Research article

Similarities and differences in cervical and thoracolumbar multisegmental motor responses and the combined use for testing spinal circuitries

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Study design: Experimental study.
Objective: To determine similarities and differences of C7 and T11–12 multisegmental motor responses (MMR) studies for the upper limbs (UL) and lower limbs (LL).
Settings: Neuroscience Lab, TWU (School of Physical Therapy, TX, USA).
Methods: C7 and T11–12 percutaneous electrical stimulations were applied while recording muscle action potentials from ULs and LLs.
Results: The procedure of cervical MMR (CMMR) was easier in application than thoracolumbar MMR (TMMR), requiring less current intensities but cause more "jolts" in the trapezius/shoulder complex, due to close proximity of the stimulation electrodes. CMMR evoked large amplitude motor responses in the millivolts range in (UL) muscles, but smaller amplitude signal in (LL) muscles (in microvolts). TMMR evoked large amplitude motor responses in both UL and LL (in millivolts). The MMR amplitude was generally larger in the UL as compared to the LL, in the distal limb muscles more than in the proximal limb muscles. CMMR and TMMR for the UL were comparable in amplitude, latencies and action potential shapes. Signal latencies were longer for distal limb muscles as compared to proximal limb muscles and were slightly longer for LL as compared to UL muscles. MMR signals were either biphasic or triphasic in shape.
Conclusion: CMMR and TMMR have similarities and differences in the methods and recording signal that must be considered during its clinical applications. Comparing the signal of the UL muscles with CMMR and TMMR could be a useful test for the integrity of the ascending and descending spinal pathways in patients with spinal cord injuries and diseases.

Keywords: Multisegmental Motor Responses, Spinal Cord, Thoracic, Cervical, Propriospinal pathways

Introduction

Spinal cord stimulation using surface electrical current while recording limb muscles has recently been reported citing the possible use in patients with spinal cord injuries and diseases.[1–3] Thoracolumbar stimulation with recording from the lower limbs (LL) has been the commonly published reports.[4–6] However, recently we have been able to electrically stimulate the cervical and thoracic spinal segments and record multisegmental motor responses (MMR) from the upper limb (UL) muscles.[7,8] The methods and application for both procedures (cervical and thoracolumbar) have similarities and differences that could be useful for scientists and clinicians during application of those tests. Furthermore, the clinical application of these procedures might differ due to the possible pathways involved.[9]

Spinal cord injuries and diseases are common clinical pathologies in the 21st century. Motor accidents, longer time periods spent on computer and internet, and lack of exercises are central causes for vertebral and spinal disorders. Spinal cord diseases, such as syringomyelia, spina bifida, transverse myelitis, and multiple sclerosis,

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are common spinal cord diseases that need more objective testing procedures to identify the pathological site and extent of the pathology as well as the circuitries involved. Electrophysiological testing procedures may be those promising testing protocol. MMR is new procedure that might be useful for spinal cord testing protocol. The development of cervical MMR (CMMR)[7] and the new findings of recording UL MMR with T11–12 stimulation opened the door to test pathways that have not been tested before.[8]

Comparing UL muscular signal to cervical and thoracic spinal stimuli may explain the direct (cervical stimuli to UL muscles) and ascending (thoracic stimuli to UL muscles) pathways. Similarly comparing LL muscular signal to thoracic and cervical spinal stimuli may explain the direct (thoracic stimuli to LL muscles) and descending (cervical stimuli to LL muscles) pathways. Charting these pathways in healthy subjects would be necessary standard for pathological spinal cord injuries and diseases.

Imaging testing of spinal cord injuries and diseases might identify the location and extent of the cellular disruptions. But it does not specify the pathophysiology of the injury or the disease. Clinical testing, besides being subjective it might miss those pathways or cellular structure that are at the fringe of activity.[2] Electrophysiological tests, especially the MMR finding, might complement those gold standard procedures, namely clinical testing and imaging procedures.

These MMR testing procedures are useful also for experimental physiologists who test integrated arm and LL functions.[6,10] Thoracolumbar MMR (TMMR) has been tested during locomotive function[3,6,11] as well as postural[12] changes.

The purpose of this report is to discuss the similarities and differences between CMMR and TMMR in healthy subjects. A second purpose is to show muscular responses of the LL using cervical spinal stimulation with new methodological approach or different electrode configuration. A third purpose is to present comparative data of cervical and lumbar spinal stimulation in UL and LLs and discuss the possible use of testing spinal cord circuitries.

Disruption of these circuitries, or some of it in spinal cord injuries and diseases, can then be evaluated and monitored resulting in better care for those patients in setting up more effective treatment strategies.

The clinical implication of the similarities and differences between the CMMR and TMMR is tremendous and may affect the interpretation of the results of pathological disorders. Example of these implications will be discussed at different sections of this report.

Material and methods

Human subjects

A total of 36 subjects were tested in this study, 19 females and 17 males with age range 18–65 years. Some subjects completed all phases of the tests while others completed some phases only. All subjects were healthy with no neck, back, leg, or arm pain or radiculopathy during the previous 12 months and could tolerate electric pulses to the cervical or thoracolumbar spine. Subjects were excluded if they had metabolic or neurological diseases, arthritis, or radiculopathy of the cervical spine or cancers. Table 1 presents the demographic characteristics of the study subjects as well as the number of subjects tested for different experimental phases. Subjects signed informed consent forms approved by the IRB-Texas Woman's University, to participate in the study.

Electrical stimulation and recording

The C7 or T11–12 vertebral segment was electrically stimulated using 1 millisecond square wave pulses at 0.2 pps and at the maximum muscular responses. The C7 or T11–12 segment was located by palpation during flexion/extension of the neck or the trunk, and a cup electrode was affixed to the skin overlying the intervertebral space of the selected spinal segment. For effective stimulation, the electrode (cathode) was kept snug, at the vertebral site, by the operator during testing. Spinal levels were marked and all electrode positions were identified by the same researcher for each subject. The reference electrode (anode) was a $5'' \times 9''$ cm, pre-gelled flexible pad (similar to those used with transcutaneous electrical nerve stimulation; TENS), and the pad was applied on top of the clavicle-upper trapezius between the acromion and the border of the neck (for UL recording in both cervical and T11–12 stimulation). It was also applied on the anterior superior iliac spine (ASIS) (for LL recording both in cervical and T11–12 stimulation). Stimulation technique was the most critical step of the current experimental procedures. In this study, two different stimulation intensities (maximal and sub-maximal) were used. This study was carried in two phases/experiments:

