

Video Article

# Assessment of Neuromuscular Function Using Percutaneous Electrical Nerve Stimulation

Vianney Rozand<sup>1</sup>, Sidney Grosprêtre<sup>1</sup>, Paul J. Stapley<sup>2</sup>, Romuald Lepers<sup>1</sup>

<sup>1</sup>INSERM U1093, Faculty of Sport Sciences, Univ. Bourgogne Franche-Comté

<sup>2</sup>Neural Control of Movement Laboratory, School of Medicine, Faculty of Science, Medicine and Health, University of Wollongong

Correspondence to: Vianney Rozand at [vianney.rozand@u-bourgogne.fr](mailto:vianney.rozand@u-bourgogne.fr)

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## Abstract

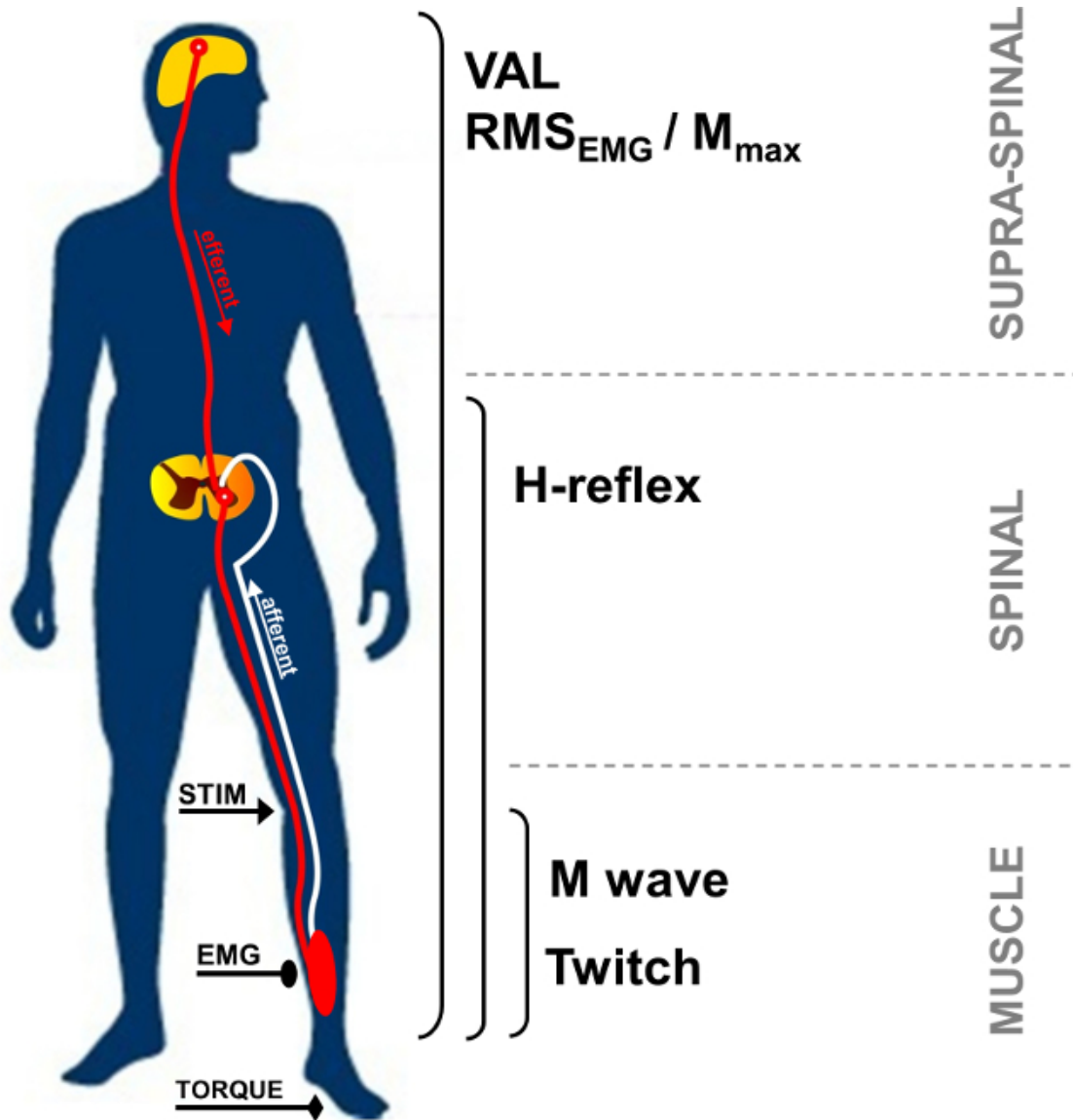
Percutaneous electrical nerve stimulation is a non-invasive method commonly used to evaluate neuromuscular function from brain to muscle (supra-spinal, spinal and peripheral levels). The present protocol describes how this method can be used to stimulate the posterior tibial nerve that activates plantar flexor muscles. Percutaneous electrical nerve stimulation consists of inducing an electrical stimulus to a motor nerve to evoke a muscular response. Direct (M-wave) and/or indirect (H-reflex) electrophysiological responses can be recorded at rest using surface electromyography. Mechanical (twitch torque) responses can be quantified with a force/torque ergometer. M-wave and twitch torque reflect neuromuscular transmission and excitation-contraction coupling, whereas H-reflex provides an index of spinal excitability. EMG activity and mechanical (superimposed twitch) responses can also be recorded during maximal voluntary contractions to evaluate voluntary activation level. Percutaneous nerve stimulation provides an assessment of neuromuscular function in humans, and is highly beneficial especially for studies evaluating neuromuscular plasticity following acute (fatigue) or chronic (training/detraining) exercise.

