Cervical Spinal Stimulation at Different Levels Evoked Multisegmental Motor Responses in the Lower Limbs

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Abstract

Objective: To report on the effect of electrical stimulation of the cervical spine while recording muscular signal from lower limb muscles with Cervical Multisegmental Motor Responses (MMR).

Design: Experimental Study

Methods: C1, C3, and C7 vertebral levels were electrically stimulated using 1 msec. 0.2 PPS square wave pulses applied at response maximum while recording muscular signal from right leg Vastus Medialis (VMO) Medial Hamstrings (MH), Tibialis Anterior (TA), and soleus (SOL) muscles simultaneously.

Outcome Measures: Peak-to-Peak amplitude and deflection latency of the MMR.

Results: Large signal amplitudes were obtained in all tested muscles with the largest amplitude in the VMO and SOL followed by the MH and TA. Signal latency increased with distal limb muscles. Signal threshold, maximum intensity levels were comparable between all stimulation levels and the visual analogue noxious level showed the three procedures to be comfortable.

Conclusions: These procedures can be used for testing descending pathways for patients with spinal cord injuries and diseases.

Keywords: Cervical Spinal Cord; Multisegmental Motor Responses; Descending Spinal Pathways; Spinal Cord Injury; Spinal Circuitries

Introduction

The multisegmental motor responses (MMR) from upper limbs has been recently reported using cervical and thoracic vertebral stimulation [1,2]. These MMR records could be used for non-invasive testing of ascending and descending spinal pathways integrity between the cervical and lumbosacral spinal segments [3]. Upper and lower limb MMRs have been recorded using stimulation at the T11–12 vertebral segments and have suggested strong ascending and descending propriospinal pathway functioning, originating from the lumbar spinal segment [2,4]. On the other hand, cervical spinal stimulation has been reported to generate MMR in the upper limbs only, with minimal responses in the lower limbs [2]. Other studies have reported MMRs in the lower extremities with thoracic epidural or percutaneous stimulation during walking but none has reported using cervical stimulation [5-7].

Although there are some human studies that used stimulation over the base of the skull (the cervicomedullary junction) to activate the descending motor pathways and generate evoked responses from leg muscles, most of these studies used high-voltage stimulations and reported some difficulties evoking responses in the leg muscles [8-10]. The lack of lower limb signal upon cervical spinal stimulation in human is incompatible with the known descending pathways from the cervical spinal segments to the lower limbs. In our previous reports, we showed that the stimulation intensity and placement of the reference and ground electrodes play a critical role in recorded upper or lower limb signal quality [3,4]. Therefore, the existing gap in lower extremity MMR recordings after cervical stimulation may be due to methodological limitations, which we address herein.

The current study evaluates the specific responses (amplitude and latency) of the progravity and antigravity muscles to the brain stem or spinal stimulation in healthy subjects. This may be useful for neurophysiologic studies of the integrative functions of lower limb muscles during locomotion, cycling or other leg activities.
Evaluation of lower limb signal latency from C1, C3, and C7 spinal segments stimulations are also necessary to assess the characteristics of neural conduction of the descending pathways from the cervical spine to the lower limb. The differential values in signal latency could be a useful clinical parameter for segmental pathology of the cervical spine.

The clinical application of MMR findings could be useful in assessing the location and extent of spinal and brain stem pathologies. Cervical MMR (CMMR) can determine the neurophysiologic control of lower limb muscles with the descending pathways of the cervical spinal region. Patients with syringomyelia, spinal canal stenosis, transverse myelitis, multiple sclerosis and cervical and lumbosacral radiculopathy could benefit from such testing protocols. MMR (both cervical and thoracic) capabilities could lead, eventually, to the development of standardized electrophysiological tests for neurology patients and could serve as an objective supplement to current clinical and imaging testing methods.

Comparing lower limb–evoked responses threshold and noxious sensation to C1, C3 and the C7 spinal segment is a secondary aim of this study.

