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ORIGINAL COMMUNICATION

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The amplitude of lower leg motor evoked potentials is a reliable measure when controlled for torque and motor task

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Abstract *Objectives* Motor evoked potential (MEP) amplitudes have the disadvantage of a high variability when repeatedly assessed. This affects the reliability of MEP amplitude measurements taken during the course of motor incomplete spinal cord injury (iSCI). The study investigated the reliability of anterior tibial (TA) MEP measures controlled for dorsal flexion torque and motor task. *Methods* TA MEPs were recorded at 10, 20, 40 and 60% of maximal voluntary contraction (MVC) during a static and dynamic (isometric increase of dorsal flexion torque) motor task. To determine reliability, 20 healthy and five chronic iSCI subjects were tested twice (≥7 days) by the same investigator. Intraclass correlation coefficients (ICCs) were calculated. MEP amplitudes and latencies were compared between 20 healthy and 29 iSCI subjects. *Results* The reliability of MEP amplitude was in general good (ICC ≥ 0.52) and was highest during the static task at 40% MVC (ICC = 0.77). The increased facilitation by the dynamic motor task showed the best reliability at 20% MVC (ICC = 0.48). The reliability was good to excellent for MEP latency ($0.46 \leq ICC \leq 0.81$), MVC (ICC ≥ 0.90) and for the TMS threshold required to evoke a MEP response (ICC ≥ 0.77). The torque generated by the MEP response ($-0.02 \leq ICC \leq 0.55$) and the duration of the silent period ($0.07 \leq ICC \leq 0.50$) were not reliable. Both MEP amplitudes and latencies differed significantly between healthy and iSCI subjects. *Conclusions* Controlling for torque generation and motor task establishes a reliability of TA MEP amplitudes that is sufficient for longitudinal assessments in motor incomplete SCI.

Key words TMS · MEP · repeatability · reference value · latency · cumulative sum · spinal cord injury

Introduction

After an incomplete spinal cord injury (iSCI), clinically relevant improvement in functional outcome can be observed, for both upper and lower extremity motor tasks. As voluntary motor control in humans is largely mediated by direct corticospinal (CS) pathways (e.g., [1, 2]), one would expect that improvement in function correlate well with recovery of CS transmission. Indeed in stroke patients, it appears that over time, the latency of motor evoked potentials (MEP) decreases, while hand function improves [3]. In contrast, after spinal cord lesions, MEP latencies

(i.e., spinal conductivity) remain unchanged, although function significantly improves (e.g., [4, 5]). This might be caused by several factors. For example, during rehabilitation, patients may be trained to perform daily life tasks using different movement strategies. Such strategies allow the patient to perform tasks by alternative muscles synergies using other neuronal circuits. In addition, existing neural structures and pathways might be retrained. Motor units can become larger by training muscle fibers, and subsequently, larger motor units can generate more force, enabling the patient to perform a task again.

However, besides such phenomena, the weak relationship between MEP measures and functional impairment could also be caused by the insensitivity of the neurophysiological recordings applied after iSCI. At present, the impaired CS transmission is evaluated using MEP latency, as this measure shows the lowest variability when repeatedly assessed. Inclusion of MEP amplitude might provide additional information about CS transmission, as it reflects not only the integrity of the CS tract, but also the excitability of motor cortex (especially without activation of the target muscle) and subcortical (brain stem to spinal motoneuron pools) structures. However, MEP amplitudes vary considerably from trial-to-trial [6–8].

The aim of this study was to evaluate the test–retest reliability of several MEP parameters recorded from the anterior tibial muscle (TA). MEPs were recorded at several levels of maximal muscle torque in different conditions, as previously described [9]. The question was whether a protocol controlling for torque and motor task allowed for a reliable assessment of TA MEP amplitude, as well as that of other measures such as latency, the torque generated by the MEP and the duration of silent period. In addition, MEP amplitudes and latencies were compared between healthy and iSCI subjects.

Methods

Subjects

Twenty healthy, young (24 ± 3 years) subjects participated in this study (11 females). On purpose, subjects were included who varied widely in body height between 1.58 and 1.92 m (mean ± SD: 1.76 ± 0.09 m). Body weight was 67 ± 12 kg. Most subjects were right handed (18/20) and right footed (18/20; "With which foot do you kick a ball?"; see [10]).

In addition, reliability was tested in five chronic incomplete spinal cord injured (iSCI) subjects. Table 1 shows several characteristics of these patients. Besides personal characteristics, information about the cause, location and severity of the SCI lesion was provided. Sensory vibration threshold was tested using a Reidel-Seiffer tuning fork [11, 12]. Strength of the foot dorsal flexors was tested using a torque measurement device (see Methods), while walking capacity was tested using 10 m walk tests [13] at preferred and maximum speed, as well as the revised version of the Walking Index for Spinal Cord Injury (WISCI II; [14]).

Subjects were not included when they had any contraindications against the use of TMS (epilepsy, migraine, cardiac pacemakers and ferromagnetic parts in the head), or had orthopedic, neurological or cardiovascular problems (except neurological ones in the case of the iSCI of the patients). All subjects were informed by the investigators personally and in writing and gave written informed consent prior to the experiments. The experiments were approved by the local ethics committee and conformed to the Declaration of Helsinki.