Experiment 1: (Sub-maximal stimulation intensity-SI): In order to reach maximum action potential with sub-maximal level stimulus (65–75 mA), a smaller sized reference electrode was used. A 2-inch square reference electrodes (anode) were used instead of $5'' \times 9''$ cm. Using small reference electrodes provided smaller signal amplitude than the large reference electrode that was used in experiment 2. Stimulus intensity was then increased until maximum possible action

Table 1 Demographic characteristics of subjects tested for CMMR and TMMR of the ULs and LLs

Experiments	Methods	Sex	Age (years)	Height (cm)	Weight (kg)
Experiment 1 Stimulus intensity: sub-maximal	UL: CMMR and TMMR LL: TMMR (*N*: 13)	5 M 8 F	27.6 ± 5.6	168 ± 14.2	65.3 ± 5.2
Experiment 2	UL: CMMR and TMMR (*N*: 20)	9 M 11 F	35.4 ± 14	167 ± 10.2	71.8 ± 14.7
Stimulus intensity: maximal	LL: TMMR (*N*: 22)	10 M 12 F	36.6 ± 14	167 ± 9.9	71 ± 14

potentials were recorded from the tested muscles at a comfortable level.

Experiment 2: (Maximal stimulation intensity, MI): Using 5 mA increments, the stimulation intensity was increased from threshold to 100 mA or the maximum tolerable intensity and maximum action potentials were recorded from the tested muscles. All UL and LL CMMR and TMMR measurements were carried out in both limbs.

Muscular responses/action potentials were recorded using the four-channel Cadwell EMG unit (Cadwell Lab., Kennewick, WA, USA). Surface silver–silver chloride bipolar bar electrodes with an inter-electrode distance of 30 mm with gel were applied on the muscles using 3M hypoallergenic tape. A round metal ground electrode (2 cm diameter) with gel was applied to the subject's lateral epicondyle (for UL recording), and the head of the fibula (for LL TMMR recording) or scapular spine (for LL CMMR recording). Recording electrode placements were carried out using the method of Sabbahi and Sengul.[7,8] Both CMMR and TMMR action potentials were recorded from the motor points of the following muscles in both upper-extremities: abductor pollicis brevis (APB), flexor carpi radialis (FCR), biceps brachii (BB), and the lateral head of triceps brachii (TB) muscles. For CMMR, action potentials were recorded from the right LL of the following muscles: the Vastus medialis oblique's (VMO), medial hamstrings (MH), Soleus (SOL) and tibialis anterior (TA) muscles. TMMR were recorded from the same muscles of the right and left lower extremities. Fig. 1 shows the stimulation-recording electrode set-up. In the first experiment, the signal was recorded from the abductor digiti minimi (ADM) instead of TB and was recorded from the gluteus medius (GM) instead of the medial hamstring and the flexor hallucis brevis instead of TA (Tables 2–5). These recordings from different muscles were carried out in order to study the MMR signal of larger number of muscles in the ULs and LLs. Recording parameters were 100–1000 μv/div with a sweep speed of 5 milliseconds/div, using 10 Hz–10 kHz band pass of Butterworth filter. All signals were recorded using 38.4 kHz sampling rate per channel with 16 bit A/D converter.

Experimental procedures

After signing the informed consent, subjects were seated in an armless chair with the forearm rested on a pillow in the lap. Stimulation and recording electrodes sites were cleansed using alcohol and dried and then the electrodes were applied in place. In the first experiment, cervical spinal stimulation was carried out first (step 1) while recording muscular signal from the right and left ULs in random order. This was followed by T11–12 vertebral stimulation while recording both UL signals (step 2) and followed by T11–12 electrical stimulation while recording both LL signals (step 3). Cervical stimulation was then performed while recording muscular signal from the right LL (step 4). CMMR-ULs, TMMR-UL and LL tests were carried out in random order.

Subjects tolerated the electrical stimuli, especially when given rest periods (5 minutes) between different steps of the test. For each step, five traces were recorded. Subjects were told to relax during the testing periods and to refrain from head turning, arm or leg movements. At the end of the experiment, electrodes were removed, skin was cleansed, and the subject was dismissed.

Signal and statistical analyses

Five traces were recorded and averaged for each muscle tested in all different steps. The peak-to-peak amplitude and deflection latency were the dependent parameters for the CMMR and TMMR. Signals were pooled for all subjects using descriptive statistics, with the mean value and standard deviations analyzed using the SPSS-19.0 statistical package. Differences in the MMR muscles amplitude variables (APB, FCR, BB, and TB) were tested using MANOVA with the following factors: (1) vertebral level (C7 and T11–12); (2) limb Side (right and left). Tukey's *post hoc* analyses were used to identify specific variations within factors. A Wilcoxon's signed rank test was used for comparisons

