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Original Research

Design and Optimization of a Novel Method for Assessment of the Motor Function of the Spinal Cord by Multipulse Transcranial Electrical Stimulation in Horses

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ABSTRACT

Compared to transcranial magnetic stimulation (TMS), transcranial electrical stimulation (TES) more specifically assesses the motor function of the spinal cord and excludes reproducibility errors from coil repositioning. Objective: to assess the applicability of multipulse TES in horses and retrieve optimal TES parameters to elicit muscular motor-evoked potentials in the m. extensor carpi radialis (ECR) and the m. tibialis cranialis (TC) in a scouting study. This is a prospective observational study in five healthy horses based on TES as a novel alternative to TMS to assess the motor function of the spinal cord for clinical diagnosis in search for optimal settings of stimulation parameters. After sedation, a subcutaneous anesthetic ring block was placed on the forehead around bilateral TES needle electrodes. In each step of a specific parameter optimizing protocol, one parameter was varied while leaving others at default values: TES motor threshold $+30$ V, n $= 3$ pulses/train (ppt), interpulse interval (ipi) $= 1.3$ ms, and 0.1 ms/phase biphasic pulses. Variable parameters were TES voltage (0–200 V), n (1–5 ppt), and ipi (0.5–4.5 ms). A multipulse facilitation factor (MPFF) quantified the motor neuron recruitment gain by multipulse stimulation. Mean latency times, MPFF, optimal ipi, and n for the ECR muscles were, respectively, 18.6 (1.26) (mean[SD]) ms, 7.1 (3.4), 1.25 (0.21) ms, 3.0 (1.4) ppt (left) and 18.4 (1.10) ms, 4.3 (1.4), 1.9 (0.7) ms, 3.5 (1.3) ppt (right) and for the TC muscles, respectively, 34.5 (0.96) ms, 5.3 (2.4), 1.2 (0.28) ms, 3.3 (1.0) ppt (left) and 33.4 (1.52) ms, 17.5 (21.2), 1.3 (0,17) ms, 3.3 (0.5) ppt (right). Optimal multipulse TES parameters were n $= 3$ ppt and ipi $= 1.2$ to 1.3 ms. Multipulse TES is well tolerated and an attractive alternative to TMS. Transcranial electrical stimulation is expected to be a more robust technique than TMS for evaluation of spinal motor function in horses. A better reproducibility of repeated stimulations is expected due to fixed electrodes, and a reduced sensitivity to hyperpolarizing effects of sedatives is expected.

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1. Introduction

Transcranial magnetic stimulation (TMS) has been used to assess the motor function of the spinal cord for clinical diagnosis in horses [1–3]. Compromise of the spinal cord

can be detected by significant increase of motor latency times and decrease of muscular motor-evoked potential (MEP) amplitudes. Basically, TMS creates a magnetic pulse by a short strong current in a circular or figure-of-eight coil. This induces electrical currents in the motor cortex of the brain that are capable to activate axons [4]. The elicited action potentials are processed by cortical neurons of which upper motor neurons (UMNs) form the gateway to the corticospinal tract of which the segmental motor neurons (LMNs) are connected to muscle fibers by peripheral nerves. Transcranial magnetic stimulation may also activate directly a small fraction of corticospinal axons [5,6].

In contrast, the route via the motor cortex of the brain is skipped when transcranial electrical stimulation (TES), as introduced by Merton and Morton [7], is applied. This method predominantly relies on direct activation of corticospinal axons [4]. The absence of the synaptic delays of cortical connecting neurons and UMN is expected to (1) reduce the latency times of TES-induced muscular MEPs and (2) reduce the sensitivity of TES to cortical function and thus anesthetics and sedatives. Both these TES-specific features could enhance its accuracy over that of TMS and may render TES a more robust technique.

Like in TMS, TES has to be performed in sedated horses [8,9]. Hyperpolarizing effects on motor neurons exerted by anesthetics or sedatives may reduce or even block the synaptic transmission through motor neurons, which in turn could deteriorate the success rate of single pulse TES or TMS [10–14]. Because cortical neurons are also affected by sedatives, TMS is considered inappropriate for monitoring during surgical procedures in humans [15]. Under extreme hyperpolarizing conditions, which are inevitably linked with full anesthesia, even single pulse TES often fails to activate LMNs. Double pulse TES stimulation [16] improves the ability to generate muscular potentials, whereas high-frequency multipulse stimulation [17] can markedly improve the success rate for obtaining muscular potentials in adults. In very young children, in whom corticospinal axons are immaturely myelinated, even multipulse TES has a low success rate. In these circumstances, additional facilitation by double-train TES [18–20] or peripheral stimulation [21] is required.

The objective of this scouting study is to design a protocol for application of TES in horses by optimizing several multipulse stimulation paradigms and by exploring the characteristics of TES-induced MEPs bilaterally in the m. extensor carpi radialis (ECR) and the m. tibialis cranialis (TC) in five healthy horses.

2. Material and Methods

The animal ethics committee of the University of Groningen, The Netherlands, approved the study protocol (DEC6440A). In advance of procedure, the horses were clinically examined. Before, after electrode placement and at 2/3 of the measurements, sedation was performed in all horses (n = 5), each time by IV administration of detosedan (AST Farma B.V., Oudewater, The Netherlands) and butomidor (AST Farma B.V., Oudewater, The Netherlands) (both 1.5–2.0 mcg/kg in total). Subsequently, horses were stimulated transcranially using a human intraoperative neurophysiological monitoring system (Neuro-Guard JS Center, Bedum, The Netherlands) [22]. For electrical stimulation, a central point (Cz) on the forehead of the horse was delineated at the junction of two lines drawn, respectively, from the left ear base to the right eye medial canthus and from the right ear base to the left eye medial canthus. Subsequently, a subcutaneous ring block anesthesia was performed in a wide area surrounding Cz, using 300 to 400 mg lidocaine 2% + adrenaline (Alfasan, Woerden, The Netherlands). Two needle electrodes (L 35 mm, diameter 0.45 mm) were placed subcutaneously in a sagittal direction with their middle points 2.5 cm bilateral from the central location Cz. Transcranial electrical stimulation was performed using biphasic multipulse trains administered through these electrodes. Muscular motor-evoked potentials (MEPs) were recorded bilaterally from subcutaneous needle electrodes in the ECR (10 and 20 cm above the os carpi accessorium), the TC (10 and 20 cm above the medial malleolus), in the trapezius muscles (interspaced 15 cm), and unilateral at the right side in the caninis and oral orbicularis muscles (both interspaced 2 cm). These electrodes were connected to the differential inputs of the physiological amplifiers of the measuring system. A ground needle electrode was placed subcutaneously in the neck at the right side of the horse. Only the muscle groups in all limbs are considered in this study.