## Video Link

The video component of this article can be found at <https://www.jove.com/video/52974/>

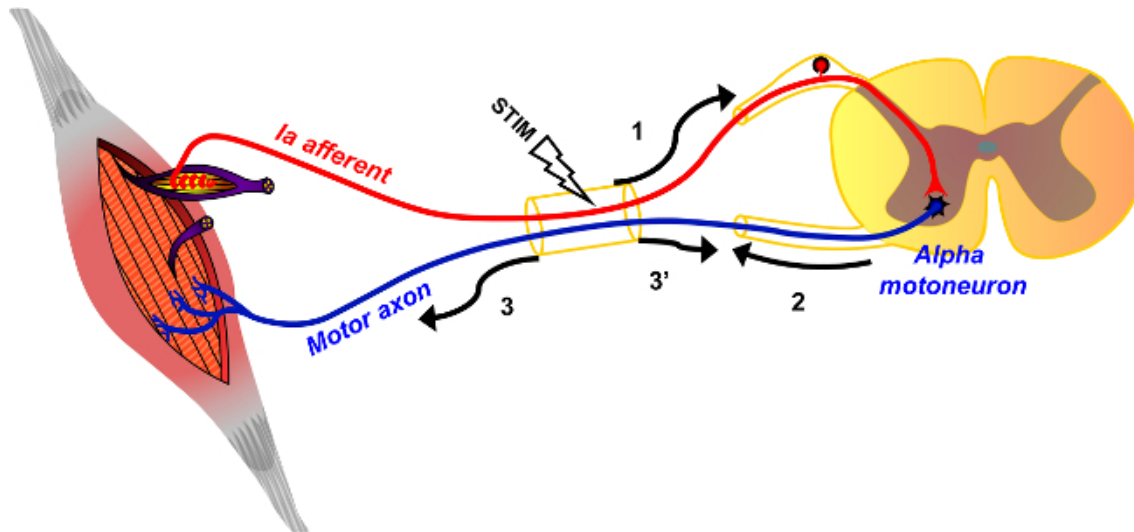
## Introduction

Percutaneous electrical nerve stimulation is widely used to assess neuromuscular function<sup>1</sup>. The basic principle consists of inducing an electrical stimulus to a peripheral motor nerve to evoke a muscular contraction. Mechanical (torque measurement) and electrophysiological (electromyographic activity) responses are simultaneously recorded. Torque, recorded at the considered joint, is assessed using an ergometer. The electromyographic (EMG) signal recorded using surface electrodes has been demonstrated to represent the activity of the muscle<sup>2</sup>. This non-invasive method is not painful and more easily implemented than intramuscular recordings. Both monopolar and bipolar electrodes can be used. The monopolar electrode configuration has been shown to be more sensitive to changes in muscle activity<sup>3</sup>, which can be useful for small muscles. However, bipolar electrodes have been shown to be more effective in improving the signal-to-noise ratio<sup>4</sup> and are most commonly used as a method of recording and quantifying motor unit activity. The methodology described below will focus on bipolar recordings. EMG activity is an indicator of the efficacy and integrity of the neuromuscular system. The use of percutaneous nerve stimulation offers further insights into neuromuscular function, *i.e.* changes at muscular, spinal, or supra-spinal level (**Figure 1**).



**Figure 1: Overview of the neuromuscular measurements.** STIM: nerve stimulation. EMG: Electromyography. VAL: Voluntary Activation Level. RMS: Root Mean Square.  $M_{max}$ : Maximal M-wave amplitude.

At rest, the compound muscle action potential, also called M-wave, is the short-latency response observed after stimulus artefact, and represents excitable muscle mass by the direct activation of motor axons leading to the muscle (**Figure 2**, number 3). M-wave amplitude increases with intensity until reaching a plateau of its maximal value. This response, called  $M_{max}$ , represents the synchronous summation of all motor units and/or muscle fiber action potentials recorded under the surface EMG electrodes<sup>5</sup>. The evolution of the peak-to-peak amplitude or wave area is used to identify alterations of neuromuscular transmission<sup>6</sup>. Changes in the mechanical responses associated with the M-wave, *i.e.* peak twitch torque/force, may be due to alterations in muscle excitability and/or within the muscle fibres<sup>7</sup>. The association of  $M_{max}$  amplitude and peak twitch torque amplitude (Pt/M ratio) provides an index of electromechanical efficiency of the muscle<sup>8</sup>, *i.e.* mechanical response for a given electrical motor command.



**Figure 2: Motor and reflexive pathways activated by nerve stimulation.** Electrical stimulation of a mixed (motor/sensory) nerve (STIM) induces a depolarization of both motor axon and Ia afferent firing. Depolarization of Ia afferents towards the spinal cord activates an alpha motoneuron, which in turn evokes an H-reflex response (pathway 1+2+3). Depending upon the stimulus intensity, motor axon depolarization evokes a direct muscular response: M-wave (pathway 3). At maximal M-wave intensity, an antidromic current is also generated (3') and collides with reflex volley (2). This collision partially or totally cancels the H-reflex response.

The H-reflex is an electrophysiological response used to assess changes in the Ia- $\alpha$  motoneuron synapse<sup>9</sup>. This parameter can be assessed at rest or during voluntary contractions. H-reflex represents a variant of the stretch reflex (**Figure 2**, number 1-3). The H-reflex activates motor units monosynaptically recruited by Ia afferent pathways<sup>10,11</sup>, and can be subjected to peripheral and central influences<sup>12</sup>. The method of evoking a H-reflex is known to have a high intra-subject reliability to assess spinal excitability at rest<sup>13,14</sup> and during isometric contractions<sup>15</sup>.