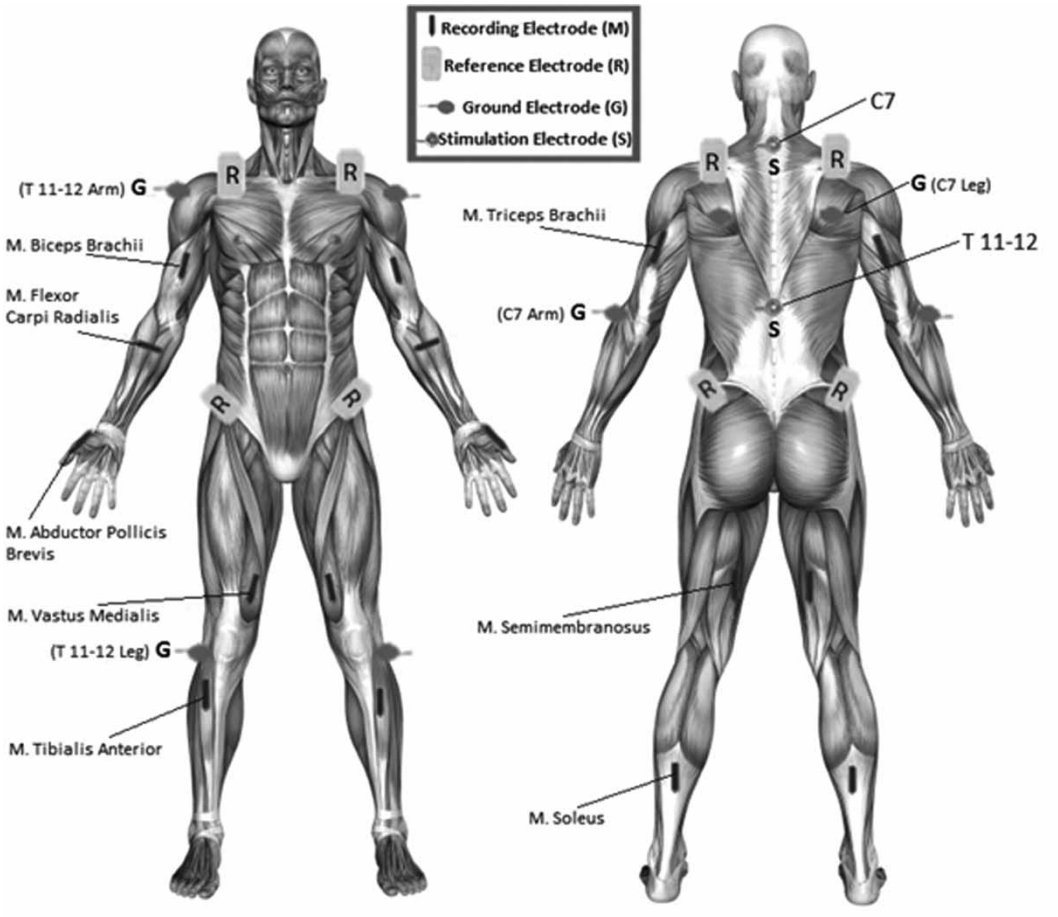

Figure 1 Recording set-up for cervical MMR and T11–12 MMR in healthy subjects.

between CMMR and TMMR variables (amplitude, latency) for the right leg muscles. Correlations between TMMR and CMMR were calculated using Pearson's method. The stimulus threshold for the smallest MMR signal was recorded and compared for C7 and T11 stimulation. Threshold was measured when all five traces were visible on the screen. Also the maximum stimulus intensity for eliciting a plateau MMR, as well as the subjective noxious sensation, was compared for C7 and T11 stimulation, using *t*-test. A significance level of $P \leq 0.05$ was used to identify statistical significance.

Results

Similarities and differences in methodology

Both CMMR and TMMR require superficial electrical stimulation at C7 and T11–12 intervertebral space levels. Identification of the most effective stimulation point differs between these two procedures. Cervical spinal stimulation of C7 can be located by palpation of the cervical spinal process, while the subject positions his head in flexion, until the vertebral level of C7 is located. Electrical stimulation of such spot is easily obtainable and can be effectively stimulated without manipulation of the cathode

Table 2 Comparison of C7 and T11 stimulation amplitude and latency values for UL muscles with sub-maximal stimulation intensity

CMMR andTMMR UL results with the sub-maximal stimulus intensity (*N*: 13)						
	Amplitude (mV)			Latency (ms)		
Muscles	C7	T11–12	P value	C7	T11–12	P value
ADM	1.1 ± 1.5	0.56 ± 0.6	0.01	13.6 ± 1.9	14.0 ± 2.1	0.63
APB	1.0 ± 1.1	0.8 ± 1.1	0.07	13.7 ± 1.5	14.1 ± 1.8	0.33
FCR	0.3 ± 0.3	0.3 ± 0.3	0.11	8.5 ± 1.05	7.8 ± 0.6	0.10
BB	0.3 ± 0.4	0.9 ± 0.8	0.02	6.1 ± 1.4	5.4 ± 1.1	0.10

Table 3 Average amplitude and latency results in the sub-maximal/maximal stimulus intensities for TMMR upper and lower extremities

	Amplitude (mV)	Latency (ms)
Leg muscles TMMR sub-maximal stimulus intensity (*N*: 13) (65–75 mA)		
FHB	0.6 ± 0.9	27.0 ± 4.3
SOL	4.0 ± 2.9	18.9 ± 3.5
VMO	1.8 ± 2.1	11.2 ± 1.9
GM	1.9 ± 1.4	7.7 ± 1.0
Leg muscles TMMR maximal stimulus intensity (*N*: 22) (90–100 mA)		
TA	1.7 ± 1.8	14.7 ± 2.2
SOL	6.3 ± 4.5	15.7 ± 2.3
VMO	3.8 ± 2.2	9.7 ± 2.2
MH	4.1 ± 3.2	8.6 ± 2.3

electrode. T11–12 stimulation site is a challenging procedure with regard to identification of the stimulation site. The rib cage with the 12th rib must be identified and followed to its vertebral origin. This could be carried out in both right and left side, of the spine, for double-checking of the correct electrode site. Once T12 vertebral spinal process is located the operator must move his/her finger one segment cranial to reach T11 vertebral segment. The cathode should be applied on the skin of the T11–T12 inter-vertebral space for effective stimulation. This is rather challenging in overweight or more muscular subjects. In this case, slight adjustment of the cathode electrode toward the cranial or caudal

Table 4 Average values for MMR latencies (ms) for sub-maximal (SI) (*N*: 13) and maximal stimulation intensity (MI) (*N*: 20) levels in the C7 and T11–12 stimulation for right UL

	C7 stimulation latency(ms) Mean ± SD		T11–12 stimulation latency (ms) Mean ± SD	
UL muscles	MI	SI	MI	SI
ADM		13.6 ± 1.94		14.00 ± 0.6
APB	11.9 ± 2.3	13.69 ± 1.49	11.5 ± 2.5	14.15 ± 1.82
FCR	7.9 ± 0.9	8.46 ± 1.05	7.6 ± 1.4	7.77 ± 0.60
BB	5.3 ± 0.7	6.08 ± 1.44	5.3 ± 1.3	5.42 ± 1.08
TB	5.3 ± 0.7		5.1 ± 1.5	

Table 5 CMMR and TMMR amplitude (A) and latency (B) for the right and left ULs

MMR amplitude value for UL with C7 and T11 maximal stimulation intensity (*N*: 20)									
UL Muscles		Right Mean ± SD (mV)	Left Mean ± SD (mV)	Vertebral level		Limb side		Vertebral–limb interaction	
				F value	P value	*F* value	P value	*F* value	P value
(A)									
APB	C7	9.3 ± 4.9	8.4 ± 4.5	0.181	0.672	0.741	0.392	0.001	0.975
	T11–12	8.8 ± 4.8	8.0 ± 4.1						
FCR	C7	5.2 ± 2.4	4.3 ± 2.3	1.206	0.276	0.545	0.463	1.270	0.263
	T11–12	4.1 ± 1.6	4.3 ± 2.1						
BB	C7	7.2 ± 3.7	6.3 ± 3.5	11.020	0.001**	0.052	0.821	0.804	0.373
	T11–12	3.9 ± 4.2	4.4 ± 3.5						
TB	C7	6.8 ± 4.2	5.9 ± 2.7	2.185	0.143	0.014	0.905	0.816	0.369
	T11–12	4.9 ± 3.8	5.5 ± 3.4						
(B)									
MMR latency value for the UL with C7 and T11 maximal stimulation intensity									
UL		Right				Left			
Muscles		Mean ± SD (ms)	P value			Mean± SD (ms)	P value		
APB	C7	11.5 ± 2.5	>0.05			11.5 ± 2.6	>0.05		
	T11–12	11.9 ± 2.3				11.7 ± 2.5			
FCR	C7	7.6 ± 1.4				7.3 ± 1.4			
	T11–12	7.9 ± 0.9				7.7 ± 1.1			
BB	C7	5.3 ± 0.7				5.4 ± 1.6			
	T11–12	5.3 ± 1.3				5.4 ± 1.7			
TB	C7	5.1 ± 1.5				5.0 ± 1.7			
	T11–12	5.3 ± 0.7				5.2 ± 0.8			

Note: No statistically significant difference was recorded between C7 and T11–12 stimulation latency period for UL (P > 0.05).

direction while recording maximum UL or LL muscular signal would help identifying the optimum site.

