (Cox *et al.*, 1995)..

Developmental syndromes (malformations, osteochondrosis) are rarely encountered in the thoracolumbar vertebral column of horses (Johnson *et al.*, 1997). Still, variable degrees of scoliosis, kyphosis or lordosis have been described (Stecher, 1962; Rooney, 1969; Kirberger and Gottschalk, 1989). The fact that in patient two (donkey) clinical signs of the malformation only occurred after 8 to 9 months of life, makes a traumatic event superimposed on the malformation very likely. Degenerative disease of the articular processes, and spondylosis deformans are common incidental findings, and occasionally are causes of back pain but much less, if ever, in pelvic limb ataxia (Cauvin, 1997; Jeffcott and Wade, 1998; Haussler *et al.*, 1999). However, Mayhew (1999) presented clinical syndromes of acute ataxia and acute paraplegia in horses diagnosed with discospondylosis. He hypothesized that trauma from strenuous exercise and injuries due to falls causes damage to caudal cervical and thoracolumbar intervertebral disks and associated subchondral bone plates and epiphyseal cortical bone.The resulting hemorrhagic and/or ischemic necrosis of disks, fractures of the vertebral bodies and a foreign body reaction to fibrocartilage in the epiphyseal bone causes degrees of progressive spondylosis and instability of intervertebral joints, which is self-perpetuating if exercise continues.

Lesions of the vertebral bodies are less common in horses (Jeffcott, 1980) and cannot be imaged ultrasonographically in the thoracolumbar area (Denoix, 1998).

Thoracic vertebral fractures occur rather commonly in foals and are usually related to significant trauma, electric shock, or lightening strike (Jeffcott and Whitewell., 1976). Minimal vertebral fracture displacement is usually found with subsequent spinal cord compromise (Haussler, 1999). In humans, these thoracic fracture-dislocations nearly always cause important neurological deficits (Shapiro *et al.*, 2002). Complete paraplegia is possible in 80% of cases (Bohlman *et al.*, 1985).

Vertebral body osteomyelitis (Rooney, 1966; Markel *et al.*, 1986; 1988; Giguère and Lavoie, 1994; Olchowy, 1994), mainly described in foals, and diskospondylitis in adult horses (Markel *et al.*, 1986), are rare and life-threatening affections.

Space-occupying masses within the vertebral canal (e.g. abscess or neoplasia) can cause displacement or compression of the spinal cord (Allison and Moeller, 2000; Patterson-Kane *et al.*, 2001; Spoormakers *et al.*, 2001). Neoplasia is extremely rare, and vertebral abcesses/osteomyelitis are much more common in young domestic ruminants than in horses (Healy *et al.*, 1997).

Intervertebral disk herniation is rare in horses in comparison to dogs or humans, due to a rudimentary developed intervertebral disc and nucleus pulposus (Yovich *et al.*, 1985). These affections cause mild clinical signs if the disease is in the early phase.

The clinical neurological signs of spinal cord disease may not always correspond to the anatomical localisation of lesions detected by diagnostic imaging techniques. Incidental diagnostic imaging abnormalities on the other hand without clinical signs or symptoms are rather common, but electrophysiological studies are important to determine which anatomic abnormality is eventually relevant. Radiographs of the summits of the dorsal spinous processes of the thoracolumbar vertebrae from Th1 to approximately the third or fourth lumbar vertebrae (L3/4) are diagnostic (Ranner *et al.*, 1999), but give no information about the vertebral canal and are often of insufficient diagnostic quality. Scintigraphy is a more sensitive technique but has poor intrinsic anatomical resolution and is non-specific (Weaver *et al.*, 1999). Electromyography is the most widely used electrophysiological technique and can be used to look for denervation potentials. Recently, Wijnberg and co-workers (2002a, 2002b) described an electromyographic motor unit action potential analysis in horses allowing discrimination between myogenic and neurogenic problems. However, an electromyographic examination gives only information about lower motor neuron lesions. Furthermore, it may be falsely negative if performed either too early or too late in the course of a neuropathy or myopathy. Motor evoked potentials elicited by magnetic stimulation, on the other hand, allow examination of central motor pathways and can therefore be considered as a valuable technique for the functional assessment of the motor pathways in the spinal cord. In large breed dogs with cervical spinal cord disease (Poma *et al.*, 2002) and in dogs with thoracolumbar intervertebral disc disease (Sylvestre *et al.*, 1993), the severity of the neurologic signs is correlated with the MMEP latencies and amplitudes. In dogs with neck pain (Poma *et al.*, 2002) or back pain (Sylvestre *et al.*, 1993) and without any other neurologic deficits, MMEP latencies and amplitudes were significantly abnormal, reflecting an altered function of the spinal cord at the cervical or thoracic level. In our limited number of horses, such a correlation was not immediately evident. TMS could differentiate between a cervical and a thoracolumbar spinal cord lesion. The test is very sensitive and indicates abnormal conduction along the motor tract even in acute stages of the disease. In chronic, stabilised, mild cases the reduced conduction time can be objectively evaluated. This is especially interesting in differentiating chronic mild stabilised cases of wobbler syndrome (cervical spinal ataxia) from cases of thoracolumbar ataxia. In the latter, abnormal MMEPs will only be recorded in the hind limbs.