Materials and Methods

A total of 20 subjects, 12 male and 8 female aged 18-65 years, signed an informed consent approved by the IRB of Texas Woman's University to participate in the present study (Table 1). This is a cross sectional study to evaluate the possible recording of lower limb muscles signals called CMMR. Thirteen subjects completed all phases of the experimental procedures (CC, C3, and C7 stimulation), 20 subjects completed C7 stimulation protocol. All subjects were healthy and could tolerate electric pulses to the cervical spine. Subjects were excluded if they had any metabolic or neurologic disease, arthritis, or radiculopathy of the cervical spine or cancer.

<table>
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<th>N: 20 (12 male, 8 female)</th>
<th>Min</th>
<th>Max</th>
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<tr>
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<td>65</td>
<td>39.0 ± 12.7</td>
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<tr>
<td>Height (cm)</td>
<td>145</td>
<td>183</td>
<td>166.2 ± 10.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>43</td>
<td>117</td>
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</tr>
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Table 1: Demographic Characteristics

Electrical Stimulation and Recording

The CC junction, C3 and C7 vertebral segments were electrically stimulated with 1-msec square wave pulses at 0.2 pps at the maximum muscular response (muscle action potential, MAP) level. The CC junction was identified by palpating the space between the external occipital protuberance (EOP) and the posterior arch/tubercle of the atlas (first cervical vertebrae, C1). The C3 and C7 segments were identified by palpating the cervical spinous processes during flexion/extension of the neck. Three, cup electrodes were attached to the space between the EOP and C1 (CC stimulation) to the intervertebral space between C2 and C3 (C3 stimulation), and to the C7 and T1 (C7 stimulation), using 3M hypoallergenic tape. For accurate and effective stimulation and recording, all electrodes were tightly attached to the skin and each location was monitored throughout the session using horizontal and vertical strips of 3M tape. The reference electrode (anode) was a 5” x 9” square, pre-gelled flexible pad (similar to those used for transcutaneous electrical stimulation), which was attached to the corresponding anterior superior iliac spine (ASIS). The anode was placed on the medial border of the scapula or T11-12 vertebrae in initial tests. Such anode site showed smaller signals in lower limb muscles. Therefore, we decided to use ASIS to obtain such visible signal. A metal ground electrode (3-cm diameter) with gel was applied to the subject’s scapular spine of the right side.

Maximum MAP was recorded using a four-channel Cadwell EMG unit. Surface silver-silver chloride cup electrodes with gel were applied on the muscles using 3M hypoallergenic tape. Signal-to-noise ratio was improved by gentle skin abrading with light-grade sandpaper then cleaned with alcohol at the electrode sites. Signal artifacts were minimized by positioning the ground electrode between the stimulation and recording sites and, if necessary, using the split-screen function of the Cadwell EMG unit. Muscular potentials of the right lower limb were recorded from the motor points of the vastus medialis oblique (VMO), medial hamstrings (MH), soleus and Tibialis anterior (TA). Recording parameters were 100–1000 μv/div (sensitivity) with sweep speed of 5msec/div and the signal was band-passed filtered using a 10Hz–10 KHz Butterworth filter.

Stimulation was the most critical step of this experiment. Stimulus intensity was increased until maximum compound action potentials were recorded from the tested muscles at a comfortable level using 0.2 pp stimulation rate. The maximum MAP was determined by increasing the strength of the electrical stimulus until the signal amplitude increased and reached a plateau at a tolerable level. All signals were recorded using 38.4 KHz sampling rate per channel with 16-bit A/D converter. It is important to note that electrical stimulation on the side of the vertebral location (paraspinal muscles) did not result in recording any motor action potential in any of the tested muscles. This indicates that the recorded muscle signals in the lower limb were transmitted signal via the spinal cord pathways to the lower limb muscles.
Experimental Procedures

The subject signed the informed consent form, then his/her skin was cleaned with alcohol and dried and stimulation and recording electrodes sites were attached to the motor points of the muscles using 3-M hypoallergenic tape. We instructed the subject to stand with feet apart at shoulder width. We found this to be the preferred posture for cervical MMR signal recording. The subjects were asked to relax during the testing periods and to refrain from head turning and arm or leg movements. Testing started with C7 stimulation, then C3, and then CC junction. C7 stimulation recorded from both lower limbs to compare right and left lower limb responses, one limb at a time, four recording muscles was tested for each limb. We changed the order of the limb tested between subjects in order to reduce the order effect of the intervention (stimulation). The subjects were given a 1-3 min resting period after each trial and additional rest period upon request. Electrodes were removed, sites were cleaned and subjects were dismissed at the end of the testing. Figure 1 illustrates the stimulation and recording sites.