Transcranial electrical stimulation multipulse trains are characterized by stimulation intensity (V), pulse width (pw) and shape (mono or biphasic), number of pulses in a train (n), interpulse interval (ipi), and intertrain interval. Based on experience with TES in anesthetized human patients, a single train stimulation protocol was chosen. The human TES paradigms [15,23] were adapted for the sedated horses, defining default values of n = 3 biphasic pulses per train (ppt), pw 0.1 ms/phase, and ipi = 1.3 ms [20]. Motor-evoked potential amplitudes and delay times were subsequently measured in triple, in an optimization protocol as function of one selected parameter (voltage vs. ipi vs. n) with the other parameters set at their default values, yielding a voltage curve, an ipi curve, and an n curve. In all curves, MEP amplitudes are plotted semilogarithmically and delay times linearly. Motor-evoked potential wave forms are characterized as either monophasic, biphasic, triphasic, or polyphasic. Motor-evoked potentials from direct nerve stimulation, M waves, from extracranial currents can occur in the vicinity of the stimulation electrodes like in the facial muscles [24] and may spread to neck and trapezius muscles at greater stimulation intensities. Th_M denotes the M wave threshold stimulation voltage.

2.1. Voltage Curve

In the voltage curve, the varying parameter is the stimulation voltage. A stepwise increasing voltage was applied (0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, and 200 V). When transcranial motor thresholds th_{TES} were reached for all muscle groups, stimulation was continued to th_{TES} +50 V or otherwise stopped at 200 V. From here, the default value for TES intensity for the

multipulse facilitation and ipi curves was defined at th$_{TES}$ +30 V.

2.2. ipi Curve

The selected parameter of the ipi curve was increased starting at 0.5 ms, to 2 ms in 0.1 ms steps, continued to 4.0 ms in 0.2 ms steps, and concluded by 4.5 ms.

2.3. Multipulse Facilitation Factor and N Curve

A multipulse facilitation factor (MPFF) is defined as the division of the MEP amplitudes at n and at single pulse stimulation. The n curve is the multipulse facilitation as function of n = 1, 2, 3, 4, and 5 ppt.

After measuring, the horses were taken back to their stable where a muzzle was put on until they were full awake again. A short clinical examination was performed before they were discharged.

2.4. Statistical Analyses

This study is an exploring observational study to yet unknown neurophysiological effects on MEPs at varying TES parameters to check if the default settings are reasonable choices amenable for statistical studies with a larger number of horses. All graphs from one horse are taken as representative examples while obtained numerical data of TES–MEP latency times: t$_{TES}$ and amplitudes: MEP$_{ampl}$, the mean maximum MPFF at n$_{max}$, and optimum ipi with the highest MEP amplitude ipi$_{max}$ are given as descriptive statistics including the number of cases, n, mean values and standard deviation (SD) written as: mean (SD).

3. Results

The study group comprises five horses (two geldings and three mares) aged between 3.6 and 16.1 years, 10.2 (5.5) mean (SD) years with a height at withers of 166.6 (7.8) cm.

All horses tolerated the procedure well. Only on one occasion, one horse showed signs of inconvenience at the end of the ipi curve construction protocol. In that horse, the amount of ppt was limited to 4. All graphic plots are taken from the first studied horse. These represent the results in all five horses.

Fig. 1 shows MEP landscape plots of the ECR (A) and TC (B) at stepwise increments of the TES intensity from 0 to 150 V (top to bottom). Late MEPs become visible at thresholds of Th$_{SENS}$ of 35 and 40 V, respectively. The latency times, t$_{SENS}$, decrease gradually with increasing TES intensity. When th$_{TES}$ is reached, the MEPs show a jump-wise reduction of the latency time and additional three-phasic wave in the ECR and biphasic wave in the TC appear. The extracranially elicited MEPs remain present. The extracranial stimulation threshold, Th$_{SENS}$, is lower than th$_{TES}$ in all horses. The latency times of the transcranial MEPs show a further slight decrease by a few milliseconds. M responses are absent in both muscle groups. Pulse trains of 3 ppt and ipi = 1.3 ms were used.
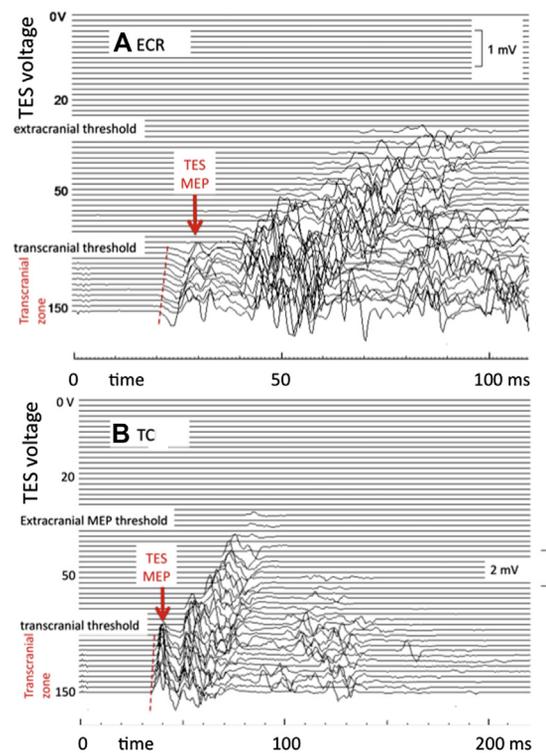