Assessment of torque generation

The subjects lay in a supine position on an examination table. Dorsal flexion torque of the upper ankle joint was assessed by a custom-made device (Lutz Engineering, Rüdlingen, Switzerland, Fig. 1A), with the foot fixed in a slightly plantar flexed position (105°). The device was fixed to the frame of the table. A three degrees-of-movement ball joint allowed a comfortable adjustment of the subject's foot, but prevented any movement at the ankle joint (up to 60 Nm). A cushion was placed under the lower leg (calf) to support its weight. The position of the foot and lower leg was kept constant throughout the experiment. The device was constructed in a way that only dorsal and plantar flexion torque could be exerted. Torque generated along the longitudinal axis of the leg was not recorded. Care was taken such that proximal muscle activation had no influence on the torque recording. Thus, the subject was able to perform isolated isometric dorsal flexion torques in two motor tasks.

Static and dynamic motor tasks

To achieve comparable inter-individual levels of TA activation, both motor tasks were performed at similar levels of background torque. The maximal voluntary contraction (MVC) of the TA was determined during several trials using the custom-built torque measurement device combined with Soleasy software (ALEA Solutions, Zurich, Switzerland). When three trials differed less than 5%, the highest MVC of these three trials was set at 100% MVC. In all subjects, a recruitment curve of force generation was achieved by exertion of both motor tasks at 10, 20, 40 and 60% of MVC. Five recordings were performed at each contraction level separated by relaxation for at least 10 seconds, for both "static" and "dynamic" motor tasks. Additional interruptions of at least 1 minute were allowed between the conditions.

In the "static" motor task, the subjects were instructed to increase the level of muscle torque to the required level and to keep it at that level for over 3 seconds (isotonic–isometric contraction). The exerted torque (in yellow) was displayed on a monitor in front of the subject next to the required torque (in red). As soon as the exerted torque was equal to the requested torque (±10% of the required torque), the color changed to red. Then the transcranial magnetic stimulus was released. Five recordings were made at each torque level. The static 10% condition is referred to as S10, static 20% as S20 etc.

During the "dynamic" motor task, subjects were instructed to perform a continuously increasing isometric (non-isotonic) TA contraction. The slope of this increase was set at 20% of MVC per second. Again, the required torque was displayed on the monitor and the subjects were asked to increase their torque accordingly. The transcranial magnetic stimulus was automatically released when the required torque level was achieved. The recordings and trials corresponded to those of the static task. The dynamic 10% condition is referred to as D10, dynamic 20% as D20 etc.

Table 1 Characteristics of the iSCI patients included in this study

ID	1[a]	2[a]	3		4		5	
Leg tested	Right	Right	Right	Left	Right	Left	Right	Left
Gender	Male	Male	Male		Male		Male	
Age (years)	33	62	60		39		52	
Body height (m)	1.70	1.86	1.82		1.82		1.73	
Weight (kg)	65	107	81		84		76	
Lesion height	C6	C5	C3		C6		C3	
Cause of lesion	Trauma	Stenosis	Tumor		Trauma		Trauma	
AIS	C	D	D		C		D	
Duration SCI (years)	12	2.5	4		5		8	
Days between tests	12	7	7		96		28	
Dominant hand	Right	Left	Right		Right		Right	
Dominant leg	Right	Left	Right		Right		Right	
Vibration med mall	8/8	3/8	5/8	6/8	5/8	7/8	8/8	8/8
Vibration MTP I	7/8	0/8	0/8	6/8	7/8	7/8	8/8	8/8
DF torque (Nm)	9.9	32.7	24.1	23.0	13.7	24.5	28.4	26.4
10M preferred (s)	–	9.6	7.9		10.2		7.8	
10M maximum (s)	–	7.0	5.7		8.0		5.1	
WISCI II	1	18	20		15		20	

[a]Only one leg was or could be tested

Abbreviations: AIS, American Spinal Injury Association Impairment Scale; SCI, spinal cord injury; med mall, medial malleolus; MTP I, first metatarsophalangeal joint; DF, dorsal flexion; 10M, 10 m walk test, performed at preferred and maximum speed; WISCI II, revised Walking Index for Spinal Cord Injury

Transcranial magnetic stimulation

A single-pulse TMS was performed using a MagPro X100 Magnetic Stimulator (DANTEC Medical A/S, Skovlunde, Denmark). For all measurements, a figure-of-eight coil was used. The coil position for stimulation of the TA was just lateral to the midline, close to Cz (Vertex), contralateral to the target muscle and moved in small increments until the location with lowest threshold was determined. The duration of the biphasic single-pulse stimulus (more effective than a monophasic pulse [15]) was set at 200 μs. Placement and stimulation threshold were identified for each subject at the beginning of the recordings during static muscle contraction at 10% of the MVC torque. The stimulus threshold was expressed as the percentage of stimulator output that evoked a MEP amplitude of at least 50 μV amplitude in approximately 50% of 10 consecutive stimuli (cf. [16]). The stimulation intensity was set at 1.2 times motor threshold [17, 18] and was kept constant throughout the experiment.