Signal and Statistical Analyses

Five representative traces were recorded for each muscle at the 5-sec intervals necessary to assure consistent traces. This was carried out at the CC junction, C3, and C7 spinal level, resulting in a total of 60 responses (5 traces, 3 levels, 4 muscles). The five traces were averaged for each spinal level stimulated and compared using descriptive statistics. The peak-to-peak amplitude (negative-to-positive peaks) and deflection latency (from baseline) were the dependent parameters for the cervical MMR. The mean value and standard deviation were performed using the SPSS 20 software (IBM SPSS Statistics for Windows, V. 20, and Armonk, NY).

The Wilcoxon signed-rank test was used to compare the right and left leg muscles for the C7 MMR variable (amplitude, latency, and threshold) and Visual analog Scale (VAS). We conducted the Kruskal-Wallis one-way analysis of variance on rank in order to examine the differences between the CC junction, C3 and C7 vertebral levels on the leg muscles (VMO, MH, soleus and TA). Signal amplitude, latency, and threshold, as well as the VAS value, were the dependent parameters. The spinal level (CC, C3, and C7) was the independent variable. A statistically significant level of p < 0.05 was used (SPSS 20) to indicate significant level.

Results

Results showed large amplitude signals in all lower limb muscles (e.g., soleus, VMO, Medial hamstring and TA) with electrical stimulus at the CC junction, C3, and C7 vertebral levels.

Upper, Middle, and Lower Cervical Stimulation

Cervical spinal stimulation at the CC, C3 and C7 resulted in large amplitude signals with a comparable shape, latency and amplitudes across the five traces (Figure 2).
The average amplitude values of the recorded signal ranged from 0.24–0.81 mV for CC, 0.26–0.92 mV for C3 and 0.26–0.88 mV for C7 (Table 2). It shows that the largest peak-to-peak amplitude was recorded for the soleus and the smallest for the MH. Signal amplitude showed high inter-subjects’ variation. Signal latency ranged from 8.84 to 14.8 ms for C3 to C7 (Table 2). It’s important to note that, four subjects were of 143-155 cm in height resulting in the averaged shorter latency recorded in this study.

There were no statistical differences between C1, C3, and C7 amplitude and latencies. As expected, latency value was shorter for proximally located muscles, such as VMO and MH, and longer for distally located muscles, soleus and TA, with smaller inter-subjects’ variation (Figure 3) (Table 2).
Stimulation Threshold and Noxious Sensations

Stimulation of CC, C3 or C7 showed a comparable threshold value for eliciting the lower limb signal. A stimulus intensity of 57.2–58.7 mA could elicit a visible signal in the VMO or soleus muscles of the lower limb. The noxious sensation was also comparable among the three stimulation sites by VAS. VAS values were described by the subjects as being comfortable and did not preclude them from continuing the experiment. There was no statistically significant difference between the stimulation thresholds and VAS for the three stimulation sites (p > 0.05). Intersubject variability was high for both stimulation threshold and reported noxious sensation (Figure 4).
Stimulation Comparison of the Three Levels

Results showed no significant differences in peak-to-peak amplitude for the upper, middle and lower cervical stimulations (Table 2 and 3). C3 stimulation showed slightly larger signal amplitude in almost all lower limb muscles (0.28-0.92 mv) although the difference was not statistically significant (Figure 5) (Table 2). Signal amplitude ranged from 0.24-0.81 mv for CC stimulation; 0.26-0.92 for C3 stimulation. 0.26-0.88 for C7 stimulation Signal shape was biphasic (VMO and MH), polyphasic (Sol and TA) (Figure 2).