Fig. 1. TES–MEP landscape plots of two muscles, left, case 1 stimulation parameters: n = 3 ppt, ipi = 1,3 ms, and pw = 100 ms/phase. (A) M. extensor carpi radialis (ECR) and (B) m. tibialis cranialis (TC). From top to bottom, stepwise increasing TES intensities from 0 to 150 V. First, MEPs appear at Th$_{SENS}$, likely originating from extracranial activated sensory showing gradually decreasing latencies starting at 70 to 80 ms. The transition to the transcranial activation is recognized by a stepwise reduction of the latencies at th$_{TES}$. A dashed line shows a slight latency time reduction. ipi, interpulse interval; MEP, motor-evoked potential; ppt, pulses per train; pw, pulse width; TES, transcranial electrical stimulation.

Fig. 2A shows the amplitude–voltage curve of the left TC. A time window between 20 and 45 ms excludes the extracranial elicited MEPs with their higher latency times. The semilogarithmic amplitude–voltage curve is s-shaped with th$_{TES}$ = 80 V. A supramaximal level is reached at about 100 V. The course of the MEP latency times of the left ECR and TC as a function of voltage is shown in Fig. 2B. The latency jumps at th$_{TES}$ can clearly be noticed in both muscle groups. Note a subsequent gradual decrement of a few milliseconds in both muscle groups to 150 V.

Fig. 3 is a representative example of the ipi curve. A survey of ipi values at maximum MEP amplitude with the number of observations where a clear determination of the maximum was possible is given in Table 1 for muscle groups in the four limbs.

The multipulse facilitation curve in Fig. 4B is derived from the MEP series in Fig. 4A. Maximum facilitation is at n = 3 ppt. The MPFF at n$_{max}$ is 40.9. This is markedly greater than the MPFF of the same muscle group in the other three cases with mean MPFF = 7.33. The mean MPFF is 17.5 when including all four cases (see Table 1).

Descriptive statistics of the TES–MEP parameters of all horses are shown in Table 1. Definitions of symbols are
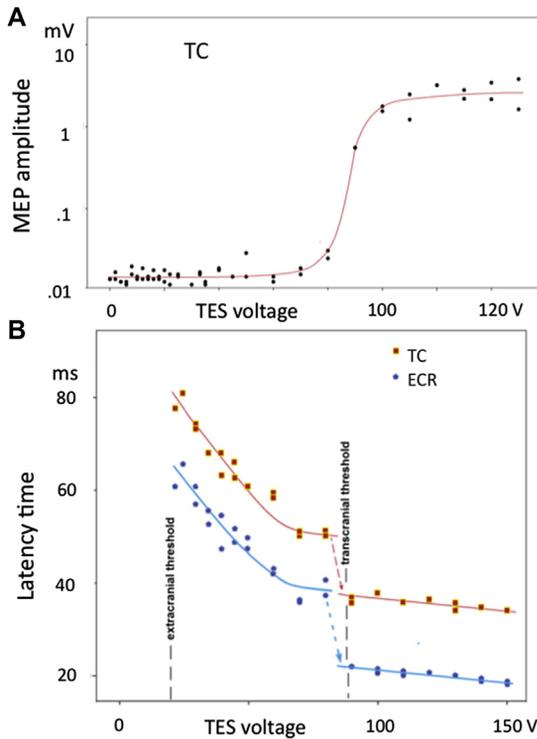
Fig. 2. MEP amplitudes and latency times as functions of TES voltage, case 1, left side. Stimulation parameters: n = 3 ppt, ipi = 1.3 ms, and pw = 100 ms/phase. (A) Amplitude–voltage curve of the left cranial tibial muscle, case1. Peak-to-trough amplitudes are obtained between 20 and 45 ms. The s-shaped curve starts at th_{TES} = 80 V and reaches a supramaximal level (−10% level/logarithmic scale) at 100 V. (B) Latency times of TC and ECR as a function of TES voltage. Like in Figs. 1A and 1B, both muscle groups have equal threshold voltages and show decreasing latency time courses. ECR, m. extensor carpi radialis; ipi, interpulse interval; MEP, motor-evoked potential; ppt, pulses per train; pw, pulse width; TC, m. tibialis cranialis; TES, transcranial electrical stimulation.

explained in the legend. The number of observations was five for th_{TES}, t_{TES}, and MEP_{ampl}. Because the ipi and n curves were aborted in one horse, the number of observations of N_{max} and MPFF was 4. In ipi_{max}, n was smaller than 4 because no modal shapes were distinguishable in some ipi curves.

4. Discussion

The objective of this study was to introduce a novel transcranial stimulation method to assess spinal motor function in horses. It is a first step to explore its characteristics, judge its clinical applicability, and find optimum values of stimulation parameters.

4.1. General Aspects of TES

Thanks to the work of Merton and Morton [7], TES rapidly became used as a powerful modality for monitoring the integrity of motor tracts in the spinal cord during surgical procedures in humans [15]. Transcranial magnetic stimulation was introduced a few years later by Barker et al
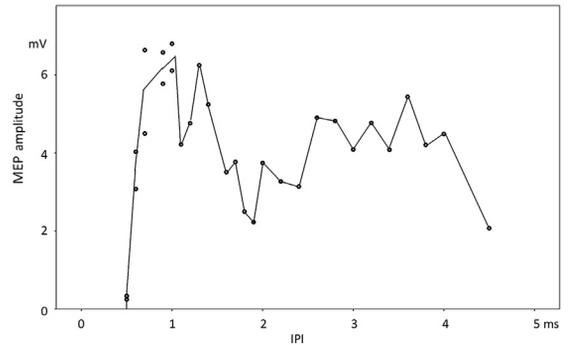

Fig. 3. Ipi curve of the right TC as a function of ipi, case 1. Stimulation parameters: n = 3 ppt, TES voltage 120 V, pulse width (pw) = 100 ms/phase. The bimodal-shaped curves show maximum values in 0.8 to 1.4 ms (absolute maximum) and 2.6 to 4 ms regions. ipi, interpulse interval; ppt, pulses per train; TC, m. tibialis cranialis; TES, transcranial electrical stimulation.