EMG recordings and analysis

For the EMG recordings, silver/silver – chloride surface electrodes were placed with an inter-electrode distance of 2 cm over the TA muscle belly, at the proximal one third of the distance between the apex of the patella and the talocruralis joint. EMG recordings were pre-amplified, sampled at 2,000 Hz and saved on hard disc for offline analysis. The recording started 500 ms prior to MEP trigger and lasted 500 ms after the MEP trigger.

Analysis was performed using Soleasy software (ALEA Solutions, Zurich, Switzerland). The mean TA EMG amplitude was calculated over a time window of 200 ms preceding the TMS trigger. The mean amplitude was subtracted to reset the EMG offset to zero. Then, the raw EMG signals were rectified and band-pass filtered (30 Hz–1 kHz). The mean trajectory was calculated from the five measurements of each condition.

MEP analysis

To determine more reliably the onset and offset of the MEP response and silent period, the cumulative sum method (Cusum; see [19–21]) was included in the analysis (see Fig. 1B, C). The Cusum was calculated by adding the difference of the EMG from the pre-stimulus mean to the preceding value of the Cusum. The pre-stimulus mean was calculated over a time window of 200 ms [22].

The MEP amplitude was determined by calculating a RMS value over a time window of 20 ms from the onset of the MEP response [9, 23]. TA MEP responses were accepted for analysis when the amplitude was at least 50 μV above background EMG and were followed by a silent period.

The net MEP amplitude was calculated by subtracting background EMG from the MEP amplitude. The background EMG was not calculated over a time window of 200 ms prior to the MEP trigger as done earlier [9], but over a time window 20 ms prior to the MEP trigger. This was done, because in the dynamic condition, the background activity was greater when calculated over 20 ms prior to the MEP trigger (D10: 44 ± 17 μV; D20: 64 ± 26 μV; D40: 106 ± 30 μV and D60: 154 ± 51 μV) compared to 200 ms (D10: 47 ± 23 μV, $p = 0.11$; D20: 59 ± 22 μV, $p = 0.046$; D40: 98 ± 29 μV, $p = 0.002$ and D60: 145 ± 35 μV, $p < 0.001$). As such differences were not found in the static condition, we corrected only for the increase in background activity in the dynamic situation.

The latency was determined from the onset of the measurement to the onset of the MEP response (Fig. 1B, C); the duration of the silent period from the offset of the MEP response to the offset of the silent period (Fig. 1B, C). Furthermore, the net MEP response obtained during the static condition was subtracted from the one from the dynamic condition and the torque exerted by the MEP response was analyzed (Fig. 1D).

Comparison of MEP parameters between healthy and iSCI subjects

MEP amplitudes and latencies were compared between the healthy subjects and 29 iSCI subjects (50 measurable legs; six females). All iSCI subjects were motor incomplete (ASIA C and D). The iSCI was of traumatic or non-traumatic (e.g., ischemic) origin and the levels of lesion varied from cervical to lumbar. The mean (±SD) age was 51 ± 17 years and the body height was 1.75 ± 0.10 m.

To compare MEP amplitudes between the groups, the net amplitude was divided by the background EMG activity (normalized MEP amplitude). As MEP latencies depended on body height, reference values were calculated for the healthy subjects by dividing the latency by body height.

Statistical procedures

Reliability

In the healthy subjects, both legs were evaluated and considered to be independent. In two SCI subjects, only one leg could be recorded from (see Table 1). The same investigator assessed all subjects twice. For the healthy subjects, the mean (±SD) time between the assessments was 14.0 ± 11.4 days and varied between 7 and 56 days (median: 10 days). The iSCI subjects were evaluated when they were in hospital for an ambulant check-up. Therefore, the time between their two assessments varied (see Table 1). For each condition, the test–retest reliability for the parameters was calculated using an intraclass correlation coefficient (ICC) as advocated by Rousson et al. [24]. An ICC between 0 and 0.25 was interpreted as none to little, between 0.25 and 0.50 as fair, between 0.50 and 0.75 as moderate to good and above 0.75 as very good to excellent [25]. An ICC increased when the variance between subjects was large (similar to a normal correlation coefficient; see also [26]).

The variability of MEP measures (such as the latency) can be expected to be small in healthy subjects. This variability is expected to increase when data from SCI subjects is analyzed. However, as only a small number of iSCI subjects were tested, the ICC was calculated for the healthy subjects ($ICC_{healthy}$) and for the healthy and iSCI subjects grouped (ICC_{all}).

The ICC was calculated using the variance component option under the linear regression methods in SPSS version 11.5 for windows (measurement and subject were random factors in a main-effect only model). Differences in MEP measures between static and dynamic condition and between the torque levels were analyzed using a two-way ANOVA for repeated measures, which was calculated using a mixed model in SAS version 9.11 for windows. Pair-wise comparisons were corrected for multiple comparisons using Bonferroni's correction.

Comparison of MEP measures between healthy and iSCI subjects

Differences in normalized MEP amplitudes between healthy and iSCI subjects were analyzed using Student's t-test.

Results

Test–retest reliability

In Fig. 2, the net MEP amplitudes of the first and second measurements are shown in scatter-plots. Points closer to the diagonal line indicate better test–retest reliability. In general, the net MEP amplitude ICCs were good. For the static condition at 40% of MVC, the ICC was excellent. Higher ICC_{all} than $ICC_{healthy}$ values for the MEP amplitude were not observed.