<table>
<thead>
<tr>
<th>Muscles*</th>
<th>Amplitude (mV)</th>
<th>Latency (ms)</th>
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<tbody>
<tr>
<td>VMO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>0.79 ± 0.49</td>
<td>8.27 ± 1.10</td>
</tr>
<tr>
<td>Left</td>
<td>0.76 ± 0.52</td>
<td>8.30 ± 0.86</td>
</tr>
<tr>
<td>MH</td>
<td>0.22 ± 0.11</td>
<td>8.42 ± 1.20</td>
</tr>
<tr>
<td>Right</td>
<td>0.24 ± 0.12</td>
<td>8.30 ± 0.99</td>
</tr>
<tr>
<td>Left</td>
<td>0.24 ± 0.12</td>
<td>8.30 ± 0.99</td>
</tr>
<tr>
<td>Soleus</td>
<td>0.43 ± 0.37</td>
<td>13.70 ± 2.50</td>
</tr>
<tr>
<td>Right</td>
<td>0.30 ± 0.13</td>
<td>13.86 ± 2.45</td>
</tr>
<tr>
<td>Left</td>
<td>0.30 ± 0.13</td>
<td>13.86 ± 2.45</td>
</tr>
<tr>
<td>TA</td>
<td>0.22 ± 0.09</td>
<td>13.60 ± 2.42</td>
</tr>
<tr>
<td>Right</td>
<td>0.26 ± 0.16</td>
<td>13.58 ± 2.48</td>
</tr>
<tr>
<td>Left</td>
<td>0.26 ± 0.16</td>
<td>13.58 ± 2.48</td>
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Table 3: Amplitude and latency values of C7 MMR of right and left lower limb muscles

Figure 5: Comparison of Three Levels of Cervical spine Stimulation Amplitude and Latency Values
The CMMR leg latencies were obtained under the maximum tolerable level of intensity and this might result with short CMMR signal latencies. Previous MMR studies showed that, if electric stimulation intensity was increased, MMR latencies usually get shorter [3,4,16]. Signal latency was slightly shorter for C3 stimulation for MH, soleus and TA, although the difference was not statistically significant (Table 2). The latency difference between CC and C3 was 0.11 msec and C3 to C7 was 0.31 msec, supporting the fast-conducting nature of these activated pathways that may serve coordinated motor functions. The Wilcoxon signed-rank test gave similar results on C7 MMR between right and left leg muscles. There was no significant difference between right and left C7 MMR (p > 0.05) (Table 3). The noxious sensation was slightly less for C3 stimulation than C1 and C7 levels, although it was not statistically significant (Figure 4). These MMR latencies might appear to be too short for activation of the spinal tracts with increased intensity of spinal stimuli. However, these values were comparable to previously published reports [15].

Discussion

The lower limb large amplitude signal recorded in response to cervical spinal stimulation was probably due to direct activation of the descending pathways to the cervical spine motor nuclei with motor axons converging on the different lower limb muscles. These pathways could be either the propriospinal or the corticospinal. It would be a speculation to try to pinpoint the origin of spinal pathways activated with MMR, and animal studies are necessary for confirmation. The main intent of this report was to identify the CMMR causing lower limb muscular activity. It was not intended to specify which tract could be the vehicle for such signal transmission.

Upper, Middle and Lower Cervical Stimulation Evoked Muscular Signals in Lower Limb Muscles

The lower limb muscular signals with CC, C3 and C7 stimulation was probably due to direct, strong input from the brainstem and cervical spinal nuclei onto the lumbar spinal nuclei sending motor axons to activated muscles. It is possible that these signals traveled along the descending corticospinal or propriospinal pathways between the cervical and lumbar spinal cord nuclei. These propriospinal pathways function in the coordinated movements of upper and lower limbs. Corticospinal pathways could activate lower limb muscles and were suggested in previous reports to be the most likely pathway causing the lower limb signal [8,11]. The larger amplitudes of the Soleus and VMO muscles, both antigravity muscles, would support the possibility of corticospinal tract activation in this study.

Activation of the propriospinal pathway could not be ruled out in this experiment, a possibility that has been supported in previous reports [12]. Studies have emphasized the integrative function of the propriospinal pathways between upper and lower limbs [13]. However, as mentioned above either tracts carrying these signals is a speculative venture. This detail needs to be the focus of future reports.

The CC junction is located 6-7 spinal segments above the largest section/center of the cervical nuclei. This indicates that the propriospinal pathway may extend several segments above most of the cervical spinal motor neuron clusters (cervical enlargement) [8,11]. In this experiment, we tested the descending section of the propriospinal pathway.