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The wobbler signs are especially seen in young horses. In some of these horses the clinical symptoms may be very mild and unobservable for an unexperienced eye. Such horses are often categorised as being "weak due to fast growth". They may be so favourably advised in a purchase examination at the onset of the disease and the start of a sporting career may be a disaster. In these cases TMS is extremely helpful because the objectively measured onset latencies and peak-to-peak amplitudes and the configuration of the MMEPs can undisputably demonstrate a lesion along the cervical or thoracolumbar spinal cord.

However, a limitation of the technique is the fact that the absence of or the delayed MMEPs recorded from the cranial tibial muscle do not demonstrate the vertebral level at which the lesions are located. Therefore, in the future, it would be interesting to examine recordings from the paravertebral muscles by needle electromyography and by TMS. An important feature of the paravertebral muscles is their segmental innervation, corresponding to the vertebral level. A second advantage is that the paravertebral muscles are close to the spinal cord, and their peripheral motor nerve components are short (McIntosh *et al.*, 1986). In humans, the usefulness of paravertebral MMEP investigation in localizing lesions in the spinal segments in most of the patients with thoracolumbar cord lesions was demonstrated (Ertekin *et al.*, 1998; Hashimoto *et al.*, 2000; Misawa *et al.*, 2001). Van Ham (1995b) already recorded reproducible MMEPs at multiple cervical, thoracic, lumbar and sacrococcygeal levels with the recording needle placed in the midline between the spinous processes in adult neurologically normal, anaesthetized dogs. This test would be especially helpful in horses, since neuroimaging methods such as spinal nuclear magnetic resonance and spinal computed tomographic scanning currently are only possible in patients up to 150 kg.

CONCLUSION

In conclusion, TMS can differentiate mild cervical spinal cord lesions from thoracolumbar spinal cord lesions in horses with bilateral hind limb ataxia and can be used as a complementary test in the examination of subtle gait abnormalities. In our limited experience, TMS was very useful to come to conclusions in purchase examinations of horses with discrete hind limb ataxia. But however useful the technique may be as a diagnostic tool, the clinical outcome in most of these horses is however poor.

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CHAPTER 6

GENERAL DISCUSSION

Equine neurological diseases and the various diagnostic techniques have been extensively described (Mayhew, 1989). However, in some cases of cervical spinal cord disease subtle to subclinical lesions can be present and radiographic findings, if present, do not always correlate with the actual cord lesions. In thoracolumbar spinal cord disease currently available imaging techniques are usually not precise enough to clearly identify significant lesions. For these reasons better methods to quantify dysfunction of sensory and/or motor pathways in the spinal cord are welcome, provided they are sufficiently sensitive. Therefore, in this thesis the development of a workable protocol for transcranial magnetic stimulation (TMS) is described in horses. With this technique it is possible to monitor the integrity of the descending motor tracts. Since it is non invasive and almost painfree, it appears to be an attractive method to complement the neurological examination in the horse. Also it can be performed on standing sedated horses. This eliminates the risks of recovery related problems after general anaesthesia. These problems are more likely in ataxic horses and are a limiting factor in performing spinal cord contrast studies in these horses. Although this technique has been introduced in veterinary medicine more than 10 years ago (Konrad *et al.*, 1987; Heckmann *et al.*, 1989; Sylvestre *et al.*, 1992), very little work has been done to develop it further (Sylvestre *et al.*, 1993; Van Ham *et al.*, 1994, 1995a, 1996a, 1996b; Poma *et al.*, 2002). This is particularly true in horses where only one publication mentioned the possible application of TMS in normal ponies (Mayhew and Washbourne, 1996).

The most important parameters evaluated with TMS are onset latency and peak-to-peak amplitude.