The MEP response is facilitated during the dynamic compared to the static task [9]. Therefore, the reliability was determined for the difference in MEP amplitude between dynamic and static tasks. The best ICC values were observed for the 20% of MVC conditions (ICC_{all} = 0.48; $ICC_{healthy}$ = 0.46). A fair

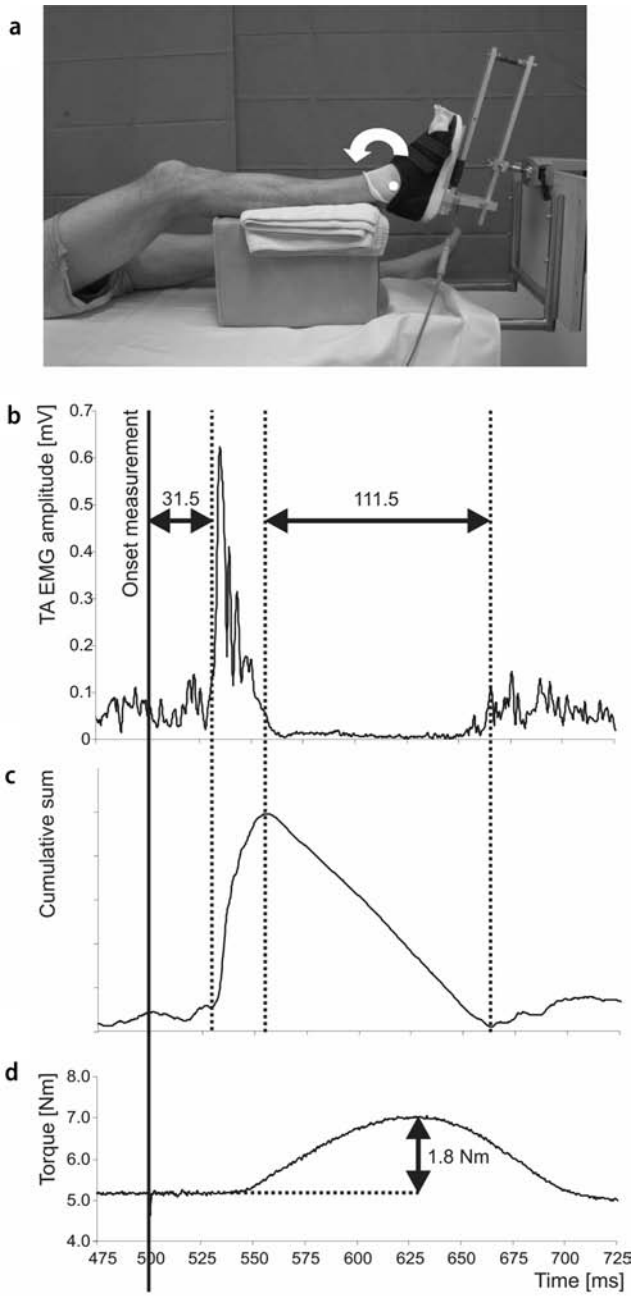

Fig. 1 Experimental set up and data analysis. (**a**) Custom-made torque measurement device, used to assess maximal voluntary isometric dorsal flexion torque, as well as the amount of background torque required for the transcranial magnetic stimulation. (**b**–**d**) Analysis of MEP measures obtained from a healthy female tested in the static condition at 20% MVC. The latency (31.5 ms) of the (**b**) tibial EMG amplitude was quantified using (**c**) the cumulative sum. The cumulative sum was also used to determine onset and offset of the silent period (duration 111.5 ms). The difference between (**d**) background torque and torque generated by the MEP response was calculated (1.8 Nm). Abbreviations: MVC, maximal voluntary contraction

reliability was found for the other conditions (10% MVC: ICC_{all} = 0.29; $ICC_{healthy}$ = 0.28; 40% MVC: ICC_{all} = 0.35; $ICC_{healthy}$ = 0.37; 60% MVC: ICC_{all} = 0.22; $ICC_{healthy}$ = 0.28).

The MEP latency ICC_{all} values were considerably higher compared to the $ICC_{healthy}$ values, although both varied between fair and excellent (Table 2).

The ICCs calculated for the MVC were excellent: $ICC_{healthy}$ was 0.90 and ICC_{all} was 0.93. For the stimulation intensity, which was 1.2 times the stimulation threshold, the ICCs were very good ($ICC_{healthy}$ = 0.77; ICC_{all} = 0.84). A similar high reliability was found for the distance between the tragus and the center of the coil ($ICC_{healthy}$ = 0.70; ICC_{all} = 0.83), while it was fair to good for the distance between the root of the nose and the center of the coil ($ICC_{healthy}$ = 0.39; ICC_{all} = 0.63).

The reliability of the torque generated by the MEP response was poor in the conditions with small (10% and 20% MVC) voluntary torque (see Table 2), while it increased in the 40% and 60% MVC conditions. We found slightly higher ICC_{all} than $ICC_{healthy}$ values. Finally, the reliability of the duration of the silent period was poor to moderate (Table 2).