Action Potentials Amplitude and Potential Shape

The comparable MMR signal amplitudes among CC, C3 and C7 spinal level stimulations indicate a possible stimulation of nearby clusters of pathways/neurons that simultaneously feed to most of the lower limb muscles. Similar to CC stimulation, C3 and C7 resulted in the highest signal amplitude in the VMO and soleus muscles followed by the MH and TA, indicating the preferential control of antigravity muscles by the spinal cord central neurons. The larger amplitude signal of the Soleus and VMO after C3 stimulation, more than C1 and C7 could be attributed to the Soleus and VMO's larger sizes. Does this also mean that C3 segment exhibits better control of lower limb muscles than do the CC and C7? This would be an interesting question for future studies.

The similarities in the muscular signal amplitudes for CC, C3, and C7 stimulation indicate an equal richness of cervical neural supplies to lower limb muscles This may indicate strong inter-limb coordination between the upper and lower limbs and could be tested using cervical MMR to the lower limbs.

The comparable C7 MMR signal amplitude and latency in the both lower limbs here and in our previous study indicates the symmetric nature of such coordinated neural supplies for both lower limbs, with equal dominance. Such CMMR could also be recorded using transcranial magnetic stimulation (TMS) a non-invasive method. With the limited access to TMS magnetic stimulation, electrical C7 stimulation could be the potential alternative [2].

Signal amplitude was larger in Soleus and VMO muscles because of the weight bearing functions of these muscles. This was pronounced in the soleus muscle more than VMO CMMR signal. Soleus muscle, being in smaller size than VMO and carry more body weight than more proximal (VMO) muscles necessitate a better neural control. On the contrary, CMMR for the MH and TA were smaller in amplitude due to lack of such postural functional demand. These results indicate that the cervical spinal cord drive to the antigravity postural muscles of the lower limb is stronger than in some other limb muscles. Such functional significance could be interpreted as an essential function in the bipedal human during erect standing posture.
Action potential shapes were biphasic or polyphasic indicating conversion more than divergence of the neural signal on these muscles. It also indicates the homogeneous nature of these lower limb muscles for functional purposes.

It is important to note that these procedures resulted in synaptic activation of the spinal motoneurones due to the fact that subjects were standing during testing. Our earlier experience with CMMR for the lower limbs showed higher amplitudes (%H160) during standing than sitting or lying positions [15]. This study was carried out after our previous report eliciting thoracic MMR while recording lower limb muscle activation [1,2]. In both studies viable CMMR and TMMR were recorded in lower limb musculatures.

**Action Potentials Latency**

The latency value increased with CC>C3>C7 at the most distal soleus and TA over the VMO and MH. This was probably due to the distance between the stimulation site and target muscles. These same results have been reported in conduction velocity studies [14]. The short latency between the stimulation sites and lower limb muscles indicate fast conducting pathways from the cervical to the lumbar nuclei and lower limb. This is supported by the lower threshold activation of the pathways at the three cervical spinal levels. The fast-conducting nature of the pathways is further supported by the small latency difference (CC: 9.06, C7: 8.64) between upper and lower cervical stimulation. A difference of 0.42 msec calculated between CC and C7 traveling signal for a distance of approximately 16 cm would result in a conduction velocity of 31 m/sec.

**Latency Changes with Cervical MMR**

There was minimal signal latency increase, as stimulation proceeds from C7 toward the cortical level (C3 and CC). This was expected with the small increase in distance between the stimulation and recording sites. The latency difference between CC and C3 was 0.11 msec, between C3 and C7 was 0.31 msec, and CC to C7 was 0.42 msec. Such minimal latency increments also indicate a fast-conducting pathway (with large diameter axons) traveling down the neuraxis that served this coordinated interlimb movements. Shorter latency and a faster-conducting signal of the thoracolumbar MMR to the same muscles have been reported [3,4,15,16].

Latencies for the CMMR were almost equal to half of the soleus and VMO H-reflexes latency that travel the electrical signal in an afferent-efferent direction, despite the fact that H-reflex is usually evoked using lower stimulation intensity compared to these CMRR. Higher stimulation intensity results in shorter latencies of the action potential [15,17,18].