In both cervical and thoracic stimulation, the anode (reference) should be applied on the clavicle-upper trapezius between the acromion and lateral border of neck instead of the top of the acromion.[7,8] The reference electrode should be applied on the ipsilateral side of UL of the recorded muscular signal (MMR). Most of the subjects felt less jolting effect in the shoulder and more comfortable sensation with this reference electrode position.

LL MMR signals (CMMR and TMMR) were best recorded with the reference anode applied on the anterior superior iliac spine (ASIS) of the ipsilateral limb.

In both cervical and thoracolumbar stimulation a large ($5'' \times 9''$ cm) self-adhesive electrode was found to be more effective in eliciting larger amplitude MMR signal than 2 inch square electrode. Further optimization of recording set-up for CMMR and TMMR will be the focus of future study.

Similarities and differences in evoked responses

C7 stimulation evoked large amplitude motor responses in the muscles of the ULs (in the millivolts range) (Fig. 2A, Fig. 3A).

It also evoked small motor responses in the VMO, MH, SOL and TA of the LLs (in the microvolts range) with maximal stimulation (Figs. 2D and 4). No visible or recordable muscular signal was recorded in most distal LL muscles (e.g. FHB) with sub-maximal cervical spinal stimulation. T11–12 stimulation evoked large amplitude motor responses in both ULs and LLs (in the millivolts range) (Figs. 2B, 2C, 3 and 4).

Similarities and differences in potential shapes and amplitudes

C7 maximal stimulus intensity produces larger amplitude MMR in the UL when compared to T11–12 stimulation and LL MMR (Fig. 2A–D). Tables 5A and 6 present the average amplitude values for the MMR for distal and proximal limb muscles with C7 and T11–12 stimulation.

Vertebral level main effects were significant only for BB muscle ($F = 11.0201$; $P = 0.001$). There were no significant effects for the other muscles ($P > 0.05$).

Limb side main effects were not significant for muscles amplitude ($P > 0.05$).

Vertebral level and limb side interaction effects were not significant for all muscles amplitude values ($P > 0.05$) (Table 5A).

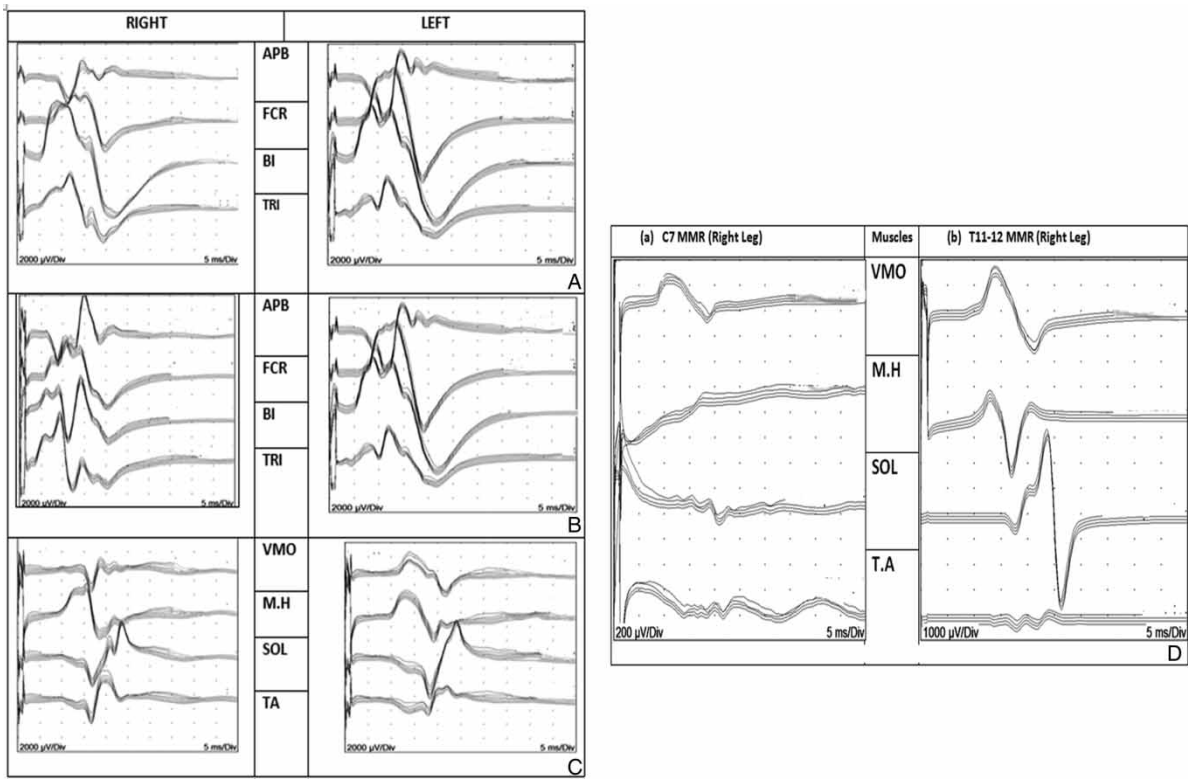

Figure 2 (A) Sample traces of C7 UL MMR (APB: abductor pollicis brevis, FCR: flexor carpi radialis, BB: biceps brachii, TB: triceps brachii muscle). (B) Sample traces of T11–12 stimulation UL MMR. (C) Sample traces of T11–12 LL MMR (VMO: vastus medialis obliques, M.H: medial hamstring, SOL: soleus, TA: tibialis anterior). (D) Sample traces for (a): C7; (b): T11–12 LL MMR from right leg muscles.

As we expected, MMR amplitude value increased with maximal intensity compared to sub-maximal intensity for the same muscle group (Table 3).[13]

MMR in the UL muscles with T11–12 maximal stimulus intensity resulted in equal to or slightly smaller amplitude than C7 stimulation with MMR recorded from the same muscles (Fig. 2B).