Task-specific facilitation

In line with previous studies [9, 27, 28], the net MEP amplitude of healthy subjects was larger in the dynamic compared to the static condition at 10% and 20% MVC, but not at 40% and 60% MVC. An ANOVA for repeated measures indicated a significant difference in the net MEP amplitudes assessed during the first measurement between the eight conditions [$F(7,273) = 29.85$; $p < 0.001$]. Pair-wise comparisons showed that the amplitudes were larger in the dynamic than in the static condition at 10% (D10: 259 ± 118 μV vs. S10: 185 ± 97 μV; $p < 0.001$) and 20% (D20: 306 ± 133 μV vs. S20: 227 ± 119 μV; $p < 0.001$), but not at 40% (D40: 320 ± 129 μV vs. S40: 298 ± 140 μV; $p = 1.0$) and 60% (D60: 374 ± 134 μV vs. S60: 369 ± 132 μV; $p = 1.0$). Similar results were obtained for the second measurement.

Furthermore, we found significant differences between the latencies calculated for the different conditions in the healthy subjects during the first measurement [$F(7,273) = 4.39$; $p < 0.001$)]. Pair-wise comparisons showed that the latency calculated for the static condition at 20% of MVC (S20: 31.1 ± 2.8 ms) was longer compared to the latencies calculated for the static condition at 60% of MVC (S60: 30.1 ± 2.2 ms; $p = 0.046$), the dynamic condition at 40% of MVC (D40: 29.9 ± 2.5 ms; $p = 0.003$) and the dynamic condition at 60% of MVC (D60: 29.7 ± 2.4 ms; $p < 0.001$) conditions. In addition, the latency was longer for the static condition at 10% of MVC (S10: 30.8 ± 2.5 ms) than for the dynamic condition at 60% MVC (D60; $p = 0.021$). However, these results were not confirmed at the second measurement [$F(7,273) = 0.87$; $p = 0.53$)].

Comparison of MEP parameters between healthy and iSCI subjects

As the reliability was best for the static 40% MVC condition, normalized MEP amplitudes were compared that were assessed in this condition. In the 40% static condition, we had data from 27/29 iSCI subjects (45 measurable legs). The normalized MEP amplitudes varied widely between the healthy (3.71 ± 1.72; see Fig. 3) and iSCI subjects (1.44 ± 0.86). Values of young (≤40 years; n = 11, mean age: 32 ± 8 years) and elderly iSCI subjects (n = 18, age: 63 ± 10 years) are presented separately in Fig. 3 (and Fig. 4). For better comparison between iSCI and healthy subjects, we matched for MVC (Fig. 3A) and background EMG activity (Fig. 3B). For example, when selecting subjects with a MVC between 25 and 35 Nm (grey area, Fig. 3A), the MVC did not differ between the iSCI (29.5 ± 2.8 Nm; n = 15) and healthy subjects (30.1 ± 3.0 Nm; n = 22; $p = 0.55$), while the MEP amplitudes did (iSCI: 1.16 ± 0.49; healthy: 3.57 ± 1.33; $p < 0.001$). Similarly, when selecting subjects with a background EMG activity between 60 and 100 μV (grey area Fig. 3B), the background activities did not differ between the iSCI (83.5 ± 10.1 μV; n = 16) and healthy subjects (80.3 ± 10.7 μV; n = 26; $p = 0.35$), while the amplitudes did (iSCI: 1.22 ± 0.57; healthy: 3.56 ± 1.90; $p < 0.001$).

Figure 4A, shows that the MEP latencies of the healthy subjects, determined in the 20% dynamic condition, increase as a function of body height (linear regression equation: latency [ms] = 17.4 × body height [m] − 0.2; the explained variance (R^2) was 0.39). When the latency [ms] was divided by body height [m] and plotted as a function of body height (Fig. 4B), the resulting value did not depend on body height (latency/body height = 8.7×10^{-3} × body height + 17.3; $R^2 = 5 \times 10^{-7}$). Pathological latencies could easily be recognized using the upper limit (19.4 ms/m) of the 95% confidence interval (mean = 17.3 ms/m; lower limit = 15.2 ms/m). The upper limit of the 95% confidence intervals was consistent for all conditions (dynamic and static; 10, 20, 40 and 60% MVC) and varied between 18.8 and 20.5 ms/m (mean: 19.5 ms/m). The upper limit of the 95% confidence interval could distinguish well between healthy and iSCI subjects, as 41 out of the 50 iSCI legs were defined pathological (sensitivity of 82%; see Fig. 4B).