In normal horses, onset latency is a very reliable parameter, having a very small standard deviation in horses of the same height. Also, this parameter is very sensitive to even minor compression of the descending motor tracts and is therefore important in evaluating subtle pathology. Onset latency is the time from the application of the magnetic stimulus to onset of electromyographic (EMG) activity in the target muscle and is made up of two components: (1) the time to activate spinal alpha-motor neurons (central motor conduction time; CMCT), and (2) the time from activation of spinal motor neurons to the muscular response (peripheral motor conduction time, PMCT). In that way both upper and lower motor neurons are tested. However, in humans, the increased latency of transcranial elicited MMEPs compared to reference values was more prolonged with upper motor neuron (UMN) lesions than with lower motor neuron (LMN) lesions (Herdmann *et al.*, 1992). This indicates that onset latency

is much more influenced by spinal cord lesions than by peripheral nerve lesions. The same authors, who compared recording of MMEPs of patients with a circumscript lesion of the UMN with recordings from patients with a circumscript lesion of the LMN, concluded that patients with UMN lesions showed MMEPs of significantly smaller amplitude ratio (ratio of MMEP amplitude to M-wave amplitude) and distinctly higher number of phases than MMEPs in control subjects or in patients with LMN lesions.

In humans, CMCT is often used and is calculated by the subtraction of the PMCT from the MMEP latency obtained in the target muscle after transcranial magnetic stimulation. In that way, a more correct representation of the UMN tracts is presented. The peripheral conduction time can be estimated either by the F-wave technique or by using electrical or magnetic stimulation of the cervical or lumbar spinal nerve roots at a point where they exit the intervertebral foramen. The F-wave results from a centrifugal volley in an alpha motor neuron, following antidromic excitation of the same nerve cell body in the ventral horn of the spinal cord after a single electrical impulse delivered on a peripheral nerve (Weber, 1997). Preliminary experiments on magnetic spinal nerve root stimulation in horses (unpublished observations) shows that magnetic stimulation is possible at the cervical level in most horses and at the lumbar level only in some very small and light horses, even when butterfly-shaped coils with a more focal stimulation site were used. This problem even occurs in humans, where motor responses are not consistently elicited upon applying magnetic stimulation to the lumbar root in healthy subjects (Claus, 1990). Therefore, the recording of F-wave latency is usually preferred. This technique is difficult to apply on horses since the peripheral nerves lie very deep between the muscles and because there is an absence of musculature to record from in the distal portion of the limbs.

The peak-to-peak amplitude has a larger standard deviation than the onset latency, even in the same horse. In humans, it is suggested that this variability is caused by constant, rapid, spontaneous fluctuations in corticospinal and segmental motor neuron excitability states (Kiers *et al.*, 1993; Ellaway *et al.*, 1998). Consequently, amplitude measurements have low sensitivity to detect central motor conduction failure due to the broad range of normal values. Only if all recordings in the same horse have a very small amplitude (around or <1mV) can a lesion be suspected. In man sometimes the ratio of the MMEP amplitude versus the compound muscle action potential (CMAP) amplitude, measured as the amplitude of the M-wave evoked after supramaximal peripheral stimulation of the nerve, is estimated (Weber and Eisen, 2002). For the above mentioned reasons, this is practically impossible in the horse.

Moreover, even in human patients this ratio is also very variable, ranging in normal subjects from 10 to 100% (Weber and Eisen, 2002). A recently developed triple stimulation technique, combining transcranial with peripheral electrical stimulation, provides a more accurate and less variable estimate of upper motor neuron activation, but has the disadvantage to be uncomfortable for patients (Magistris *et al.*, 1998, 1999) and is therefore not useful in horses.

When a new diagnostic technique is proposed, specificity and sensitivity have to be evaluated. In the first part of this study, clinically healthy horses were tested, and the small standard deviation for onset latency allowed us to conclude that false positive results were unlikely to occur. An additional proof for the specificity of the test can be deducted from the examination of mild cases of ataxia where the clinical examination performed by several examiners can lead to different conclusions concerning the localisation of any lesion. The possibility to differentiate between cervical or thoracolumbar spinal cord lesions by evaluating MMEPs in horses with complaints of hind limb ataxia (second part of chapter 5) shows that horses suspected of thoracolumbar spinal cord lesions only have abnormal MMEPs recorded in the hind limbs. The examination of 65 ataxic horses with both minor to major clinical evidence of cervical spinal cord lesions in the first part of chapter 5 of the study demonstrated a high sensitivity of the test protocol. In fact, only two of these horses showed normal MMEPs, probably indicating a purely sensory ataxia.