[25] and became increasingly important as a diagnostic tool in clinical neurophysiology. Although TMS was initially used in spinal surgery [26,27], to date, TMS is considered as a less reliable technique for intraoperative monitoring [24]. Transcranial electrical stimulation would be the first choice for assessing the UMN function in the spinal cord; however, its poor toleration in unsedated humans precludes clinical diagnostics use.

In 1996, Mayhew and Washbourne [1] first published a TMS study in ponies. The sensations from TMS, the sudden clicking noise and evoked muscular contractions in the studied horses forced these investigators and later on in 2002 Nollet et al [9] to use sedatives. Likewise, sedatives were administered to all horses in the present study.

4.2. TES Tolerance

Transcranial electrical stimulation was well tolerated at parameter settings that are relevant for diagnostic purposes. Side effects that may cause discomfort in TES are comparable with those described with TMS [9,28]. The lidocain ring block is an additional measure to prevent any

Table 1
Descriptive statistics (mean [SD]) of TES–MEPs describing parameters in the four limbs.

	N	ECR		TC	
		Left	Right	Left	Right
Th_{TES} (V)	5	90 (21.2)	90 (21.2)	108 (25.9)	108 (25.9)
t_{TES} (ms)	5	18.6 (1.26)	18.4 (1.10)	34.5 (0.96)	33.4 (1.52)
MEP_{ampl} (mV)	5	1.18 (1.55)	2.52 (2.86)	1.51 (1.21)	1.89 (1.83)

	N		N		N		N	
n_{max}	4	3.0 (1.4)	4	3.5 (1.3)	4	3.3 (1.0)	4	3.3 (0.5)
MPFF	4	7.1 (3.4)	4	4.3 (1.4)	4	5.3 (2.4)	4	17.5 (21.2)[a]
ipi_{max} (ms)	2	1.25 (0.21)	2	1.9 (0.70)[a]	2	1.2 (0.28)	3	1.3 (0,17)

Abbreviations: ECR, m. extensor carpi radialis; ipi_{max}, interpulse interval with largest MEP amplitude; MEP, motor-evoked potential; MEP_{ampl}, MEP amplitude; MPFF, multipulse facilitation factor; N, number of observations; n_{max}, number of pulses/train with largest MEP amplitude; SD, standard deviation; TC, m. tibialis cranialis; TES, transcranial electrical stimulation; t_{TES}, latency time; th_{TES}, TES threshold voltage.
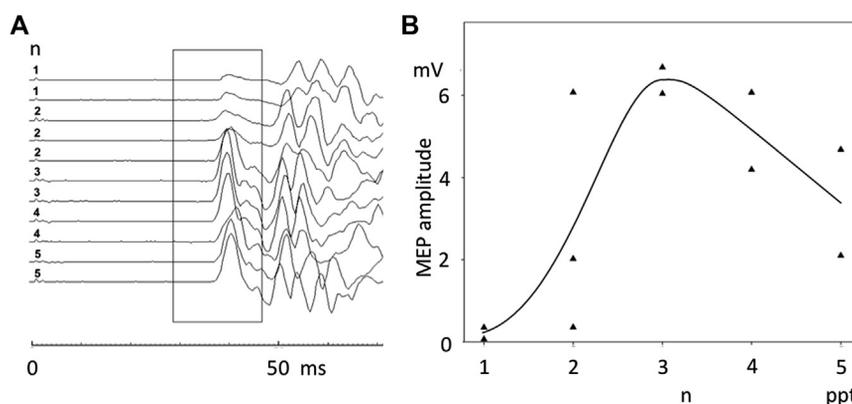[a] Increased variance and mean from an outlier.

Fig. 4. Example demonstrating a marked facilitating effect of multipulse TES on the MEP amplitude of the right anterior tibial muscle of case 1 as function of the number of pulses, n. TES intensity 120 V, ipi = 1.3 ms, and pw = 100 ms/phase. (A) Waterfall plot of the MEPs indexed by n. Peak-to-trough amplitudes are analyzed between 28 and 47 ms. (B) Semilogarithmic MEP amplitude as function of n. The response is weak at n = 1, whereas maximal at n = 3. MPFF at n = 3 is 40.9. ipi, interpulse interval; MEP, motor-evoked potential; MPFF, multipulse facilitation factor; pw, pulse width; TES, transcranial electrical stimulation.

activation of unmyelinated pain fibers at high electrical field gradients of the pulse paradigms near the TES electrodes. When compared to TMS, more extracranial conduction of stimulation currents may occur with TES, especially when high stimulation intensities are applied. At those high intensities, jerking contractions of the neck musculature may then become apparent. The present scouting study has demonstrated that disturbing extracranial conduction can be avoided by application of modest strong multipulse stimulation, yielding MEP responses of good quality.

4.3. Specific Characteristics of TES

Unlike TMS, TES predominantly targets direct stimulation of the corticospinal tract. Corticospinal axons in humans and primates are oriented perpendicular to the cortical surface. Anodal transcranial stimulation depolarizes corticospinal axons directly and has a lower stimulation threshold than cathodal stimulation [4,29–31]. Elicited action potentials can be recorded epidurally as d waves along the pyramidal tract. Upper motor neurons are bypassed. Modulating inputs from the cortex of the brain are therefore surpassed for a great deal [7,23,32,33]. This direct input to the corticospinal tract where neuron connections are precluded renders d waves resistant against the hyperpolarizing effects of sedatives and analgesic agents. This is in contrast to the predominant present epidural i waves from TMS (see below) [15].