During a voluntary contraction, the magnitude of the voluntary neural drive can be assessed using the amplitude of the EMG signal, generally quantified using the Root Mean Square (RMS).  $RMS_{EMG}$  is commonly used as a means of quantifying the level of excitation of the motor system during voluntary contraction (**Figure 1**). Because of the intra- and inter-subject variability<sup>16</sup>,  $RMS_{EMG}$  has to be normalized using the EMG recorded during a muscle-specific maximal voluntary contraction ( $RMS_{EMGmax}$ ). In addition, because changes in EMG signal may be due to alterations at peripheral level, normalization using a peripheral parameter such as M-wave is required to assess only the central component of EMG signal. This can be done by dividing the  $RMS_{EMG}$  by the maximal amplitude or the  $RMS_{Mmax}$  of the M-wave. Normalization using  $RMS_{Mmax}$  (i.e.  $RMS_{EMG}/RMS_{Mmax}$ ) is the preferred method as it takes into consideration the possible change of the M-wave duration<sup>17</sup>.

Motor commands can also be evaluated by calculating the voluntary activation level (VAL). This method uses the twitch interpolation technique<sup>18</sup> by superimposing an electrical stimulation at  $M_{max}$  intensity during a maximal voluntary contraction. The extra-torque induced by stimulating the nerve is compared to a control twitch produced by identical nerve stimulation in a relaxed potentiated muscle<sup>19</sup>. To evaluate maximal VAL, the original twitch interpolation technique described by Merton<sup>18</sup> involves a single stimulus interpolated over a voluntary contraction. Recently, the use of paired stimulation has become more popular because the evoked torque increments are larger, more readily detected, and less variable compared to single stimulation responses<sup>20</sup>. The VAL provides an index of the capacity of the central nervous system to maximally activate the working muscles<sup>21</sup>. Currently, VAL evaluated using the twitch interpolation technique is the most valuable method of assessing the level of muscle activation<sup>22</sup>. Furthermore, peak torque assessed using an ergometer is the most properly studied strength testing parameter applicable of use in research and clinical settings<sup>23</sup>.

Electrical nerve stimulation can be used in a variety of muscle groups (e.g. elbow flexors, wrist flexors, knee extensors, plantar flexors). However, nerve accessibility makes the technique difficult in some muscles groups. The plantar flexor muscles, especially triceps surae (soleus and gastrocnemii) muscles, are frequently investigated in the literature<sup>24</sup>. Indeed, these muscles are involved in locomotion, justifying their particular interest. The distance between stimulation site and recording electrodes allows the identification of the different evoked waves of the triceps surae muscles. The superficial part of the posterior tibial nerve in the popliteal fossa and the large number of spindles make it easier to record reflex responses compared to other muscles<sup>24</sup>. For these reasons, the currently presented reflex methodology focuses on the triceps surae group of muscles (soleus and gastrocnemius). The aim of this protocol is therefore to describe percutaneous nerve stimulation technique to investigate neuromuscular function in the triceps surae.

## Protocol

The experimental procedures outlined received Institutional ethical approval and are in accordance with the Declaration of Helsinki. Data were collected from a representative participant who was aware of the procedures and gave his written informed consent.