The absence of a "gold standard" precludes at this moment a more statistical quantification of sensitivity and specificity. The limited availability of ancillary diagnostic techniques and the lack of post mortem data on most of our patients do not allow us to localise the lesion unequivocally. In human medicine it is described that the technique is very sensitive and that abnormalities can be seen immediately after the onset of the lesion. The question remains however if the technique is sensitive enough to identify very mild motor deficits in horses. Therefore experimental studies could be used where the MMEPs from horses with a variety of standardized compression on a specific spinal cord level are characterized. These studies already exist in laboratory animals (Dimar *et al.*, 1999) where neurological clinical scores and TMS are used to evaluate the effect of spinal cord narrowing and the timing of decompression on neurologic recovery after a standardized spinal cord injury in a rat model. Two groups (each subdivided in 4 subgroups) were tested: a control group that received no contusion injury, but only a spacers to narrow the vertebral canal by 20%, 35% or 50% and an injury group where beside a standardised contusion injury a spacer also was placed reducing the vertebral canal by 20%, 35% or 50%. The authors described that during preparatory studies, it

became clear that even with a very mild (not specified) spinal cord injury, there was too great a reduction in the amplitude of the MMEPs, making it sometimes impractical to record amplitudes. In the control group abnormal clinical neurological scores were seen only in the group with 50% vertebral canal narrowing. Further, the 50% control group regained good locomotor function by week 6 post surgery. However, abnormal MMEPs (especially decrease in amplitude) were already recorded in the group with only a 35% vertebral canal narrowing, indicating the sensitivity of the technique. The spacers were removed six hours after surgery and the MMEPs of the 35% control group returned to normal levels at 6 weeks post surgery. Also, histologic analysis of the spinal cord in the region of spacer insertion demonstrated no significant damage within the control groups until the 50% spacer was inserted. In all injured groups a significant deterioration of MMEPs (complete loss of responses or prolonged onset latency and decreased amplitude) was demonstrated. The authors also noticed that MMEPs were less reliable in predicting neurologic recovery because of their sensitivity.

Clinical studies also indicate the sensitivity of the technique. In humans with asymptomatic spondylotic cord compression (Travlos *et al.*, 1992; Tavy *et al.*, 1999) and in dogs with neck pain or back pain only due to cervical spinal cord disease (Poma *et al.*, 2002) and thoracolumbar intervertebral disc disease (Sylvestre *et al.*, 1993) respectively, transcranial magnetic stimulation can indicate motor tract dysfunction. In horses, the role of spinal cord dysfunction in the "back pain syndrome" is not often mentioned and still is unclear. Moreover, in equine medicine, there exist no useful methods to diagnose UMN dysfunction in that region. Even in these subtle cases, the evaluation of several parameters such as onset latency, peak-to-peak amplitude, shape of the MMEPs and left-to-right difference of onset latency could well give some indications of the ongoing pathology. Therefore, TMS could be used in the future as an additional diagnostic tool in the examination of horses referred with this complex and sometimes obscure complaint.

A first limitation of TMS in horses is that a precize indication of the vertebral level of the lesion is still impossible. In humans the diagnosis of spinal cord lesions has been made more easy and accurate by the development of imaging techniques, such as magnetic resonance imaging (MRI). Availability of MRI units in veterinary medicine, especially in equine medicine, is very limited. Since the bore of the magnet is often quite small and the tables have size and weight limitations, the human MRI systems are not practical for the adult horse. But a new open sided unit has been developed and the use of MRI in large animal medicine might therefore increase in future. However, also in humans, there have been patients in whom the

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clinical motor deficit level does not agree with the imaging diagnosis, and, on the other hand the site of the main lesion can be obscure because of multiple imaging lesions. Recently Wijnberg and co-workers described motor unit action potential analysis (MUAP) in horses where pathological MUAPs can be the result of disease processes in muscle fibres or in the LMN (Wijnberg *et al.*, 2002a; 2002b; 2003). Combining this technique with TMS in horses would be very interesting in future since in that way both UMN and LMN tracts can be assessed, and a better discrimination between myogenic and neurogenic problems may be obtained. Moreover, this MUAP analysis might indicate the localisation of the lesion more precisely when simultaneously local LMN lesions are present. One drawback of the MUAP analysis is the delay period of two to three weeks before abnormalities can be seen. Another possibility to identify more exactly the site of the lesion would be paravertebral recording of MMEPs after transcranial magnetic stimulation. In man, limited but promising studies already exist describing this technique (Ertekin *et al.*, 1998; Hashimoto *et al.*, 2000; Misawa *et al.*, 2001). Also Van Ham (1995b) already recorded reproducible MMEPs at multiple cervical, thoracic, lumbar and sacrococcygeal levels with the recording needle placed in the midline between the spinous processes in adult, neurologically normal, anesthetized dogs and showed the possible clinical value of the technique in one dog with paraplegia due to thoracolumbar disc disease. Therefore we also plan to investigate paravertebral recordings of MMEPs in normal and ataxic horses.