Transcranial magnetic stimulation is less specific because the induced currents follow the direction of the electrical currents in the coil parallel to the cortex surface. Therefore, TMS primarily activates cortical axons. These are directly or indirectly connected to UMNs before action potentials are sent to the corticospinal tract. They appear in epidural recordings indirectly as i waves. Being mediated by indirect activation of the corticospinal axons, typically, i waves arrive delayed, when compared to the small d waves, which may result from direct stimulation a few corticospinal axons [30,31]. The i waves contributing to muscle MEPs are sensitive to the hyperpolarizing effects of

anesthetic agents and due to involvement of the motor cortex. Also motor cortex function has its influence [14,23]. Therefore, i waves have a greater variability than d waves.

Multipulse TES likely generates MEPs with a greater reproducibility than TMS and is more successful at generating MEP's. (1) The fixed position of stimulation electrodes excludes repositioning errors of the TMS coil over the dome-shaped forehead of the horse, (2) TES predominantly stimulates the corticospinal tract and is therefore less sensitive to modulating effects of motor cortical functions and sedation, (3) multipulse stimulation reaches supramaximal stimulation at lower stimulation intensities and (4) offers a marked greater success rate when compared with single pulse MEPs as shown by the high mean values of the MPFF in Table 1. Transcranial electrical stimulation offers a broader variety of applicable stimulation parameters.

The corticospinal tract is most likely involved. Previous studies by Mayhew and Washbourne [1] and Nollet et al [2,3,8] support the pivotal role of the corticospinal tract in horses. Also, extrapyramidal routes as, for example, the cortico-rubo-spinal tract may contribute as well. Although other studies in the past (both physiological and anatomic) have questioned the significance of the corticospinal tract in ungulates [34,35], later neurophysiological insights also in animals earlier in the evolution, confirm that the corticospinal system is still pivotal in control of movement, whereas the propriospinal system plays a more important role [36].

4.4. Side Effects From Extracranial Stimulation Currents

Transcranial electrical stimulation and TMS generate electrical fields in the scalp that are stronger than at intracranial depths. These may also elicit MEPs already below transcranial stimulation thresholds. These may interfere with the intended transcranial MEPs. Extracranial stimulation pertains to motor and sensory axons yielding, respectively, direct motor responses and reflex MEPs.

4.4.1. Direct Motor Responses

Action potentials from extracranial motor axons are directly conducted to muscles and result in M waves. Their

latency times are short because of the small distance between the stimulus and muscle. M waves are generated in vicinity of the stimulation electrodes and can be distinguished from transcranial MEPs by their latency times, t_M, that are significantly shorter than t_{TES} of MEPs from transcranial stimulation [24]. We observed M responses in the two facial muscles and at greater TES intensities in the trapezius muscles, however, none in limb muscles.

4.4.2. MEPs From Sensory Stimulation

A surprising finding in this study is the appearance of late MEPs in all eight muscle groups in each horse with stimulation thresholds, Th_{SENS} well below th_{TES} (Fig. 1). Most probably, these late MEPs are elicited by extracranial sensory axons because cortical stimulation is then excluded. The extracranial axons feed neural reflex circuits in the spinal cord and brain, which in turn generate widespread MEPs as encountered in all muscle groups in all horses of this study. The latency time t_{SENS} of the reflex MEP is longer than t_{TES} due to the extra delay of the reflex arch that adds to the conduction time along the motor pathway as shown in the model of Fig. 5. Because extracranial sensory axons can both be activated by TMS and TES, reflex MEPs will apply to both stimulation methods.

Fig. 1 shows appearance of late MEPs at the hind and front limbs when stepwise increased TES voltages are applied. The observations are identical in all muscle groups. Late MEPs share one stimulation threshold voltage of $Th_{SENS} = 30$ V, which is far below $th_{TES} = 80$ V and a common input to reflexes to many muscles. The late MEP latency times of the ECR and TC muscles in Fig. 1 decrease to limit values, from about 80 to 52 ms for the TC and from about 65 to 38 ms for the ECR muscles (Fig. 2B). Very remarkable is the consistent difference Δt of latency times of t_{TC} and t_{ECR} of about 12 ms. Similar consistent differences are measured in all horses in this study. This consistency is in great contrast and apparently independent on the large intraindividual variation of t_{TC} and t_{ECR} seen in this scouting study. According to the model in Fig. 5, the voltage-dependent delay variations of t_{TC} and t_{ECR} must reside in the reflex arch, which specifically accounts for the intensity dependency of the delay times because the conduction time t_{cond} along the spinal cord to the muscle groups being equal to x/v (x, motor pathway length to the ECR and TC; v, the conduction velocity) is independent of the stimulation intensity. This also applies to the delay difference $\Delta t = 12$ ms in Fig. 2B.

4.4.3. Transcranial MEPs

At stimulation voltages above $th_{TES} = 80$ V, additional MEPs appear in the landscape, whereas the late MEPs remain present. The latency transitions can be recognized in Fig. 2B by latency jumps of about 15 ms in the TC and by 17 ms in the ECR. These are transcranial elicited MEPs that follow the corticospinal route and maintain a fixed delay difference of Δt.

When disregarding extreme pyramidal signs, extracranially elicited reflex MEPs are unknown in humans. The reflexes resemble the lateral thorax reflex in horses [37]. The authors showed rostral bound reflex muscle activity at T6 and T11 levels from a mechanical stimulation at the 16th thoracic rib. Obviously, cutaneous stimulation of a dermatome also spreads out bidirectional and bilateral to target muscles at higher and lower segmental levels.