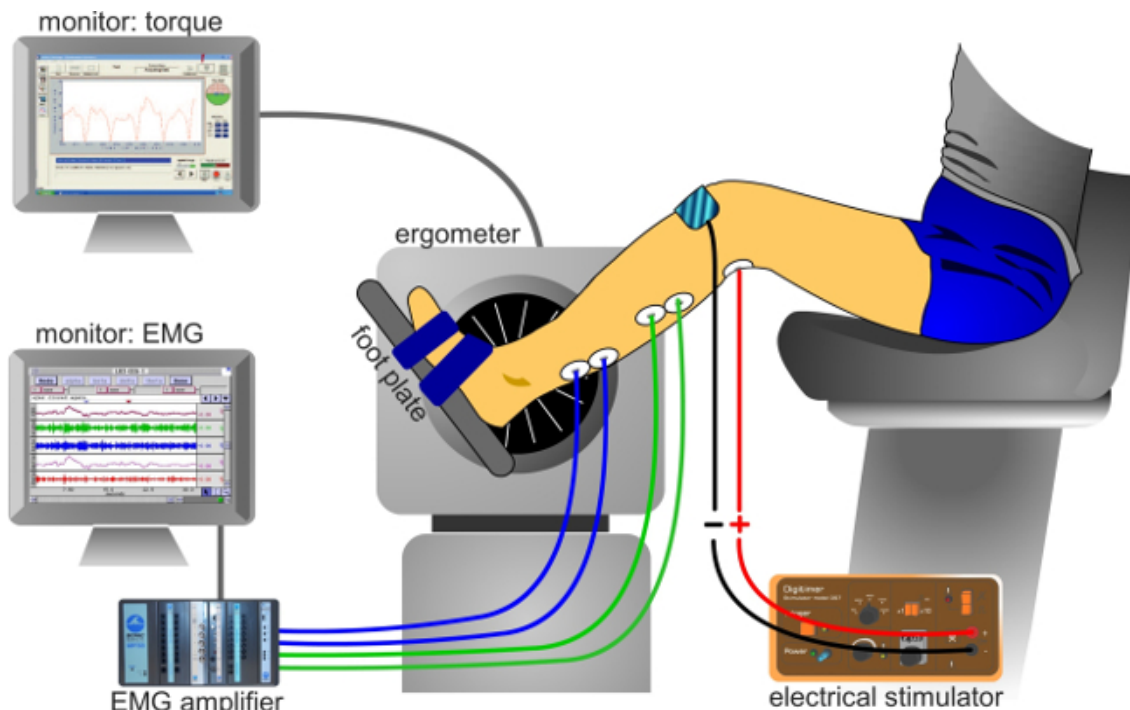
### 1. Instrument Preparation

1. Clean the skin at the electrode location by shaving, and remove the dirt with alcohol to obtain low impedance (<5 k $\Omega$ ).
2. Place two AgCl surface electrodes (recording diameter of 10 mm) at 2/3 of the line between the medial condylis of the femur to the medial malleolus for the soleus muscle; on the most prominent bulge of the muscle for the medial gastrocnemius; at 1/3 of the distance along a line

between the head of the fibula and the heel for the lateral gastrocnemius; and at 1/3 of the distance along a line between the tip of the fibula and the tip of the medial malleolus for the tibialis anterior muscle, with an interelectrode distance (center-to-center) of 2 cm, according to the SENIAM recommendations<sup>30</sup>.

Note: The soleus muscle electrodes have to be positioned under the distal insertion of gastrocnemii muscles to ensure that they are not recording activity from the heads of the gastrocnemii muscles (cross-talk).

3. Place a reference electrode in a central position on the same leg (between stimulation and recording sites).
4. Adjust the height and the depth of the chair to obtain an ankle angle of 90° (0° = full plantar flexion), so that the soleus and gastrocnemii muscles are not stretched and the H-reflex is not altered<sup>11,12</sup>.
  1. Set the knee angle at 90° (0° = full knee extension) due to the biarticular nature of the gastrocnemii muscles. However, the optimal ankle angle to perform a maximal voluntary torque of the plantar flexors is 70-80° (0° = full plantar flexion)<sup>26</sup>. Thus, ankle angle will depend on the parameter of interest (electrophysiological versus mechanical recordings).  
Note: Regardless of the chosen initial angle, it must remain constant throughout the experiment to standardize neuromuscular excitability<sup>11,12,27,28</sup>.
  2. Pay particular attention when monitoring the subjects' posture during the test to maintain constant cortico-vestibular influences on the excitability of the motor pool<sup>29</sup>.
5. Firmly strap the ankle to an ergometer, with the anatomical axis of the joint (external malleolus) aligned with the axis of rotation of the ergometer<sup>25</sup>.
  1. Have the subject exert pressure on a footplate attached to the ergometer to record plantar flexor torque. Keep the foot immobile throughout the experiment so that small changes in torque can be detected.
6. Note: Under certain circumstances, the heel may lift slightly off the force plate if the foot and ankle are not secured, which may lead to an incomplete transmission of the torque against the plate. **Figure 3** presents a description of the experimental setup.



**Figure 3: Experimental setup.** Classical experimental setup to record electromyographic (EMG) and torque signals.

6. Connect the electrodes to the amplifier with cables.
7. Set the sampling rate for torque and EMG measurements to 2-5 kHz. Record the EMG signal using an Analog-to-Digital (AD) conversion system. The signal is displayed on a monitor with a data acquisition system, which instantaneously gives values of several parameters (e.g. maximal value, peak-to-peak amplitude, duration). The spectrum of the EMG signal can range between 5 Hz and 2 kHz frequencies, but is mainly contained between 10 Hz and 1 kHz<sup>31</sup>. Thus, sampling frequency must be high enough to preserve signal shape during EMG recording. Amplify and filter EMG signals (gain = 500-100) using a bandwidth frequency between 10 Hz and 1 kHz<sup>8,21,32</sup>.
8. Place the anode for the electrical stimulation over the patellar tendon.
9. Determine the best stimulation site of the posterior tibial nerve to obtain an optimal soleus H reflex for a given intensity, using a hand-held cathode ball electrode in the popliteal fossa. Test several stimulation sites with the cathode ball electrode until a maximal value of the H reflex is reached.
  1. Record tibialis anterior EMG activity to ensure that the common peroneal nerve is not activated to avoid influence from antagonist Ia afferents<sup>12</sup>. Set the pulse width at 1 msec to provide an optimal activation of the nervous fibers, especially afferent fibers<sup>10</sup>.
10. Place a self-adhesive AgCl cathode at the location of the stimulation site to ensure constant stimulus condition (e.g. pressure, orientation).  
Note: All of these parameters (subject position, electrode location and stimulation site) do not change for the assessment of the different electrophysiological measurements. Only the intensity of the stimulation and the condition (rest versus contraction) vary.