A second limitation of TMS in horses is that, in contrast to some findings in humans (Di Lazzaro *et al.*, 1992) and in dogs (Sylvestre *et al.*, 1993; Poma *et al.*, 2002), there was no correlation between the MMEP onset latency and the clinical grade of ataxia in horses. This is especially true in grade I, II and III ataxia, since in severely ataxic horses (grade IV) or horses in lateral recumbency, MMEP responses can be markedly reduced in amplitude so that they are partially buried in the background EMG, which makes the onset latency difficult to recognize (grading cfr. Mayhew *et al.*, 1978). A possible interspecies difference in the pathophysiology of cervical spinal cord lesions can be one possible explanation. In dogs and humans, cervical spinal cord compression is often caused by disk herniation that is more likely to produce compression of the ventrolateral region than of dorsal columns (i.e. dorsal funiculi), with subsequent damage of especially the motor tracts (Maertens de Noordhout *et al.*, 1991; 1998). When cervical spondylotic myelopathy is the cause, the lateral corticospinal tracts are the first to suffer from minor compression (Ogino *et al.*, 1983). In horses however, cervical spinal cord lesions are more often due to malformation, subluxation, osteochondrosis

or fractures. Therefore, the localisation of spinal cord compression is more variable in horses and maybe lesions of the ascending proprioceptive tracts, with subsequent more severe signs of ataxia, are present to a greater degree in horses. Nevertheless both proprioceptive and motor tracts are damaged in most cases, so that TMS can indicate a spinal cord lesion. Pathological studies in horses confirm this also, since Yovich *et al.* (1991) reported wallerian degeneration in both the proprioceptive pathways cranial to the compressed site and motor pathways caudal to the compressed site in horses with chronic compressive myelopathy. Moreover, they also noted that the degree of deformation of the spinal cord did not correlate with the severity of clinical signs. To test the role of damage to the proprioceptive tracts in our cases, a comparative study with somatosensory evoked potentials (SSEPs) would be useful. This latter technique examines the responses recorded from scalp electrodes after electrical stimulation of a peripheral sensory nerve and gives an indication of the patency of the ascending proprioceptive pathways. However, multiple responses to stimuli must be averaged to extract the SSEP from simultaneous electroencephalographic (EEG) and EMG activity, because of the small amplitude of the SSEP. Both central factors and voluntary movement suppresses SSEPs (Nishihira *et al.*, 1991; 1997; Touge *et al.*, 1997). Reducing the EEG and EMG activity in horses is difficult, since anaesthetics have enormous depressing effects on SSEPs. Therefore this technique would not be very practical in horses. Moreover, studies in humans (Misra and Kalita, 1994. Tavy *et al.*, 1999) indicate that TMS is more sensitive than the SSEP in detecting spinal cord injury. In animal experiments it has also been reported that motor pathways are more susceptible than sensory pathways to spinal cord trauma and ischemia (Machida *et al.*, 1988; Fehlings *et al.*, 1989, Kai *et al.*, 1995); therefore, identifiable motor dysfunction commonly might be expected to precede sensory dysfunction.

A future challenge is to evaluate the informative value of TMS in other neurological syndromes with motor deficits, for example equine motor neuron disease (EMND). EMND is described as progressive weakness and muscle atrophy as a result of degeneration and death of motor neurons in the ventral horns of the spinal cord and certain cranial nerve nuclei (Cummings *et al.*, 1990). Both clinical and pathological findings resemble to human disease, amyotrophic lateral sclerosis (ALS) or Lou Gehrig's disease. However, ALS is characterized clinically and anatomically by abnormalities of both UMNs and LMNs. Lower motor neuron involvement may be documented with electromyography, whereas definite evidence of upper motor neuron involvement may be elusive. Therefore some authors reported that TMS provides a sensitive means for the assessment and monitoring of UMN function in motor

neuron disease (Rossini and Rossi, 1998; Triggs *et al.*, 1999; Pouget *et al.*, 2000). In these studies the following MMEP abnormalities were reported: TMS fails to evoke a muscle response despite high stimuli, modest prolongation of CMCT, normal CMCT in spinal muscular dystrophy and decrease of amplitude. In our experience (unpublished observations) MMEPs can also have a mild increase in onset latency or a very broad configuration (long duration) in some EMND cases. If in these horses with abnormal MMEPs also UMN involvement can be demonstrated, this would have to be evaluated in the future.