4.4.4. Early, Middle, and Late MEP Latency Times

Interestingly, Mayhew and Washbourne [1] mention the same occurrence of late MEPs after the fast MEPs induced by TMS. Likewise, middle and long latency MEPs are also visible in Fig. 1 of Nollet et al [38], Fig. 4 of Nollet et al [8], and Figs. 1A–C and 2A–C [39]. The data of Mayhew and Washbourne [1], obtained from 10 ponies, provide support to our model describing fixed latency time differences between hind and fore limb muscles. This also applies to early and midlatency MEPs. Table 1 of the authors mentions for early MEP latency times, expressed as mean (SD) of the ECR: $t_{early,ECR} = 19.0$ (2.3) and TC: $t_{early,TC} = 30.2$ (34) ms. The early MEP difference yields $\Delta t_{early} = 11.2$ ms. A similar
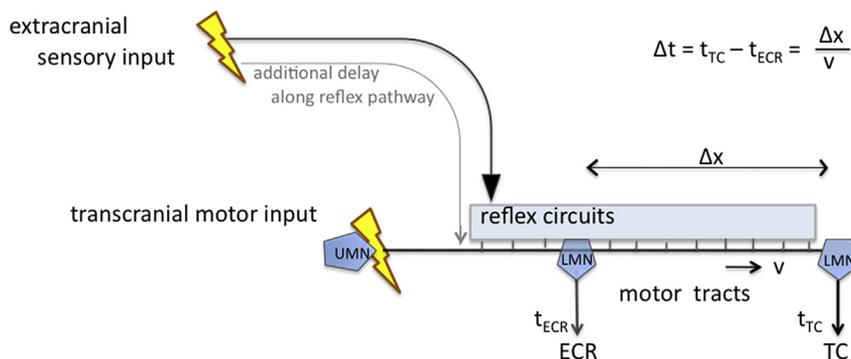

Fig. 5. Model of the conduction routes of extracranial and intracranial elicited MEPs. The extracranial route consists of sensory axons located in the scalp and may extend to head and neck regions, being the input to reflex circuits being connected to motor neurons of ECR and TC muscle groups. The motor latency time differences $\Delta t = t_{TC} - t_{ECR}$ is equal to the motor tract length difference Δx divided by conduction velocity v. For reasons of simplicity, the difference of conduction times along the peripheral motor nerves is not considered. Because TMS and TES have direct access to the motor pathway, the route along the reflex pathway does not elongate their latency times. ECR, m. extensor carpi radialis; LMN, lower motor neuron; MEP, motor-evoked potential; TC, m. tibialis cranialis; TES, transcranial electrical stimulation; TMS, transcranial magnetic stimulation; UMN, upper motor neuron.

difference for middle latency times of their data is: $\Delta t_{mid} = t_{mid,TC} - t_{mid,ECR} = 59.2\ (5.1) - 47.9\ (4.0) = 11.3$ ms. This is statistically equal to $\Delta t_{early} = 11.2$ ms.

Mayhew and Washbourne [1] assumed that all (early, mid, and late) MEPs originated from transcranial stimulation and ascribed the onset of early, middle, and long latency MEPs to different corticospinal conduction velocities. However, we dispute this assumption. When indeed the middle and late latency MEPs were to occur due to differences in corticospinal conduction velocities, then according to the formula in Fig. 5, the difference of the latency times Δt of the early, middle, and late MEPs would increase linearly with $1/v$ instead of remaining constant. The middle latency difference Δt_{mid} from Table 1 of Mayhew and Washbourne [1] would then be equal to $\Delta t_{early} \times V_{mid}/V_{early} = 11.2 \times 47.9/19.0 = 28.2$ ms. This differs from the aforementioned $\Delta t_{mid} = 11.3$ ms of their data and contradicts their hypothesis that arrival times of early latency and midlatency MEPs result from different conduction velocities.

To exclude intermingling of MEPs from extracranial origin, transcranially evoked MEPs from TES or TMS can only be reliably analyzed in a time window below t_{SENS}.

4.5. Multipulse TES Facilitation

One single cortical stimulation is able to produce multiple descending volleys of direct and indirect waves in the pyramidal tract [30,31,40]. Spatial and temporal summation of excitatory postsynaptic potentials facilitate spinal motorneurons. The number of recruited motorneurons correlates with the MEP amplitude. Multipulse stimulation causes temporal summation. The MPFF quantifies the facilitation of extra recruited motor neurons by multipulse trains as a ratio. The MPFF is most pronounced when a single pulse generates a weak MEP just above threshold when a few motor neurons are recruited like in the example in Fig. 4A and 4B. At N = 3 ppt, the MPFF is 40.9. Multipulse facilitation causes extra steepening of the amplitude–voltage curve so that a supramaximal level is approached at lower TES voltages when compared with single pulse stimulation. A representative example is the sharp increasing s shaped voltage–amplitude plot in Fig. 2A from $th_{TES} = 80$ V to the supramaximal level starting at about 100 V. Because at supramaximum most motorneurons are recruited, higher stimulation voltages are ineffective.

4.6. Transcranial Latency Time

As shown in the landscape plots of Fig. 1 and in the graphs of Fig. 2B above th_{TES}, the transcranial latency times t_{ECR} and t_{TC} of the ECR and TC muscles show a slight decrease between 85 and 150 V.

Two effects contribute to the decrease. (1) Increasing the stimulation voltage enhances spatial facilitation because more synaptic endings join and more interneurons contribute. This results in a faster increase of the motor neuron membrane potential and earlier firing. Less number of pulses in a train and interneurons are required to recruit LMNs, which decrements are composed of ipi sizes and synaptic delays. (2) Stronger stimulation intensities penetrate deeper in the brain, and activation is further down the corticospinal tract. The traveled distance to the motor neuron and thus the latency time become shorter. In humans, the penetration can reach the foramen magnum where d wave latency times are reduced by 1.8 ms [41,42].

Studies comparing the latencies of epidural responses along the spinal cord in humans have shown that both TMS and TES may cause direct activation of corticospinal neurons [4,5,28,42–44]. As the intensity of TMS was increased, the latency of d waves may show a modest decrease, but activation of the corticospinal tract remains located in the deep layer of the cortex [42]. This can be explained by the limited depth of stimulation of circular and figure-of-eight shaped coils, which are similar to those used in horses having a penetration depth being limited to about 4 cm [45,46]. Larger coil diameters have a deeper but still limited penetration depth [47]. One may expect larger latency times than for TES because of less deep activation along the corticospinal tract or from synaptic delays of the cortical neurons.