Although these latency differences were not statistically significant; it could be clinically relevant in serial studies and of single-patient studies. Latency measurement in group studies might not be as accurate as serial studies, due to high inter-subject variability, inaccurate latency value due to curved and non-steep deflection of the action potentials. It is expected that superficial cutaneous stimulation will have a different activation of superficially versus deep locating axons in the spinal cord. This may increase such lack of sensitivity, in intergroup studies, that is mostly seen in healthy subjects, as in this report. Latency values were highly variable between subjects, mostly because of the large distance between the stimulation and recordings sites and the difference in subject's height (4 subjects out of 20 had height of 143-155 cm). Such variability might result in some disagreement with some previous studies such as those of Minassian, et al. [18,19].

**Noxious Sensation and Electrical Stimulation Intensity**

The stimulation field resulted in a visible contraction of the upper trapezius muscle causing a jolting effect at the three levels of stimulation. This effect was reported and observed more often with C3 stimulation than with CC and C7. This is probably because of the larger number of descending pathways that could be stimulated (CC>C7) and the proximity of the spinal cord to the stimulation electrode, which may generate a more effective electric stimulation field. The proximity of the cervical enlargement between the C3-T1 spinal segments and Central Pattern generators (CPGs) neurons are another factor that may result in stronger electric stimulation at the cervical region. These pathways taper as the spinal cord proceeds to the caudal level, and the effect of electrical stimulation become less noxious, as in thoracolumbar MMR [2-4]. This was more pronounced than the minimal noxious sensation we previously reported in lumbosacral spinal stimulation [2,3]. Although cervical stimulation caused a shoulder girdle contraction, subjects were able to tolerate it and complete the test with no reported complications.

**Potential Clinical Application of Cervical MMR for the Lower Limbs**

Cervical MMR is a simple, noninvasive and, most important, an objective procedure to use in the clinic for evaluation purposes. Administering cervical MMR is more effective particularly for spinal cord stimulation because the cervical spine vertebrae have less muscle mass around the bony components and are; therefore, more easily palpated [3]. Cervical MMR could be useful to evaluate patients with different spinal cord pathologies, especially those with spinal cord injury [16]. Combined with thoracolumbar MMR testing, cervical MMR could help differentiate between the short and long propriospinal pathways that are activated between the upper and lower limbs [3]. This understanding could lead to more effective testing and treatment methods and improve the quality of care for patients with spinal cord injuries and diseases.

It’s important to note that such CMMR potential was a neural signal originating from the spinal cord levels. To test this hypothesis,
we stimulated the muscular paraspinal muscles as mentioned earlier. No MMR was recorded using such method.

There was some concern that electrical stimulation procedure/method utilized in this study might stimulate the subject’s heart through the electric field passing between the active stimulation electrodes including the ground electrode while recording from lower limb muscles. However, this was avoided by placing the ground electrode on the RIGHT side of the acromion or scapula away from the path of the field to the heart. We were well aware of that concern but cardiologist informed us that this recording set-up is certainly safe.

The value of recording data from multisegmental muscle sites (proximal vs distal) with electrical stimulation of three different cervical spinal locations is that; it provides, in one study, the control of multiple cervical spinal levels. This could have both physiological and clinical application.

Limitations of this study include small sample size resulting in lack of statistical significance in response signal between different stimulation sites. Another limitation is related to the specificity of the recorded signal with a reference electrode applied on the ASIS. CMMR signal travelling from cervical spine has been recorded in the lower limb with reference electrodes applied on the medial border of the scapula, T11-12 and even an L5-S1. However, the signal was smaller than those recorded in this report. It is also important to note that in a sister study comparing cervical and lumbosacral MMR during lying down, sitting and standing the CMMR was modulated similar to TMMR. This indicates a useful/valuable CMMR that was recorded in this study.

Conclusion

To our knowledge, this is the first study to report lower extremity muscular responses to cervical spinal stimulation at three cervical spinal levels. Application of cervical MMR combined with previously reported thoracolumbar levels could play a critical role in the electrophysiological evaluation and in establishing a sound and effective rehabilitation protocol based on the involved neural circuitries of the spinal cord in patients with neurological disorders.

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References