Action potential's amplitude was usually larger in the distal limb muscles more than in the proximal limb muscles whether C7 or T11–12 stimulations were carried out. Similarly, *sub-maximal* stimulus intensities resulted in larger amplitude in the distal limb muscles more than proximal, for the upper and lower extremities T11–12 MMR (Tables 2 and 3). Potential shapes were either biphasic or triphasic in the UL and LL muscles with C7 and T11–12 stimulation (Fig. 2A–D). Distal limb muscles (e.g. APB or SOL) tend to have triphasic potentials whereas more proximal muscles (BB or VMO) tend to have biphasic potentials. Potential shapes of the UL MMR were similar whether C7 or T11–12 stimulation was carried out.

Similarities and differences in potential latencies

MMR latencies for distal limb muscles showed higher value than those for proximal limb muscles. These latency changes were recorded during application of maximal stimulus intensity (Tables 5B and 6); or sub-maximal stimulus intensity levels (Tables 2–4). This was the case whether C7 or T11–12 stimulation were carried out (Fig. 4). UL MMR showed slightly higher latency values for T11–12 stimulation than C7 stimulation

Table 6 Amplitude and latency values of CMMR and TMMR for the right lower limb muscles (using maximal stimulation intensity)

Lower limb (*N*: 8)		Amplitude (right)		Latency (right)	
Muscles		Mean ± SD (mV)	P value	Mean ± SD (ms)	P value
VMO	C7	0.7 ± 0.4	0.028*	10.3 ± 1.5	>0.05
	T11–12	4 ± 2.2		8.8 ± 1.8	
MH	C7	0.4 ± 1	0.028*	9.7 ± 2.1	
	T11–12	5.2 ± 3.4		8.6 ± 1.8	
SOL	C7	0.7 ± 1	0.028*	16.0 ± 2.7	
	T11–12	8.3 ± 5.5		15.6 ± 2.6	
TA	C7	0.2 ± 0.1	0.046*	15.3 ± 2.7	
	T11–12	1.7 ± 2.2		14.4 ± 3.2	

Significant difference between C7 and T11–12 stimulations (*P < 0.05).

Table 7 Pearson's correlations coefficient table of ULs CMMR and TMMR (amplitude (A) and latency (B))

UL		C7 Amplitude			T 11–12 Amplitude		
Muscles (*N*: 20)		Mean ± SD (mV)	*r* Value	P value	Mean ± SD (ms)	*r* Value	P value
(A)							
APB	R	9.4 ± 4.9	0.867**	0.000	8.9 ± 4.7	0.674**	0.000
	L	7.8 ± 4.5			8.0 ± 4		
FCR	R	5.2 ± 2.4	0.731**	0.000	4.1 ± 1.6	0.766**	0.000
	L	4.3 ± 2.3			4.3 ± 2		
BB	R	7.2 ± 3.7	0.772**	0.000	3.9 ± 3	0.904**	0.000
	L	6.3 ± 3.5			4.4 ± 3.4		
TB	R	6.8 ± 4.2	0.769**	0.000	4.9 ± 3.7	0.559*	0.010
	L	6 ± 2.7			5.5 ± 3.3		
UL		C7 Latency			T 11–12 Latency		
Muscles (*N*: 20)		Mean ± SD (mV)	*r* Value	P value	Mean ± SD (ms)	*r* Value	P value
(B)							
APB	R	11.6 ± 2.4	0.948**	0.000	11.9 ± 2.34	0.815**	0.000
	L	11.5 ± 2.5			11.7 ± 2.4		
FCR	R	7.5 ± 1.4	0.747**	0.000	7.9 ± 0.9	0.844**	0.000
	L	7.3 ± 1.4			7.96 ± 1		
BB	R	5.2 ± 1.3	0.744**	0.000	5.4 ± 0.8	0.833**	0.000
	L	5.4 ± 0.7			5.4 ± 1.5		
TB	R	5 ± 1.5	0.878**	0.000	5.3 ± 0.7	0.877**	0.000
	L	5 ± 1.7			5.2 ± 0.8		

**Correlation is significant at the 0.01 level (2 tailed). *Correlation is significant at the 0.05 level (2 tailed)

(Table 5B) although the difference was not statistically significant.

C7 and T11–12 LL MMR latencies were comparable (Table 6, Fig. 4) and correlated (Table 7). Although C7 LL latencies were slightly longer than T11–12 stimulation (Fig. 3), there were no statistically significant differences between both stimulations. As was expected, MMR latency value decreased with maximal stimulus intensity when compared to sub-maximal stimulation for the same muscle group (Tables 3 and 4).[13,14]

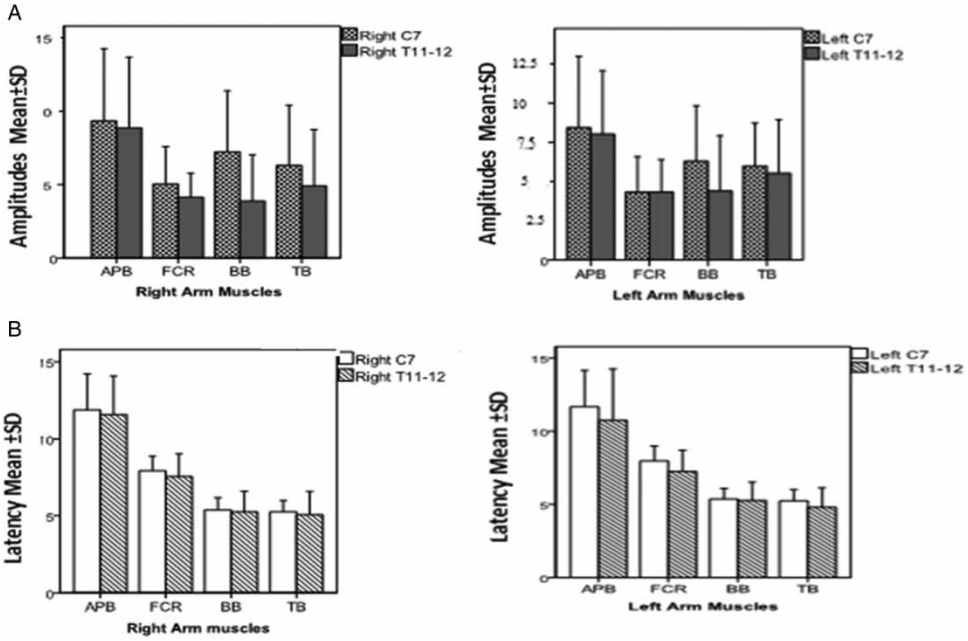

Figure 3 Amplitudes (A) and latency (B) of CMMR and TMMR for both ULs. C7 (hatched) and T11–12 (solid).