4.7. Optimum Choices for Multipulse Parameters

Multipulse stimulation parameters are the number of ppt (n) and the ipi. The MPFF depends on both parameters. Fig. 3 shows a typical bimodal course of the ipi curve. This agrees with the bimodal shape of ipi curves for the upper extremity muscle MEPs as decribed by Van Hal et al [20]. The bimodal maximums are between about 0.8 and 1.4 ms and between 2.6 and 4 ms. The second peak may result from addition of synaptic delays of interneurons of 1.5 to 2.0 ms to the ipi at the first maximum. This bimodal shape usually is best seen just above th_{TES}. An ipi of 1.2 or 1.3 ms is recommended. The optimum MEP amplitude in Fig. 4B is reached at n = 3 ppt. Table 1 shows that this is representative for most cases. It is concluded that under the given condition of sedation, n = 3 ppt is an appropriate choice as multipulse parameter. To cope with the sensitivity for stimulation intensity, TES latency times are taken at the default TES intensity parameter settings at $th_{TES} + 30$ V.

Several addressed subjects in this orienting study should be elaborated further on a larger number of cases to obtain supporting evidence of the encountered observations.

5. Conclusions

This orienting study shows that TES is alternatively to TMS suited for more specific assessment of the motor function of the spinal cord in horses. Transcranial electrical stimulation is painless and well tolerated in horses, is less sensitive to cortical function due to direct stimulation of the corticospinal tract, overcomes hyperpolarization from sedation, the fixated stimulation electrodes exclude reproducibility errors in repeated measurements, and is promising as a test of motor tract function in horses where the neurologic examination is mainly restricted to clinical evaluation.

Supplementary Data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jevs.2015.07.014.

References

[1] Mayhew IG, Washbourne JR. Magnetic motor evoked potentials in ponies. J Vet Intern Med 1996;10:326–9.

[2] Nollet H, Van Ham L, Dewulf J, Vanderstraeten G, Deprez P. Standardization of transcranial magnetic stimulation in the horse. Vet J 2003;166:244–50.

[3] Nollet H, Deprez P, van Ham L, Dewulf J, Decleir A, Vanderstraeten G. Transcranial magnetic stimulation: normal values of magnetic motor evoked potentials in 84 normal horses and influence of height, weight, age and sex. Equine Vet J 2004;36:51–7.

[4] Amassian VE, Quirk GJ, Stewart M. A comparison of corticospinal activation by magnetic coil and electrical stimulation of monkey motor cortex. Electroencephalogr Clin Neurophysiol 1990;77:390–401.

[5] Houlden DA, Schwartz ML, Tator CH, Ashby P, MacKay WA. Spinal cord-evoked potentials and muscle responses evoked by transcranial magnetic stimulation in 10 awake human subjects. J Neurosci 1999;19:1855–62.

[6] Amassian VE, Eberle L, Maccabee PJ, Cracco RQ. Modelling magnetic coil excitation of human cerebral cortex with a peripheral nerve immersed in a brain-shaped volume conductor: the significance of fiber bending in excitation. Electroencephalogr Clin Neurophysiol 1992;85:291–301.

[7] Merton PA, Morton HB. Stimulation of the cerebral cortex in the intact human subject. Nature 1980;285:227.

[8] Nollet H, Van Ham L, Deprez P, Vanderstraeten G. Transcranial magnetic stimulation: review of the technique, basic principles and applications. Vet J 2003;166:28–42.

[9] Nollet H, Van Ham L, Gasthuys F, Dewulf J, Vanderstraeten G, Deprez P. Influence of detomidine and buprenorphine on motorevoked potentials in horses. Vet Rec 2003;152:534–7.

[10] Nicoll RA, Madison DV. General anesthetics hyperpolarize neurons in the vertebrate central nervous system. Science 1982;217:1055–7.

[11] Zhou HH, Jin TT, Qin B, Turndorf H. Suppression of spinal cord motoneuron excitability correlates with surgical immobility during isoflurane anesthesia. Anesthesiology 1998;88:955–61.

[12] Zentner J, Albrecht T, Heuser D. Influence of halothane, enflurane, and isoflurane on motor evoked potentials. Neurosurgery 1992;31:298–305.

[13] Zentner J, Thees C, Pechstein U, Scheufler KM, Würker J, Nadstawek J. Influence of nitrous oxide on motor-evoked potentials. Spine (Phila Pa 1976) 1997;22:1002–6.

[14] Sloan TB, Heyer EJ. Anesthesia for intraoperative neurophysiologic monitoring of the spinal cord. J Clin Neurophysiol 2002;19:430–43.

[15] Macdonald DB, Skinner S, Shils J, Yingling C. Intraoperative motor evoked potential monitoring—a position statement by the American Society of Neurophysiological Monitoring. Clin Neurophysiol 2013;124:2291–316.

[16] Kalkman CJ, Ubags LH, Been HD, Swaan A, Drummond JC. Improved amplitude of myogenic motor evoked responses after paired transcranial electrical stimulation during sufentanil/nitrous oxide anesthesia. Anesthesiology 1995;83:270–6.

[17] Taniguchi M, Cedzich C, Schramm J. Modification of cortical stimulation for motor evoked potentials under general anesthesia: technical description. Neurosurgery 1993;32:219–26.

[18] Journée HL, Polak HE, de Kleuver M, Langeloo DD, Postma AA. Improved neuromonitoring during spinal surgery using doubletrain transcranial electrical stimulation. Med Biol Eng Comput 2004;42:110–3.

[19] Journée HL, Polak HE, De Kleuver M. Conditioning stimulation techniques for enhancement of transcranially elicited evoked motor responses. Neurophysiol Clin 2007;37:423–30.