## 2. Testing Procedures at Rest

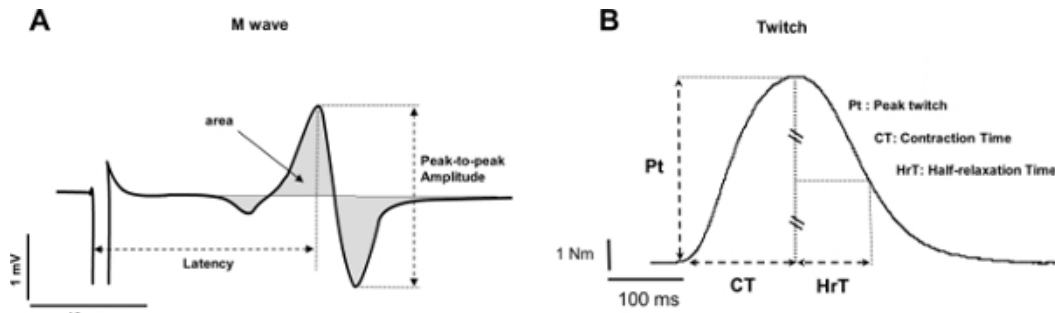
1. Instruct the subject to remain relaxed and to keep his/her muscles at rest.
2. Adjust the stimulation intensity to obtain maximal soleus H-reflex amplitude ( $H_{\max}$ ; usual range: 20-50 mA). An M-wave of the soleus muscle can be observed at  $H_{\max}$  intensity.  
Note: For repeated measurements (e.g. before and after a fatiguing protocol), the optimal intensity to obtain a  $H_{\max}$  response may vary during the session. As keeping a constant intensity can lead to an underestimation of  $H_{\max}$  amplitude, it is recommended that the experimenter regularly reevaluates  $H_{\max}$  intensity<sup>33</sup>.
3. Record a minimum of 3 soleus H-reflex responses at this intensity with a minimum interval of 3 sec to avoid post-activation depression<sup>34</sup>.  
Note: Although recording several responses is more suitable due to the particular sensitivity of the H-reflex, a single stimulation may be enough under some circumstances, for example when trying to avoid the effects of rapid recovery (e.g. during a fatiguing protocol).
4. Increase the stimulation intensity to obtain maximal soleus M-wave amplitude ( $M_{\max}$ ; usual range: 40-100 mA). Usually, set the increment in stimulation intensity at 2-4 mA, with an interval of 8-10 sec between two stimuli<sup>12,35</sup>. The desired intensity is reached when  $M_{\max}$  is obtained, and no H-reflex response can be observed.
5. Set the final intensity to 120-150% of  $M_{\max}$  stimulus intensity to ensure that the M-wave attains a plateau of its maximal value. This intensity is called supramaximal intensity in the instructions below.
6. Keep constant stimulation intensity for soleus M-wave recordings throughout the session.
7. Record 3 soleus M-waves and 3 associated twitch torques at this intensity.

## 3. Testing Procedures During Voluntary Contraction

1. As a warm-up, ask the subject to perform 10 brief and non-fatiguing submaximal contractions of the plantar flexor muscles, with a few seconds rest between each of the contractions. At the end of the warm-up, take a minimum 1 min rest to avoid any fatiguing effects<sup>11</sup>.
2. Continuously record triceps surae EMG activity. Recording soleus and gastrocnemii muscles allows the analysis of the behavior of different muscle typologies for a single stimulation site<sup>24</sup>.
3. Instruct the subject to perform an isometric maximal voluntary contraction (MVC) of the plantar flexors. The subject has to push as hard as possible against the ergometer by contracting his plantar flexor muscles. Give visual feedback to the subject during the effort, and standardized verbal encouragement<sup>19</sup>. The MVC is reached when a plateau is observed.
4. Deliver a paired stimulation (100 Hz frequency) at supramaximal intensity during the plateau of the MVC (superimposed doublet), and another paired stimulation when the muscle is fully relaxed immediately after the contraction (potentiated doublet) to evaluate the voluntary activation level. Deliver this paired stimulation through a specific device (e.g. Digitimer D185 MultiPulse Stimulator) or through a stimulation program associated with a single pulse stimulator.
5. Instruct the subject to perform a second MVC of the plantar flexor with at least 1 min rest between each trial<sup>11</sup>. If the peak torque from the second trial is not within 5% of the first, additional trials must be performed<sup>36</sup>. The greatest torque achieved by the subject is taken as the MVC torque.

## 4. Data Analysis

1. Data Analysis at Rest
  1. Select a time window including the EMG response associated with the twitch at rest (H-wave or M-wave).
  2. Measure the peak-to-peak amplitude, peak-to-peak duration, and/or the area of the waves (**Figure 4A**). If the amplitude is not directly provided by the software, subtract the minimum to the maximum values.
    1. For the duration, measure the time frame starting from the maximal peak and ending to the minimal peak. For the area, calculate the integral of EMG signal starting from the beginning of the wave and ending to the end of the wave.  
Note: Peak-to-peak amplitude can reflect: 1) neuromuscular transmission, 2) motor unit action potential amplitude and/or 3) temporal dispersion of motor unit action potential<sup>37</sup>. M-wave duration reflects neuromuscular propagation<sup>37</sup>.
  3. For multiple trials, calculate the average of the waves. If the average cannot be directly provided by the software, use spreadsheet software (e.g. the formula function in a spreadsheet program) to calculate this value from several trials (at least 3).
  4. Select the resting twitch.
  5. Measure the peak torque associated with the resting twitch (**Figure 4B**).
  6. For multiple trials, calculate the average peak torque of the resting twitches. If the average cannot be directly provided by the software, use spreadsheet software (e.g. the formula function in a spreadsheet program) to calculate this value from the several trials (at least 3).
  7. Repeat these procedures described in point 4.1.2 for the other desired parameters (contraction time or half-relaxation time). The analysis of twitch parameters provides indications as to the excitation-contraction coupling efficiency<sup>17</sup>. In particular, contraction time provides an index of contraction kinetics<sup>8</sup>, which can depend upon the chosen muscle group<sup>38</sup>.
  8. Calculate the ratio between the peak torque and the sum of M-waves using spreadsheet software (e.g. Excel), to quantify the electromechanical efficiency ( $P_e/M$ ). As the mechanical responses evoked by posterior tibial nerve stimulation correspond to the activation of the triceps surae as a whole, amplitudes of soleus and gastrocnemii M-waves must be summed<sup>39</sup>.