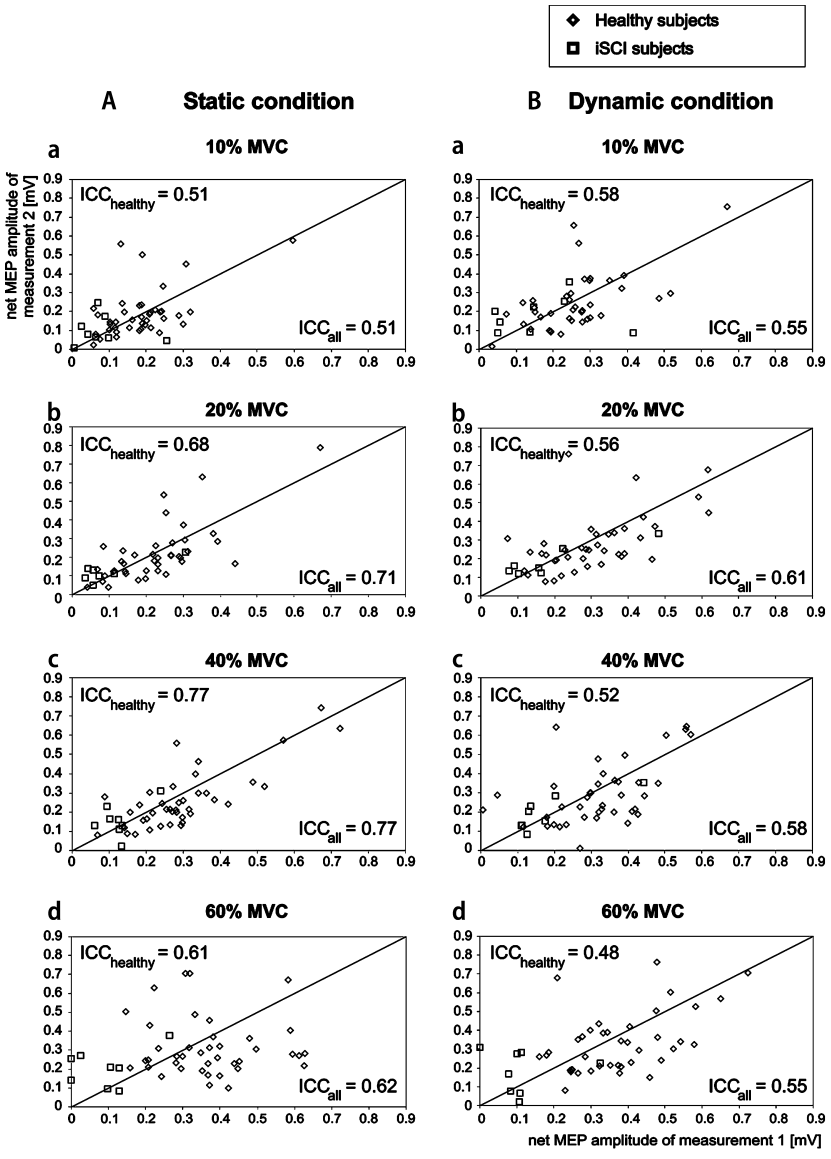

Fig. 2 Reliability of the anterior tibial MEP amplitude. Scatter-plots of MEP amplitudes during (**A**) static and (**B**) dynamic conditions. Background dorsal flexion torque was set at (a) 10, (b) 20, (c) 40 and (d) 60% of maximal torque. The closer the points are located to the diagonal line, the better the reliability. Abbreviations: iSCI, incomplete spinal cord injury; MVC, maximal voluntary contraction; ICC, intraclass correlation coefficient

Discussion

This is the first study that has used a new TMS protocol to assess test–retest reliability and reference values of TA MEP measures. For this, MEPs were assessed in static and dynamic conditions at different levels of TA torque with the following conclusions being reached. (1) Torque and task controlled MEPs allow reliable follow up recordings of amplitudes and latencies as assessed by the good test–retest reliability. (2) The reliability calculated for the difference in dynamic and static MEP amplitude was only fair, but best for the 20% MVC condition. (3) In line with previous studies [9, 27, 28] the net MEP amplitude was more strongly facilitated during a dynamic compared to a static motor task at low percentages of MVC. (4) MEP amplitudes matched for torque and background EMG were smaller for iSCI subjects compared to healthy ones. (5) Reference values for TA MEP latency with a cutoff value were obtained for distinguishing between normal and pathological TA MEP latencies.

Test–retest reliability

Although research reports on TMS have increased over the years [26], only a small number have investigated the reliability of this approach. The main objective of this study was to determine the reliability of MEP amplitude for the evaluation of functional and

Table 2 Intraclass correlation coefficients for MEP measures

Condition	MEP latency		Torque MEP		Silent period	
	Healthy	All	Healthy	All	Healthy	All
S10	0.48	0.77	−0.02	0.08	0.30	0.39
S20	0.63	0.76	0.20	0.38	0.40	0.50
S40	0.55	0.74	0.28	0.34	0.16	0.29
S60	0.71	0.80	0.46	0.50	0.34	0.31
D10	0.63	0.77	0.33	0.44	0.24	0.20
D20	0.46	0.72	0.16	0.27	0.12	0.07
D40	0.62	0.81	0.39	0.44	0.30	0.25
D60	0.64	0.74	0.50	0.55	0.25	0.24

Abbreviations: S10, static condition, tested at 10% of the maximal dorsal flexion torque; D60, dynamic condition, tested at 60% of the maximal dorsal flexion torque

neurological recovery in iSCI subjects. Here, the best reliability of MEP amplitude occurred in the 40% static condition.

TMS reliability has previously been studied in the first dorsal interosseous muscle [29]. The authors plotted the MEP amplitude against the background EMG activity and used linear regression to quantify this relationship. The reliability obtained for several MEP measures of the regression equation was 0.50 for the slope and 0.53 for the y-intercept. Another study [26] investigated the reliability of TMS measures in the biceps brachii and first dorsal interosseous muscles, both in rest and active conditions. During the active conditions, ICC varied between 0.63 and 0.73. However, the reliability of MEP amplitudes in the first dorsal interosseous and flexor carpi ulnaris muscles was found to be poor ($0.16 < ICC < 0.55$; [6]).