Whether TMS will help in predicting the outcome of neurological disease in horses remains to be shown. However, since in our study MMEPs could not be recorded at the initial evaluation in some horses that afterwards could be used as leisure horses and since reproducible but abnormal MMEPs could be found in persistently tetraplegic horses, we suggest that TMS has limited or even no prognostic capabilities. In humans transcranial magnetic stimulation has been used to predict the outcome of spinal cord injury or stroke (Clarke *et al.*, 1994; Kalita *et al.*, 1998; Vang *et al.*, 1999), but the conclusions are still ambiguous. Clarke and co-workers (1994) suggested that TMS-induced MMEPs might be useful in refining the prognosis of patients who will recover to incomplete spinal cord injury if MMEPs from muscles below the level of the injury were present, even though prolonged in latencies, within 15 days of their injury. These patients showed significant recovery, but these in whom MMEPs were absent, poor recovery was observed. In another study, however, in which 25 patients were evaluated within 6 h of injury and 6 weeks postinjury, the authors concluded that in acutely injured spinal cord patients, MMEPs do not provide useful information regarding the likelihood of motor recovery (MacDonell and Donnan, 1995).
However, in equine medicine "recovery" has a different meaning compared to human medicine. In horses, a "total" clinical recovery is required for athletic performances. Till now, MMEPs of only one horse of the re-evaluated cases normalised. This horse, however, has been sold and further information on its performance is missing. Some other horses, in which both MMEPs and clinical findings improved, are being used as leisure horses. We however have to emphasize that these horses carry a higher risk for both the rider and the horse. The very slight incoordination that is still present could provoke, especially in stress situations, dangerous falls due to stumbling.

Another possible field of interest for TMS may be the follow-up after vertebral surgery. Surgical treatment by subtotal dorsal laminectomy or ventral vertebral interbody fusion,

depending on the type of vertebral abnormality present, results in clinical improvement in many affected horses (Wagner *et al.*, 1979; Nixon *et al.*, 1983; Grant *et al.*, 1985). The authors hypothesized that postsurgical neurological improvement has been attributed to a lack of continuing damage to the spinal cord, resolution of oedema, and remyelination of axons that have undergone primary demyelination (Nixon *et al.*, 1983). In equine literature, no data exists concerning the postsurgical evaluation of the motor tracts by TMS. In human medicine, however, many authors (Jaskolski et al, 1990; Maertens de Noordhout *et al.*, 1991; De Mattei *et al.*, 1995; Chang and Lin,1999) reported an improvement but no normalization of central motor conduction time in many patients with cervical spondylosis or herniated disc-induced cervical myelopathy, despite a substantial clinical improvement. Moreover, Chang and Lin (1999) showed that functional motor improvement occurred in grade I patients with mild neurological impairment after surgery, whereas no definite change was seen in grade II and grade III patients with moderate and severe neurological impairment. The presence of either a single level or a multilevel compression of the cervical cord has importance, as a statistically significant neurophysiological improvement is observed only in those patients with single level compression (De Mattei *et al.*, 1995). In these cases, MRI studies revealed that the spinal cord lesion at least partially consists of an edematous component and/or an initial demyelinization that has still a chance of recovery. In patients with multilevel damage, the compression may cause irreversible lesions (De Mattei *et al.*, 1995), but the operative stabilization of unstable cervical vertebrae can prevent further damage. Therefore, it might be useful in future, to monitor objectively the postsurgical improvement or clinical normalisation in horses by TMS.

Beside the surgical decompression, owners are told "nothing can be done" to improve function of their “athlete”. Recently more and more attention is paid to the role of medical strategies (especially 4-aminopyridine and neurotrophic factors), perhaps in addition to surgery, as treatment for spinal cord disease in humans (Wolfe *et al.*, 2001; Bregman *et al.*, 2002; Sayer *et al.*, 2002). 4-Aminopyridine is a potassium channel blocking agent that has been shown to reduce the latency and increase the amplitude of MMEPs elicited with TMS in patients with chronic spinal cord injury. Neurotrophic factors would increase recovery of function and regeneration after spinal cord injury. Maybe in future, these pharmacological agents can also be tested in the equine spinal cord injured athlete, whereby TMS can monitor their effects on motor tracts.