**Figure 4: Explanation of electrophysiological and mechanical responses.** (A) Measurement of peak-to-peak amplitude (mV), latency (ms) and area (mV.ms) of a typical M-wave. (B) Measurement of peak twitch torque (Nm), contraction time (ms) and half-relaxation time (msec) of a twitch. [Please click here to view a larger version of this figure.](#)

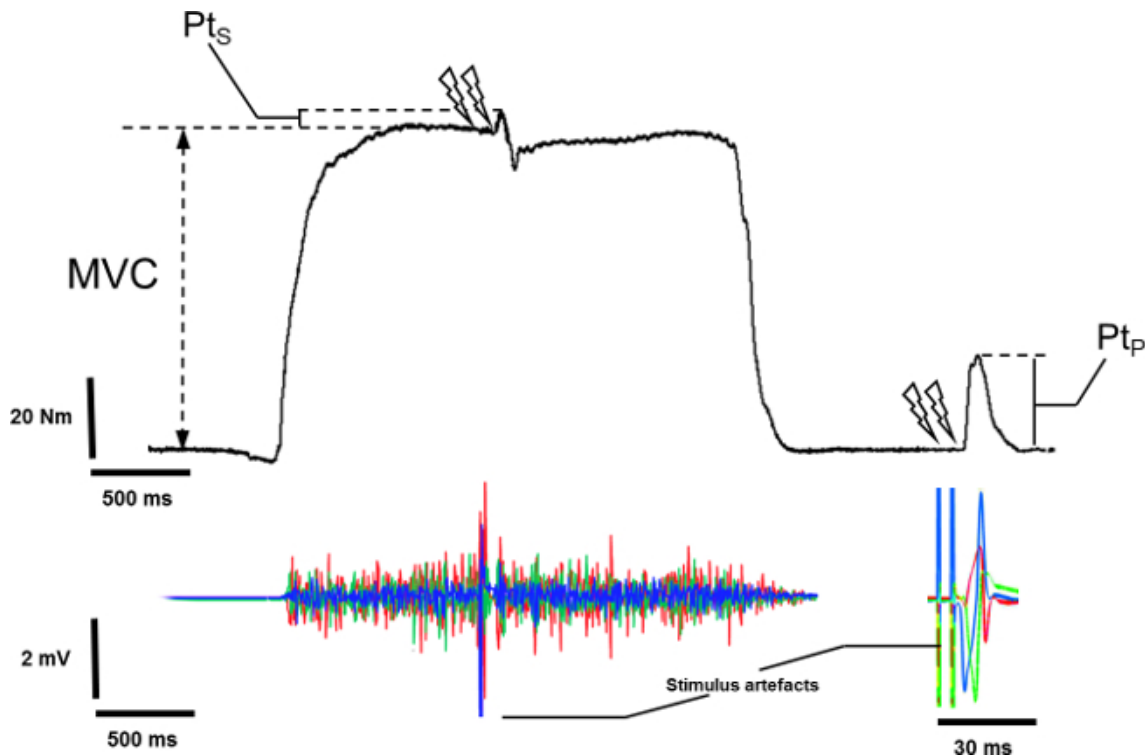
## 2. Data analysis in contraction

1. Select a 500 msec time window of soleus EMG activity during the plateau of MVC torque including the peak torque but excluding the time between the stimulation artefact and the end of the silent period of EMG. The silent period corresponds to the suppression of the ongoing voluntary EMG activity following stimulation.
2. If the root mean square (RMS) is not directly provided by the software, calculate the RMS to quantify EMG activity using the following formula<sup>40</sup>:  $RMS_{EMG}$

$$\sqrt{\frac{1}{T} \int_{T_0-T/2}^{T_0+T/2} (EMG)_t^2 \cdot dt}$$

3. Measure or calculate the RMS of  $M_{max}$  at rest over the duration of the wave.
4. Calculate the  $RMS_{EMG}/RMS_{Mmax}$  ratio using spreadsheet software.  $RMS_{EMG}$  value and  $RMS_{Mmax}$  value have to be selected from the same muscle.
5. Measure the maximal peak torque of the MVC from the baseline of torque at rest to the maximal value of MVC excluding the superimposed torque induced by the doublet stimulation (**Figure 5**).
6. Measure the superimposed torque induced by the doublet stimulation during the MVC, from the voluntary torque value at the onset of the stimulation to the peak of the evoked response (**Figure 5**).
7. Select the potentiated doublet.
8. Measure the peak torque associated with the potentiated doublet.
9. Calculate the voluntary activation level (VAL) using the following formula<sup>40</sup>:

$$VAL = \left( 1 - \frac{\text{superimposed doublet}}{\text{potentiated doublet}} \right) \times 100$$