[20] Van Hal C, Hoebink E, Polak HE, Racz I, de Kleuver M, Journee HL. Optimum interpulse interval for transcranial electrical train stimulation to elicit motor evoked potentials of maximal amplitude in both upper and lower extremity target muscles. Clin Neurophysiol 2013;124:2054–9.

[21] Andersson G, Ohlin A. Spatial facilitation of motor evoked responses in monitoring during spinal surgery. Clin Neurophysiol 1999;110:720–4.

[22] Langeloo DD, Lelivelt A, Louis Journée H, Slappendel R, de Kleuver M. Transcranial electrical motor-evoked potential monitoring during surgery for spinal deformity: a study of 145 patients. Spine (Phila Pa 1976) 2003;28:1043–50.

[23] Deletis V. Intraoperative monitoring of the functional integrity of the motor pathways. Adv Neurol 1993;63:201–14.

[24] Dong CCJ, Macdonald DB, Akagami R, Westerberg B, Alkhani A, Kanaan I, et al. Intraoperative facial motor evoked potential monitoring with transcranial electrical stimulation during skull base surgery. Clin Neurophysiol 2005;116:588–96.

[25] Barker AT, Jalinous R, Freeston IL. Non-invasive magnetic stimulation of human motor cortex. Lancet 1985;1:1106–7.

[26] Edmonds HL, Paloheimo MP, Backman MH, Johnson JR, Holt RT, Shields CB. Transcranial magnetic motor evoked potentials (tcMMEP) for functional monitoring of motor pathways during scoliosis surgery. Spine (Phila Pa 1976) 1989;14:683–6.

[27] Bartley K, Woodforth IJ, Stephen JPH, Burke D. Corticospinal volleys and compound muscle action potentials produced by repetitive transcranial stimulation during spinal surgery. Clin Neurophysiol 2002;113:78–90.

[28] Boyd SG, Rothwell JC, Cowan JM, Webb PJ, Morley T, Asselman P, et al. A method of monitoring function in corticospinal pathways during scoliosis surgery with a note on motor conduction velocities. J Neurol Neurosurg Psychiatry 1986;49:251–7.

[29] Rothwell JC, Thompson PD, Day BL, Dick JP, Kachi T, Cowan JM, et al. Motor cortex stimulation in intact man. 1. General characteristics of EMG responses in different muscles. Brain 1987;110:1173–90.

[30] Amassian VE, Stewart M, Quirk GJ, Rosenthal JL. Physiological basis of motor effects of a transient stimulus to cerebral cortex. Neurosurgery 1987;20:74–93.

[31] Amassian VE, Cracco RQ. Human cerebral cortical responses to contralateral transcranial stimulation. Neurosurgery 1987;20:148–55.

[32] Li DL, Journee HL, van Hulzen A, Rath WT, Sclabassi RJ, Sun M. Computer simulation of corticospinal activity during transcranial electrical stimulation in neurosurgery. Stud Health Technol Inform 2007;125:292–7.

[33] Merton PA, Hill DK, Morton HB, Marsden CD. Scope of a technique for electrical stimulation of human brain, spinal cord, and muscle. Lancet 1982;2:597–600.

[34] King JL. The pyramid tract and other descending paths in the spinal cord of the sheep. Quart J Expt Phys 1911;4:133–49.

[35] Bagley CJ. Cortical motor mechanism of the sheep brain. Arch Neurol Psychiat 1922;7:417–53.

[36] Lemon RN, Griffiths J. Comparing the function of the corticospinal system in different species: organizational differences for motor specialization? Muscle Nerve 2005;32:261–79.

[37] Hahn CN, Mayhew IG, Washbourne JR. Measurement of the lateral thoracic reflex latency in ponies. J Vet Intern Med 1998;12:310–2.

[38] Nollet H, Vanschandevijl K, Van Ham L, Vanderstraeten G, Deprez P. Role of transcranial magnetic stimulation in differentiating motor nervous tract disorders from other causes of recumbency in four horses and one donkey. Vet Rec 2005;157:656–8.

[39] Nollet H, Van Ham L, Verschooten F, Vanderstraeten G, Deprez P. Use of magnetic motor-evoked potentials in horses with bilateral hind limb ataxia. Am J Vet Res 2003;64:1382–6.

[40] Hess CW, Ludin HP. Transcranial cortex stimulation with magnetic field pulses: methodologic and physiologic principles. EEG EMG Z Elektroenzephalogr Elektromyogr Verwandte Geb 1988;19:209–15.

[41] Rothwell J, Burke D, Hicks R, Stephen J, Woodforth I, Crawford M. Transcranial electrical stimulation of the motor cortex in man: further evidence for the site of activation. J Physiol 1994;481:243–50.

[42] Burke D, Bartley K, Woodforth IJ, Yakoubi A, Stephen JP. The effects of a volatile anaesthetic on the excitability of human corticospinal axons. Brain 2000;123:992–1000.

[43] Edgley SA, Eyre JA, Lemon RN, Miller S. Excitation of the corticospinal tract by electromagnetic and electrical stimulation of the scalp in the macaque monkey. J Physiol 1990;425:301–20.

[44] Burke D, Hicks R, Gandevia SC, Stephen J, Woodforth I, Crawford M. Direct comparison of corticospinal volleys in human subjects to transcranial magnetic and electrical stimulation. J Physiol 1993;470:383–93.

[45] Cohen LG, Roth BJ, Nilsson J, Dang N, Panizza M, Bandinelli S, et al. Effects of coil design on delivery of focal magnetic stimulation. Technical considerations. Electroencephalogr Clin Neurophysiol 1990;75:350–7.

[46] Cohen D, Cuffin BN. Developing a more focal magnetic stimulator. Part I: some basic principles. J Clin Neurophysiol 1991;8:102–11.

[47] Jalinous R. Technical and practical aspects of magnetic nerve stimulation. J Clin Neurophysiol 1991;8:10–25.