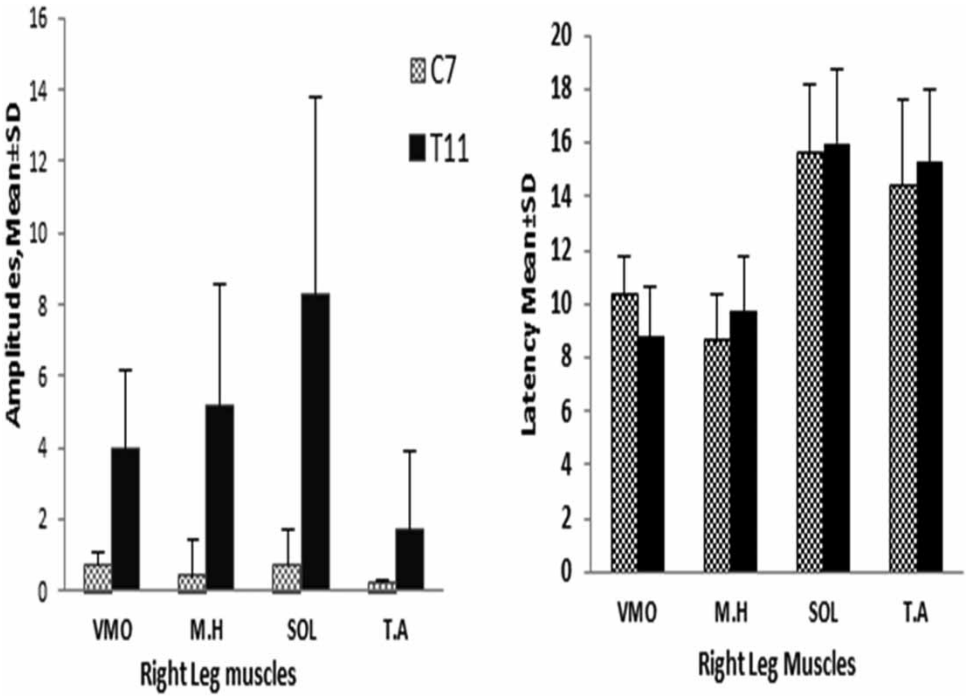

Figure 4 Amplitudes and latencies of the CMMR (hatched) and TMMR (solid) for the right LL muscles.

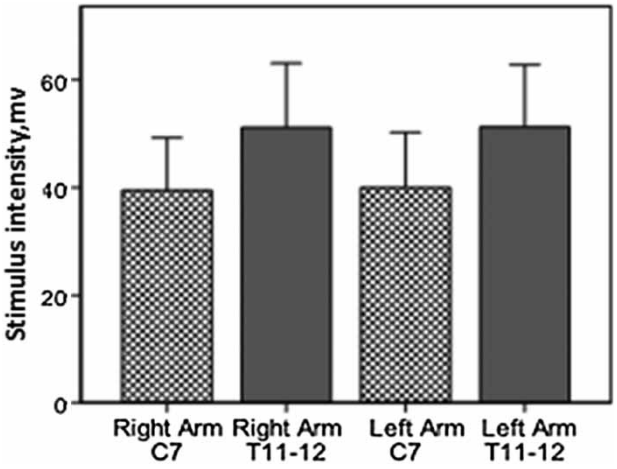

Figure 5 Mean ± values of CMMR and TMMR stimulation thresholds for both ULs.

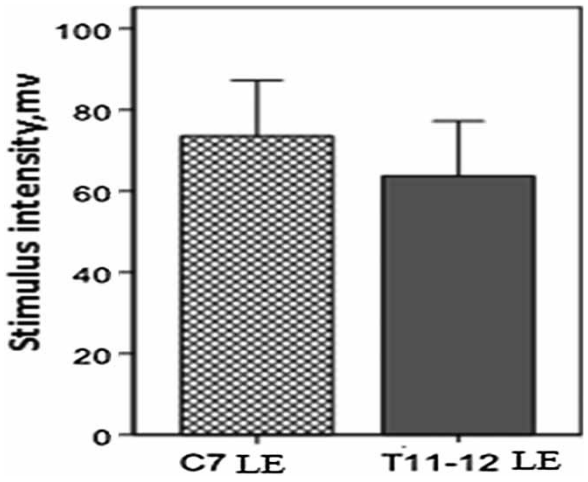

Figure 6 Mean ± values of CMMR and TMMR stimulation thresholds for LLs.

Similarities and differences in other issues (noxious sensation, stimulation intensity threshold and stimulation maximum)

C7 stimulation showed to be more noxious than T11–12 stimulation (Fig. 5, 6), VAS values are shown in Table 8, although both procedures were tolerated by all subjects. The noxious sensation of C7 stimulation was not necessarily painful but "jolty". The tolerable size "jolt" of electrical stimulation, in the upper trapezius muscle, was less pronounced with the use of large anode electrode applied on the clavicle-upper trapezius, close to the C7 stimulation site. T11–12 stimulation required higher current intensity than C7 stimulation in order to obtain threshold or maximum response amplitude (Table 9, Fig. 5). We found it to be crucial to press the cathode electrode against the vertebral spine for T11–12 stimulation, although this was not the case during C7 stimulation. We gave subjects more rest periods during T11–12 stimulation testing session that lasted for more than 60 minutes. This was not the case with C7 stimulation.

In both C7 and T11–12 stimulations electrical pulses were delivered at 0.2 PPS in order to reduce possible attenuation of the signal amplitude. Higher frequency stimulation resulted in smaller amplitude potentials in the ULs or LLs.

Percentage of C7/T11–12 and T11–12/C7

Because C7 stimulation resulted in slightly larger amplitude of UL signal than T11–12, the percentage value of C7/T11–12 was larger than T11–12/C7 value (except left APB and FCR) (Table 10). Distal limb muscles such as APB showed smaller C7/T11–12 values than more proximal limb muscles (TB and BB). Values for the right and left ULs were comparable (Table 10). These values might reflect the percentage of the ascending spinal input from the thoracolumbar segment onto UL, for distal and proximal limb muscles, in relation to the more direct or descending input from the cervical spinal cord to the same UL muscles.