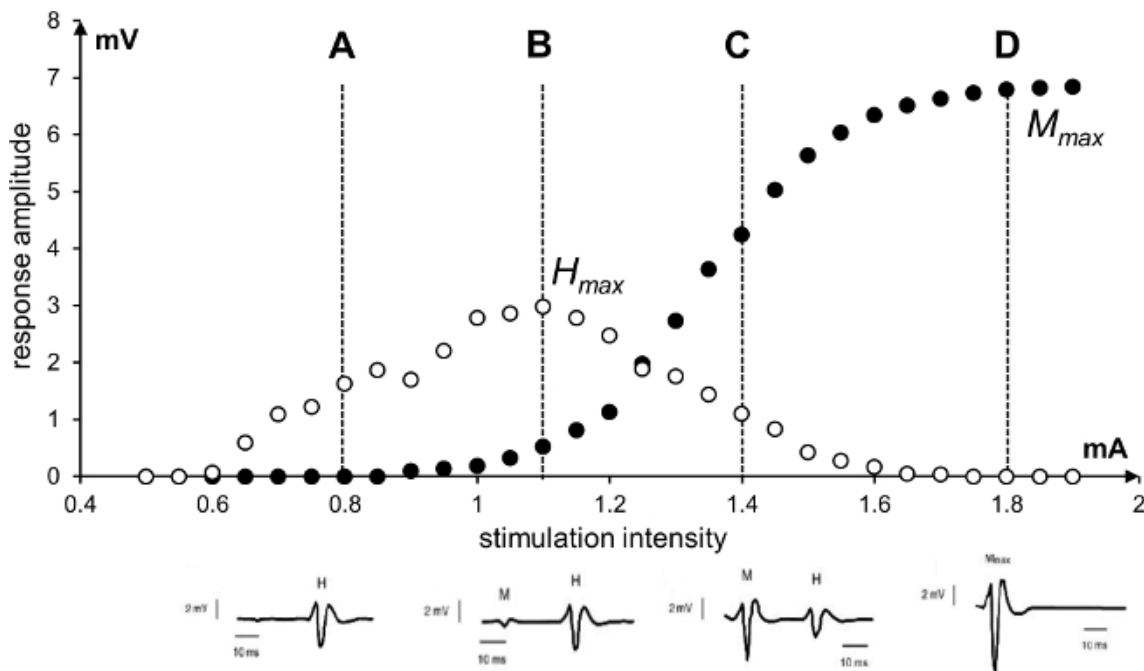




**Figure 5: Measurement of superimposed and potentiated doublet on mechanical signal.** To record the superimposed peak torque (Pts), stimulation doublet is evoked during the plateau of isometric maximal voluntary contraction (MVC). To record potentiated peak torque (Ptp), stimulation doublet is evoked at rest after the offset of MVC.

## Representative Results

Increasing stimulus intensity leads to a different evolution of response amplitudes between H- and M-waves. At rest, the H-reflex reaches a maximum value before being totally absent from EMG signal, while M wave progressively increases until reaching a plateau at maximal intensity (see **Figure 4** for a graphical depiction of the M-wave and **Figure 6** for the evolution of M-waves and H-reflex with intensity). For the soleus muscle, the latency between the stimulus onset and M-wave is about 10 msec (**Figure 4A**) and generally between 25 and 40 msec for H-wave. However, the latency will vary between the muscle groups and the subject's limb length or overall height, due to the distance between the stimulation site and the muscle. When stimulating at M-max intensity, a maximal peak twitch torque will also be observed (**Figure 4B**). M-waves, H-reflexes and peak twitch torques will vary depending on the condition. For example, these parameters tend to increase during voluntary contraction, and decrease in the presence of fatigue<sup>17</sup>.



**Figure 6: Typical recruitment curves at rest.** Amplitudes of reflex responses (H-reflex, white round) and direct muscle responses (M-wave, black round) with increasing stimulus intensity. Bottom panels present typical traces at four progressively increased intensities (from A to B). (A) weak intensity, evoking only an H-reflex response. (B) Intensity providing the maximal H-wave amplitude ( $H_{max}$ ). (C) At intensity beyond  $H_{max}$ , the collision between antidromic and reflex volleys induces a decrease in H response amplitude. (D) At  $M_{max}$  intensity, H-reflex is totally cancelled and M-wave reaches a plateau. [Please click here to view a larger version of this figure.](#)

Maximal VAL is evaluated during a MVC. **Figure 5** shows a superimposed torque induced by electrical stimulation during the MVC. The effect induced by stimulation reflects an incomplete recruitment of motor units and/or a submaximal discharge frequency of the motor units, and thus a deficit in voluntary activation (see the effect of stimulation in the middle of **Figure 5**). As previous parameters, maximal VAL varies depending on the condition (e.g. level of contraction, fatigue)<sup>21</sup>.

These different techniques have previously been validated. Indeed, recent studies demonstrated a good reliability for M wave and the associated peak twitch torque<sup>22</sup>, H-reflex<sup>14</sup> and maximal VAL<sup>41</sup>.

## Discussion

Percutaneous nerve stimulation enables the quantification of numerous characteristics of the neuromuscular system not only to understand the fundamental control of neuromotor function in healthy humans, but also to be able to analyze acute or chronic adaptations through fatigue or training<sup>17</sup>. This is very beneficial especially for fatiguing protocols, where measurements must be performed as soon as possible after exercise end to avoid the effects of rapid recovery<sup>42</sup>.

Although numerous studies have focused on the triceps surae muscles<sup>24</sup>, percutaneous nerve stimulation can be applied in other lower limb (e.g. tibialis anterior<sup>43,44</sup>, quadriceps muscles<sup>45,46</sup>) and upper limb muscles (e.g. biceps brachii<sup>32</sup>, flexor carpi radialis<sup>47</sup>, finger muscles<sup>48</sup>). However, nerve stimulation presents potential methodological limitations for some muscles. For instance, obtaining a H-reflex from biceps brachii muscle can be difficult to obtain at rest<sup>49</sup>. Furthermore, stimulating the musculocutaneous nerve over the brachial plexus leads to contraction of both agonist and antagonist muscles<sup>32</sup>, inducing the erroneous evaluation of the voluntary activation level. Recording nearby muscle activity allows the experimenter to ensure that only the target muscle is activated, or at least to limit activation of these nearby muscles. To overcome these

limitations, some authors have suggested that stimulation over the muscle belly with larger electrodes can be a reliable method to evoke M-wave and twitches<sup>32,50</sup>. However, the spatial organization of axonal terminal branches within the muscle can differ between muscles. Thus, motor units activation would vary between nerve and muscle stimulation<sup>51</sup>. Nerve stimulation activates motor units according to the size principle, whereas the recruitment order during direct muscle stimulation is more dependent upon the spatial organization of muscle fibers under the stimulating electrodes<sup>50</sup>.