Similar or better ICC values were observed in this study. This may be somewhat surprising considering that lower extremity muscles, which are known to have less direct CS projections compared to upper extremity muscles [30], were tested in the present study. Furthermore, the subjects were assessed twice after a temporal separation of at least seven days, while in previous studies, the test intervals varied between one hour [6] and about 24 hours [26, 29]. In addition, most of our healthy subjects were women, while previous studies tested mainly, or solely, men [26, 29]. Women tend to have higher trial-to-trial variability in MEP responses compared to men [31], which might be caused by changes in cortical excitability modulated by changing ovarian steroid levels during the menstrual cycle (e.g., [32]). The higher MEP trial-to-trial variability in elderly subjects [31] is unlikely to influence our findings, as the mean age of our participants was equivalent to that of volunteers in other reliability studies [6, 26, 29].

In healthy subjects, reliability of the latency was good and further improved when data of the iSCI subjects were included in the analysis. This is in agreement with previous findings that ICCs reflect inter-individual variability (e.g., [26]). The torques generated by the MEPs were small and it is likely that this influenced reliability negatively. In addition, the relationships between the net MEP amplitudes and their exerted torques varied widely (mean ± SD non-parametric Spearman's correlations (ρ) were: static, $\rho = 0.21 \pm 0.62$, median: 0.30; dynamic, $\rho = 0.06 \pm 0.61$, median: 0.0). These findings indicate that the MEP torques are of limited clinical usefulness.

Finally, the reliability of the duration of the silent period, a measure that reflects cortical inhibition, was poor. Its reliability has been investigated for testers and methods in which the same MEP response was analyzed (e.g., [22, 33]. However, as our findings indicate poor reliability of this measure when repeatedly assessed, results from studies investigating cortical inhibition might therefore be interpreted with caution.

Task-specific facilitation

The MEP amplitude reflects not only the integrity of the CS tract but also the excitability of the motor cortex (especially with no background activity of the target muscle) and nerves. In line with other reports [9, 27, 28], the present study showed that significantly more facilitation occurred at lower dynamic conditions compared to static conditions. This can be used in SCI subjects where it is difficult to record MEP from lower limb muscles. Furthermore, this difference in facilitation might represent an additional MEP measure for diagnosis and follow-up after iSCI.

Comparison of MEP parameters between healthy and iSCI subjects

The normalized MEP amplitudes were significantly reduced in the iSCI subjects, even for similar amounts of muscle strength or background EMG activity. Normalized MEP amplitudes might therefore be

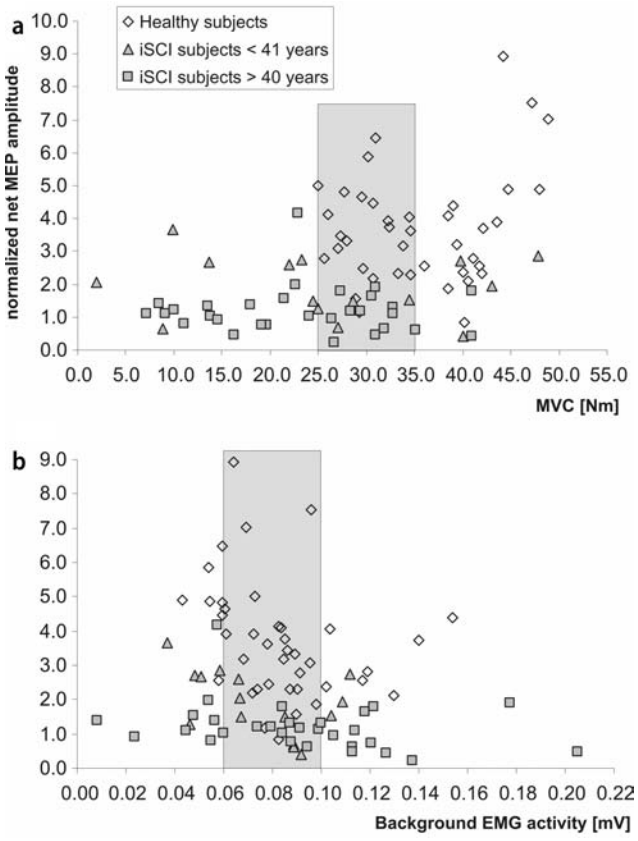

Fig. 3 Relationship between MVC/background EMG activity and MEP amplitude. The normalized MEP amplitude is plotted against (**a**) the maximum voluntary contraction (MVC) and (**b**) the background EMG activity for the healthy subjects and the young (≤40 years) and elderly incomplete spinal cord injured (iSCI) subjects. Grey areas indicate matched (**a**) MVC and (**b**) background EMG activity (see text)