Discussion

Cervical and lumbar MMR are generally simple procedures that can be employed in the clinic. The methodological similarities between CMMR and TMMR with the use of a cup stimulation electrode (cathode) applied snugly on either C7 or T11–12 and the use of 1 millisecond (in our studies) or 2 milliseconds (in other studies)[5] simplify the testing protocol. There is a need to develop more effective stimulation method (electrodes and fixtures) in order to make the stimulation technique friendlier. The differences between the use of 1 or 2 milliseconds in previous reports are of significant methodological factor. However, we believe that 1 millisecond used in our previous as well as this current

Table 8 VAS values of female and male subjects during C7 and T11–12 spinal stimulations, while recording UL and LL MMR

Sex	C7 stimulation – UL	T11–12 stimulation – UL	T11–12 stimulation – LL
Females	5 ± 1.4	4 ± 0.9	4.4 ± 1.3
Males	4.4 ± 0.8	3.5 ± 0.5	3.9 ± 1.2

Table 9 C7 and T11–12 stimulation intensities threshold and maximum current for ULs and LLs

Upper and lower limbs muscles CMMR andTMMR (threshold-maximum current level) (*N*: 37)						
			Right		Left	
			Mean ± SD	P value	Mean ± SD	P value
Threshold	UL	C7	35.9 ± 9.8	0.00**	40.0 ± 10.2	0.00**
		T11–12	51.1 ± 12		51.5 ± 11.6	
	LL	C7	73.5 ± 13.7	0.00**		
		T11–12	62.5 ± 13.1			
Maximum intensity	UE	C7	95.7 ± 12.6	>0.05	95.1 ± 12.7	>0.05
		T11–12	96.5 ± 9.4		96.2 ± 9.3	
	LL	C7	99.2 ± 2			
		T11–12	96.1 ± 8.9			

*Significant difference between C7 and T11–12 amplitude threshold levels ($P < 0.05$).
** Correlation is significant at the 0.01 level (2 tailed). *Correlation is significant at the 0.05 level (2 tailed)

Table 10 CMMR and TMMR Amplitude and latency percentages for both ULs

Muscles (*N*: 20)		Amplitudes %		Latencies %	
		C7/T11–12%	T11/C7%	C7/T11%	T11/C7%
Right	APB	131.1 ± 0.8	101.9 ± 0.6	102.6 ± 0.08	97.4 ± 0.07
	FCR	125.5 ± 0.5	79.7 ± 0.3	104.9 ± 0.2	95.4 ± 0.1
	BB	184.8 ± 2	54.1 ± 0.4	102.4 ± 0.2	97.6 ± 0.15
	TB	138.8 ± 1	72 ± 0.4	103.9 ± 0.2	96.3 ± 0.2
Left	APB	96.1 ± 0.7	104.1 ± 1.3	101.2 ± 0.11	98.8 ± 0.15
	FCR	99.6 ± 0.5	100.4 ± 0.6	109.8 ± 0.2	91.1 ± 0.15
	BB	143 ± 3.4	70 ± 0.4	98.8 ± 0.15	101.2 ± 0.2
	TB	108.3 ± 0.8	92.3 ± 0.6	105.02 ± 0.2	95.2 ± 0.2

studies are more comfortable and render no side effects due to short duration pulses. Furthermore, the location of the reference electrodes in both procedures (CMMR and TMMR), with the UL MMR using the clavicle-upper trapezius site and LL MMR using the ASIS site, is possibly related to the electric current dispersion between the cathode and anode in each procedure.

Both procedures, TMMR and CMMR, were easily recorded. However CMMR was easier (to locate the C7 spinal segment, has lower threshold of stimulation and had more visible muscular potentials) than TMMR due to the fact that cervical spine vertebrae was more palpable, more superficial with less muscle mass around the bony components. In TMMR, the landmark for the correct site was identified by palpation of the 12th rib to its vertebral connection on both sides. C7 vertebra is easily palpable with the stimulation electrode probably closer to the spinal cord/dorsal spinal roots than T11–12 vertebra. In the T11–12 procedure, the thickness of the lumbosacral muscles and fascia and the sub-cutaneous fat were major factors in the difficulty of thoracolumbar stimulation.[15] Manual pressure on the active stimulation electrode improves the coupling of the stimulation electrode to the sub-cutaneous neural structure.[6] Another important factor might be considered in the methodological difference between CMMR and TMMR included the different spatial relation between the segmental spinal cord and the vertebral bony segment stimulated. In CMMR, stimulating C7 vertebral segment resulted in electrical activity of C7 spinal cord and structure.[7,13,16] In TMMR, T11–12 vertebral stimulation may result in electrical activity of neural structures that are closer to the lumbosacral enlargement (L5) of the spinal cord.[8,17–20]

Evoked responses with TMMR and CMMR

Both MMR procedures resulted in motor responses in the UL and LL muscles. However, CMMR resulted in relatively larger motor responses in the ULs as compared to TMMR in the UL or LL. This is probably because of the difference in the circuitries/inputs activated in both procedures.[21] Our results[7,8] as well as others[6,9,16] suggested that propriospinal pathways are probably the tracts travelled by CMMR and TMMR. If this suggestion turns to be true these results indicates a stronger drive from the thoracolumbar segment to both ULs (ascending) and LLs (descending). The cervical spinal inputs were substantially stronger for the ULs than those for the LLs due to direct innervations of the

UL muscles. Furthermore it appears that the strong input to LL by cervical spinal neural structure concentrate more on the antigravity muscles such as the VMO and SOL.

Clinically, smaller amplitude of CMMR to UL muscles when compared to TMMR to the same muscles (in a patient) may indicate compromised direct pathways versus ascending spinal pathways from the thoracolumbar segment. On the other hand, smaller amplitude of TMMR to those muscles as compared to CMMR may indicate compromised ascending pathways due to possible lesion or disorder in the spinal cord between the stimulation site and cervical spinal nuclei.

Action potentials amplitude and shape

The most important differences between CMMR and TMMR are the large amplitude potentials in the distal limb muscles in CMMR versus antigravity muscles of the LL in TMMR. Large amplitude potentials were recorded in the APB, ADM more than FCR and BI. This is probably because of the functional differences between the hand and forearm or arm muscles. The dexterity function of the hand muscles requires more corticalized motor units in the hand muscles.[22,23] Proximal limb muscles such as the BB or the FCR serve forced use more than dexterity.[24] Contrary to UL results the signal amplitude for LL muscles to T11–12 stimulation was almost equal for the VMO and SOL muscles whereas signal for TA and MH were significantly smaller. These findings are probably due to functional utilization of the more antigravity muscles (SOL and VMO) versus other muscles (TA, MH).