Monosynaptic aspects of the H-reflex allow the reliable assessment of spinal excitability with nerve stimulation. However, it must be noted that Ia-alpha motoneurons synapse can be subject to numerous cortical influences, such as subject's attention<sup>52</sup>, visual environment<sup>53</sup>, head movements<sup>54</sup> or even jaw clenching<sup>55</sup>. Peripheral factors can also influence response amplitude, such as afferent feedback from muscle stretch<sup>56</sup>. The posture of the subject has also to be carefully controlled for during the experiments and through experimental sessions to minimize cortico-spinal influences<sup>29</sup>. Furthermore, familiarization sessions can reduce intersession variability, especially for novice subjects<sup>57</sup>.

Besides these physiological concerns, stimulation characteristics (e.g. intensity, location) can widely influence the results. Although  $M_{\max}$  responses reach a plateau near maximal intensity,  $H_{\max}$  is obtained for a specific intensity. Thus, intensity of stimulation to obtain  $H_{\max}$  is more susceptible to variability with conditions. To ensure good reliability under different conditions (e.g. fresh or fatigued muscle), stimulus intensity should be set to  $H_{\max}$  intensity or below, when the reflex response lies in the ascending part of the recruitment curve<sup>58</sup>. Indeed, H-reflex amplitude can be altered for intensities above  $H_{\max}$  intensity due to the collision between reflex and antidromic volleys (Figure 2, number 3' and number 2). It is also recommended that the H-reflex amplitude be normalized to the  $M_{\max}$  response ( $H/M_{\max}$  ratio). It has been shown that this method allows for reliable inter- and intra-individual comparisons<sup>59</sup>.

In terms of inferring the nature of the motor command, although the VAL technique has been shown to be a reliable technique to assess descending commands<sup>40</sup> and central fatigue<sup>19,60</sup>, this method presents some limitations. Indeed, some authors suggested that VAL overestimates maximal muscle activation<sup>61–63</sup>. It may not be sensitive enough to detect variations in activation levels during contractions above 90 % MVC<sup>62</sup>. Moreover, the use of paired stimulation to evaluate VAL can increase discomfort for subjects<sup>64</sup>. Despite the evaluation of maximal voluntary activation, this method does not provide information about cortico-spinal excitability. Transcranial magnetic stimulation could be used to assess changes at this level<sup>65–67</sup>.

The use of the  $RMS_{EMG}/RMS_{Mmax}$  ratio to evaluate voluntary activation is less sensitive than the twitch interpolation technique due to greater response variability. Indeed,  $RMS_{EMG}/M_{\max}$  ratio can remain constant whereas the twitch interpolation technique highlights a significant decrease in muscle activation<sup>68</sup>. However, the  $RMS_{EMG}/RMS_{Mmax}$  ratio allows the experimenter to evaluate the activation of the different individual muscles of the same muscle group (e.g. soleus, medial gastrocnemius and lateral gastrocnemius for the triceps surae)<sup>17</sup>.

Particular attention should be taken with percutaneous nerve stimulation regarding stimulation protocol and data analysis to avoid misinterpretation and to permit a comparison between different studies. Numerous authors have previously established methodological recommendations to record and analyze data from percutaneous electrical stimulation<sup>20,29,34,59</sup>. In particular, plantar flexor muscles appear to be a difficult muscle group to contract maximally<sup>69–71</sup>. Practice is required to ensure that the participants, especially in populations with impaired neuromuscular function, are capable of high levels of voluntary activation prior to experimental testing<sup>72,73</sup>. Thus, MVC-dependent measures such as voluntary activation will represent erroneous values that likely reflect a lack of practice or an insufficient number of isometric MVC attempts rather than an impairment or limitation of neuromuscular function. A familiarization session should be performed prior to all studies using percutaneous nerve stimulation and/or maximal efforts.

Percutaneous electrical nerve stimulation can be used to evaluate neuromuscular plasticity following acute (fatigue) or chronic (training/detraining) exercises. For instance, Lepers *et al.*<sup>74</sup> observed a decrease in central activation (voluntary activation level) and muscular parameters (peak twitch, M-wave) of the quadriceps muscle following a prolonged cycling exercise. Following chronic exercise, Duchateau and Hainaut<sup>75</sup> observed different effects of isometric and dynamic trainings on peak twitch torque properties, suggesting that skeletal muscle adapts differently to the kind of training programs. Electrical nerve stimulation is also useful to evaluate online adaptations of the neuromuscular system during various conditions, such as posture<sup>27</sup> or a concurrent mental task<sup>21</sup>. This method can be used not only in fundamental research but also in the clinical domain<sup>76</sup>. Indeed, electrical nerve stimulation has been used to investigate central drive in the elderly<sup>77</sup> and different diseases such as stroke<sup>78</sup> or Parkinson's disease<sup>79</sup>. Neuromuscular plasticity can also be assessed in pathological populations during therapy/retraining program<sup>80</sup>.

## Disclosures

The authors have nothing to disclose.

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