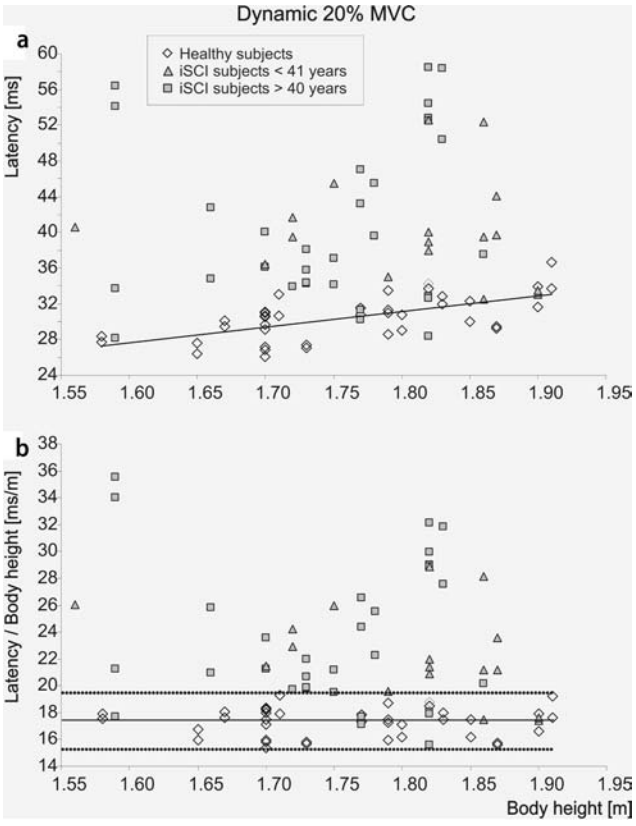

Fig. 4 Relationship between body height and anterior tibial MEP latency. Relationships between body height and (**a**) anterior tibial MEP latency and (**b**) latency divided by body height, for the dynamic condition at 20% of maximal voluntary contraction (MVC). Data points of the healthy and young (≤40 years) and elderly incomplete spinal cord injured (iSCI) subjects are presented differently. (**a**) The solid line represents the linear regression line fitted through the data of the healthy subjects. (**b**) The solid line represents the average value, while the dotted lines represent the upper and lower limits of the 95% confidence interval

sensitive enough to detect impairment in CS tract transmission in iSCI subjects with no obvious reduction in muscle strength, as has been previously discussed for the triple stimulation technique [34]. Indeed, the triple stimulation technique [34, 35] allows also to determine changes in MEP amplitudes reliably [36]. However, this technique quantifies the severity of CS tract damage, while the present study investigates the influence of facilitation caused by background activity and motor task.

No generally accepted reference values for TA MEP latency currently exist, although normal latencies appear to be around 30 ms (e.g., [32, 33, 37–40]). In line with previous reports [37, 40], a linear relationship between body height and MEP latency was found. For practical purposes, we calculated a value by dividing the latency by body height. The cutoff value of about 20 ms/m clearly indicates a pathological MEP latency. As the MEP latency includes both central and peripheral motor conduction times, it might reflect impairment of spinal tract conductivity in most iSCI subjects, as peripheral nerve conduction is not significantly affected after SCI [41, 42]. Nerve root involvement as a consequence of a SCI is common, but typically involves roots around the epicenter of the lesion [43]. Therefore, in subjects with cervical and thoracic SCI, serial measures of TA MEP latency are appropriate for following the time course of spinal tract conductivity, as suggested elsewhere [38, 44].

Clinical relevance

The present approach might add information about the recovery of CS pathways after iSCI. According to actual reports, in traumatic iSCI, MEP latencies remain unchanged even in subjects with significant improvement in functional outcome [4, 5]. Thus, clinical improvement seems to be based on compensation and plasticity, rather than on improved conductivity in CS pathways. The inclusion of MEP

amplitude as a diagnostic tool might increase the sensitivity of TMS for detecting changes over time or for functional changes related to new treatment interventions. Indeed, a recent report indicated that in chronic iSCI subjects, an increase in MEP amplitude was associated with an improvement in walking function after treadmill training [45]. Furthermore, stroke patients with a reasonable high TA MEP amplitude (≥18%, expressed as a percentage of the maximum motor response evoked by supra-maximal peripheral nerve stimulation) were able to walk at 4 months after stroke, while those with a low TA MEP amplitude (≤14%) did not [46]. Therefore, the early assessment of CS transmission and its course during clinical recovery might provide information about mechanisms underlying functional recovery.

Although it is important to monitor for EMG background activity, it appears to be less relevant whether using background EMG or torque generation. In the present study, the background torques (10, 20, 40 and 60% MVC) correlated excellently with the background EMG amplitudes, in both the static (mean Pearson's correlation coefficient ± SD: $r = 0.99 \pm 0.01$) and the dynamic condition ($r = 0.98 \pm 0.02$).

Conclusions

The present study shows that TA MEP amplitude can reliably be assessed. Based on the present findings 40% MVC provides highest reliability for longitudinal follow up recordings of corticospinal tract conductivity. Differences in task-specific facilitation of MEP amplitude might provide a sensitive measure during recovery from a SCI and can be reliably assessed at 20% MVC. The TA MEP amplitude appears to be smaller in iSCI subjects compared to healthy subjects, even when matched for the level of functional outcome or background EMG activity. A (fixed) value of about 20 ms/m (latency [ms]/body height [m]) was found to be the upper limit of normal TA MEP latencies.

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