The action potential shapes were mostly biphasic or monophasic in the CMMR. TMMR showed biphasic or triphasic potentials. This is probably related to the size and location of the electrodes in relation to the underlying orientation of the motor units. In our method of electrode placement we positioned the electrodes in parallel to the striation of the muscle fibers tested. UL muscles are generally smaller in size when compared to LL muscles.[25] The longitudinal orientation of motor units in the MH, BB, and triceps as compared to SOL and VMO muscles would be a contributing factor in signal/potential shapes. Therefore the signal travelling along the muscle fibers were fully recorded with the depolarization–repolarization phases in the LL. This was probably not the case in the smaller size muscles of the UL. The increased MMR potential amplitude with maximal stimulus intensity is expected due to the maximal recruitment of spinal pathways – motoneurons and motor units of the activated muscles when compared to sub-maximal stimulation resulting in smaller signal amplitude.

Clinically, reduced amplitude of CMMR to distal limb muscles of the right versus left ULs may indicate compromised direct input to the smaller input muscles. Furthermore asymmetric amplitude of TMMR for the right and left ULs or LLs would indicate asymmetric pathways transmission to either ULs or LLs, proximal or distal limb muscles due to spinal dysfunction in the tested patient.

Action potential latencies

Action potential latencies showed substantial similarities between CMMR and TMMR as both potentials followed the rule of distance between the stimulation site and recording muscle segment. The more distal the limb muscles, the longer the latency for the CMMR or TMMR. T11–12 stimulation resulted in slightly longer latency in UL muscles when compared to C7 stimulation and UL MMR, although the difference was not statistically significant. That was expected due to the increased travelling time from T11–12 to UL muscles. However, these latency differences were very small indicating fast conducting ascending pathways to the UL from the lumbar spinal nuclei. As expected, MMR potential latency decreased with increasing stimulus intensity with larger amplitude when compared to sub-maximal stimulation. This is mainly due to activation of all axonal pathways (large and fast conducting as well as and small diameter slow conducting) with maximal stimulation. Smaller diameter axons may conduct the signal activated at sub-maximal stimulation intensity. There is also the possibility of filtering effect of the sub-maximal stimulation intensity by the tissues underlying the skin causing changes in the amplitude and latency. A normative standard for latency of the CMMR and TMMR in a larger sample population and normalized to subject's height is necessary and will be the focus of future study.

Clinically, a prolonged latency of more than 2 milliseconds between the right and left ULs or LLs to CMMR or TMMR (for the same muscle) would indicate compromised signal transmission due to possible side-related lesions or disorders in the spinal cord.

Noxious sensation and electrical stimulation intensity

Noxious sensation was reported with CMMR more than TMMR. This is probably because of the proximity of distance between the cathode applied to C7 vertebra and the acromion/shoulder region. The stimulation field resulted in strong contraction of the upper trapezius muscle causing a "jolting" effect. This occurred

despite the relatively lower stimulus intensity used with CMMR as compared to TMMR. TMMR elicited by a cathodal stimulation to T11–12 vertebral segment with anodal electrode applied to the ASIS or the clavicle-upper trapezius site did not result in such jolty contraction due to the large distance between the two-electrode complex. This required the larger stimulus intensity reported in this study in order to elicit maximum motor responses in TMMR. Although cervical stimulation was "jolty", subjects tolerated it and accommodate to it after the first few stimuli.

The combined use of CMMR and TMMR for testing spinal circuitries

The percentage of the UL MMR amplitude for C7 to T11–12 stimulation could be an interesting approach for testing ascending thoracolumbar pathways to ULs versus the more direct input to UL muscles (or descending) cervical inputs. The ascending spinal input, possibly via propriospinal tract,[26] was equal for the right and left ULs (Table 10) but was more robust (larger amplitude) for the right more than the left upper extremities with C7 stimulation. It appears that such finding is due to the increased right-hand dominance tested by the direct cervical spinal stimulation versus thoracolumbar stimulation. This assumption would be the focus of future studies. Such comparative analysis of the ascending and direct (or descending) spinal signal could be useful for testing the integrative function of spinal circuitries that have been injured or still viable in patients with spinal cord injuries and diseases. The use of such approach has been promising in our on-going studies on subjects with spinal cord injuries. This will be the basis of future reports. In this current report we propose that the amplitude ratio could be used as an index to monitor the movement recovery throughout the rehabilitation program of patients with spinal cord injuries as reported in previous articles.[27]

The importance of such an analysis stems from the results of the previous animal studies that show higher regenerative capacities for the short more than long propriospinal tracts.[28,29] In fact, it has been shown that propriospinal neurons and axons has greater regenerative response than their supraspinal counterparts.[30] If the MMR study and analysis, using two short and direct (upper and lower) and two long (ascending and descending) pathways can evaluate these integrative function it would add valuable information related to possible effective rehabilitation strategies for treatment of spinal cord injuries and diseases. Furthermore, the fact that such a comparative analysis evaluate the inter-enlargement pathways (between cervical and lumbar spinal enlargement nuclei) add valuable integrative functional and circuitries between ULs and LLs. Loosing such circuitries and functions could lead to disruptions of intra-limb and inter-limb activities. These findings have been discussed in recent animal studies citing a strong dynamic response for the thoracic propriospinal neurons that mount strong regenerative activities post spinal injuries.[30] However, these neuronal structures that are located between the lumbar and cervical enlargements could also cause some cell death of many axotomized thoracic propriospinal neurons.[30] The balance between the regenerative and degenerative changes at these pathways and neurons has to be controlled if perfected recovery to be hoped for.

Implications for clinical testing

Cervical and lumbar MMR procedures can be used as a complementary testing method to clinical investigation. The results of clinical testing are based largely on subjective and experience aspect of the person doing the tests and it mainly evaluates the functional aspect of the disorders. On the other hand, these MMR tests evaluate the circuitries of the spinal cord causing such functional deficits/performance. These MMR tests are considered more accurate (as measured in microvolts or millivolts) as it evaluates the problem at the axonal/cellular level and not the global level. Reliability of these MMR procedures has been evaluated and was reported in our most recent publication.[31] These are some of the clinical implications of the similarities and differences between CMMR and TMMR. The list of such implication could be large with the application of these procedures clinically.

Conclusions

CMMR and TMMR procedures have similarities and differences in the technical aspect of the methods (electrode set-up, stimulation levels and noxious sensation) and signal presentations (amplitude, action potential shapes and latencies). The MMR signal amplitude in the UL muscles to C7 and T11 stimulation were comparable. The MMR signal amplitude in the LL muscles to C7 and T11 stimulation was significantly different showing larger amplitude for the latter and smaller amplitude for the former (C7 stimulation). The combined use of these two procedures and calculating percentage of signal amplitude for C7 and T11 stimulation in UL and LL muscles could be useful in evaluation of specific neural circuitries of the spinal cord, specifically ascending, descending and direct pathways, in patients with neurological disorders.

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