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In conclusion it can be stated that TMS can be used as a good complementary diagnostic tool in the neurologic examination of horses. It is a non-invasive means of stimulating the motor cortex of the brain and allows us to evaluate objectively the functional integrity of the descending motor tracts. As already noticed, there are still some limitations that maybe can be adjusted by more detailed standardization of the technique, with respect to paravertebral stimulation, paravertebral recording, evaluation of the duration of the MMEP, etc. Also, the usefulness of TMS in spinal cord disease encourages us to assess the technique as a research tool in several other neurological syndromes.

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SUMMARY

Equine neurology is mainly based on a thorough clinical examination. Only few complementary tests such as cerebrospinal fluid and blood analysis, radiography of the head and cervical vertebrae and scintigraphy of the vertebral column, are available to give additional information and to help making a differential or exact diagnosis. Techniques such as magnetic resonance imaging and to a lesser degree computed tomography are the most impressive developments in the field of neuroimaging for the elucidation of anatomical lesions in a variety of movement disorders of man and small animals, but are hardly or not applicable in adult large animals, due to technical (limited to the head and the first two cervical vertebrae) and financial reasons. Moreover, even with these techniques, the functional integrity of the spinal cord is not exactly known. Therefore, transcranial magnetic stimulation (TMS), introduced in 1985 by Barker and co-workers in man, would be of great interest as an additional diagnostic tool in the neurological examination of the horse. It is a technique that can stimulate the motor cortex through the intact skull and thus activate the corticospinal tract without causing discomfort to the patient. Since TMS was introduced, it has been clear that measuring the latency of magnetic motor evoked potentials (MMEPs) offers an excellent method for demonstrating abnormalities of corticospinal tract conduction in numerous neurologic diseases in man and to a lesser degree in small animals. TMS is a very sensitive technique since it even discloses subclinical involvement in patients with questionable signs of corticospinal tract lesions.

The limitations of the equine neurologic examination on the one hand and the excellent features of TMS on the other hand, were the basis for setting up this study. It can be divided in the following parts.

As a general introduction (chapter 1) a brief review is given on the history, basic principles, technical requirements and procedure of TMS in human medicine. Furthermore, some clinical applications in man and small animals are presented. Only one publication (Mayhew and Washbourne, 1996) mentioned the possibility to evoke MMEPs in normal ponies, but no clinical applications have been presented.

In chapter 2 the scientific aims of the study were formulated. Can TMS evoke MMEPs in horses? If yes, standardization of the technique in normal horses will be needed in order to use it as a complementary diagnostic tool in clinic. After this standardization of the technique, reference values for the measured parameters in horses should be established. Finally, the

usefulness of transcranial magnetic stimulation had to be evaluated in clinical patients; for this horses suspected to have spinal cord lesions were chosen.

Chapter 3 describes the standardization of the technique in horses.
Transcranial magnetic stimulation is described to cause only minimal discomfort in man. However, the noise generated by the production of the magnetic field and the evoked muscle contraction seems to give rise to anxiety in some horses. In contrast to man, mild sedation is therefore justified to avoid possible adverse reactions, as fear and excitation. In the first part of chapter 3, the influence of a sedative combination, detomidine (10 µg/kg of bwt) and buprenorphine (2.4 µg/kg of bwt), on MMEPs was examined in six horses. No significant difference could be observed for both onset latency and peak-to-peak amplitude measurements before sedation and measurements 10 and 30 minutes after sedation. Therefore this sedative combination can be used to facilitate the elicitation of MMEPs in horses.
In a second part (chapter 3, part 2) the influence of coil position, current direction and stimulation intensity on MMEPs recorded in the extensor carpi radialis and cranial tibial muscles was evaluated. Therefore 7 coil positions (obtained by constructing a frame on the forehead), 2 coil directions (clockwise and counter-clockwise) and 2 stimulation intensities (80% and 100% of maximal stimulator output) were studied in seven horses. The median of the forehead (position 2 and 7) seemed to be the optimal coil position for recording MMEPs in both the extensor carpi radialis and cranial tibial muscles. There was no significant difference between left and right side recordings. The direction of the current flow in the coil had no influence on the onset latency of the MMEPs. With stimulus intensity at 100 % of maximal output of the stimulator, larger MMEPs and shorter onset latencies were found than with stimulus intensity at 80 % of maximal output.

By using this standardized technique, 84 normal horses of different height (85 to 175 cm height at withers) were stimulated transcranially (chapter 4). Hence normal values for onset latency and peak-to-peak amplitude of MMEPs recorded in the extensor carpi radialis and cranial tibial muscles were set up in order to formulate a 95% prediction interval by which values obtained in clinical patients can be judged as normal or abnormal. A left-to-right difference in onset latency and peak-to-peak amplitude was not observed. In the same horse differences up to 0.82 ms and 1.53 ms for the extensor carpi radialis and cranial tibial muscle, respectively, lie within the 95% confidence limit and are considered normal. In contrast to onset latency, peak-to-peak amplitude showed a very large intra- and inter-individual

variability, even in the same muscle. Subsequently, possible influences of height, weight, ag and gender of the horses on these values were checked. No significant effects of gender we observed on onset latency and peak-to-peak amplitude. The age of the horse had only a sma but significant effect on peak-to-peak amplitude, with larger responses in older horses. Heig at the withers and weight of the horse, parameters that strongly correlate with the size of th horse, had an important and significant influence on onset latency but not on peak-to-pea amplitude.
The age of the horse and the height at the withers was used to predict peak-to-peak amplitud and onset latency respectively in normal horses. Subsequently, a 95% prediction interval fo onset latency and peak-to-peak amplitude of MMEPs recorded in the extensor carpi radial and cranial tibial muscles was calculated in order to determine the presence of abnormalitie in future clinical cases. Creation of these reference values forms the base for application o the technique as a complementary diagnostic tool in equine neurology.

In a following chapter (chapter 5), the usefulness of TMS was evaluated in clinical patients with spinal cord lesions. Therefore two clinical groups of horses were examined: 65 horses with ataxia and weakness in all four limbs or tetraplegia (chapter 5, part 1) and another group of 9 horses and one donkey suffering from hind limb ataxia (chapter 5, part 2).
The first part (chapter 5, part 1) is divided in two sections. First, a pilot study was conducted in an initial phase of the thesis in order to obtain some information on the usefulness of the technique in clinical cases. Therefore, a group of 12 ataxic horses was compared to a group of 12 clinical normal horses. The first group, where abnormal MMEPs (delayed onset latency and/or small peak-to-peak amplitude in both extensor carpi radialis and cranial tibial muscles) were recorded, was diagnosed as having cervical spinal cord lesions based on clinical, radiographic and MMEP grounds. In most horses, the configuration of the abnormal potentials was also polyphasic. In none of the horses that were re-evaluated after a period of clinical improvement, did normalisation of the evoked potentials occur, demonstrating that the technique is also able to detect lesions in horses with subtle clinical signs of incoordination. A second section describes 53 additional cases with cervical spinal cord lesions that were examined with TMS when the test was standardised for clinical use. Because the results were comparable to those found in the 12 initial cases, the results of the first two sections were discussed together. Only in 2 of these horses normal MMEPs were found, probably indicating a pure sensory ataxia. In all other horses, MMEPs recorded in the extensor carpi radialis muscles as well as in the cranial tibial muscles had prolonged onset latencies. In most horses

the peak-to-peak amplitude was smaller. Polyphasic pattern MMEPs were also observed in many of the horses. No clear correlation was seen between the clinical signs and the MMEP results, since in some ataxic horses that were still standing, no MMEPs could be recorded initially in the hind limbs or in any limb and in some tetraplegic horses abnormal MMEPs could still be recorded in all limbs. In only one of the 13 re-evaluated horses, MMEPs were normalised 6 months after the first examination. Initially this horse had only abnormal MMEPs on the left side and showed no radiographic abnormalities at the cervical vertebrae.

In the second part (chapter 5, part 2), 10 patients with bilateral hind limb ataxia were examined using TMS. By recording MMEPs in the extensor carpi radialis and cranial tibial muscles, the patients could be divided in two groups: 4 horses suspected of having cervical spinal cord lesions (abnormal MMEPs in both extensor carpi radialis and cranial tibial muscles) and 5 horses and one donkey suspected of having a lesion of the spinal cord caudal to Th2 (abnormal MMEPs only in cranial tibial muscles).

We could conclude that the TMS is a valuable ancillary test to assess the integrity of the spinal cord in horses. The technique is painless and safe and shows a good sensitivity to detect lesions along the descending motor pathways. Moreover, it can be used for differentiating thoracolumbar spinal cord disease from mild cervical spinal cord lesions, causing ataxia in hind limbs only.

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