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# H-reflex and M-wave recordings: effect of pressure application to the stimulation electrode on the assessment of evoked potentials and subject's discomfort

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### Summary

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#### Key words

discomfort; electrical nerve stimulation; Hoffmann reflex; pain; plantar flexors; spinal transmission This study aimed to compare the effect of different types of pressure applied to the stimulation electrode on assessing the efficiency of Ia- $\alpha$ -motoneuron transmission of the soleus muscle and the associated discomfort using electrical nerve stimulation. Twelve healthy young adults participated in three experimental sessions (one for each knee angle). The amplitudes of the maximal Hoffmann reflex  $(H_{max})$  and motor potential  $(M_{max})$  were recorded from the soleus muscle at 0°,  $30^{\circ}$  and  $90^{\circ}$  knee angles ( $0^{\circ}$  full extension) through three pressure applications to the stimulation electrode: no pressure, pressure with manual application and pressure using adhesive tape. The soleus H<sub>max</sub>/M<sub>max</sub> were calculated to assess the efficiency of Ia-α-motoneuron transmission during varied knee angles and pressure application to the stimulation electrode. At the stimulation intensity evoking soleus  $H_{max}$  and  $M_{max}$ , subjects were asked to orally provide a value between 'no discomfort' (0) and 'worst possible discomfort' (10). The application of pressure on the stimulation electrode, particularly using adhesive tape, decreased both the stimulation intensity needed to evoke an electrophysiological response and the associated self-reported discomfort (P<0.05), while the  $H_{max}/M_{max}$  remained constant. At the stimulation intensity evoking M<sub>max</sub>, the electrical stimulation appeared to be more painful at  $0^{\circ}$  knee angle compared with  $30^{\circ}$  and  $90^{\circ}$  angles (P < 0.01). To conclude, this study showed that a knee flexion and a pressure application to the stimulation electrode, especially using tape pressure, are recommended in the objective to reduce the patient/subjects' discomfort when eliciting evoked potentials on soleus muscle.

### Introduction

Nerve stimulation associated with electromyographic (EMG) and mechanical responses recordings is commonly used to investigate changes at the spinal level (e.g. Hoffmann reflex (H-reflex) and/or muscle level (e.g. M-wave) (Millet et al., 2011). One main limitation of the nerve stimulation is the discomfort associated with this technique (Motl et al., 2002, 2003, 2004).

The stimulation intensities evoking the maximal H-reflex  $(H_{max})$  and the maximal M-wave  $(M_{max})$  on soleus (SOL) induce discomfort in young subjects (Motl et al., 2003). Motl et al. (2003) reported that self-reported pain was greater at the  $M_{max}$  than at the  $H_{max}$  stimulation intensity, revealing an intensity-related increase in the pain experienced. It was hypothesized that the pain sensation induced by eliciting myoelectric responses could be caused by the activation of the

subcutaneous nociceptive afferent fibres of the tibial nerve (Motl et al., 2004). The discomfort induced by electrical nerve stimulation suggests that in addition to non-nociceptive type Ia and type II afferents, nociceptive afferents (Aδ and C nociceptors) are also being stimulated (Motl et al., 2002). It is well known that prolonged nociceptive feedback may affect reflex responses (Laurin et al., 2015). Nevertheless, due to the very short duration of the electrical nerve stimulation (1 ms) and the associated discomfort/pain, an instantaneous change in reflex response due to the activation of the afferent nociceptive fibres seems unlikely. However, for ethical reasons, the pain and/or discomfort associated with electrical nerve stimulation can be a limiting factor for participants in such protocols, especially in frail populations.

Accordingly, it is critical to optimize the process of electrical nerve stimulation and minimize the discomfort of the subjects, while at the same time ensuring consistent electrophysiological responses. The method commonly used to evoke electrophysiological responses by nerve stimulation in plantar flexors consists of using bipolar electrodes with the cathode placed over the nerve in the popliteal fossa and the anode on the opposite side of the limb (Hugon, 1973) so that the current passes transversely through the nerve. The stimulation protocol to elicit the maximal H-reflex and M-wave responses with minimal discomfort has already been optimized in terms of the duration of the stimulus (i.e. 0.5-1 ms) (Panizza et al., 1989). However, it is unknown whether the pressure applied on the stimulation electrode influences the evoked potentials and the pain experienced by subjects. This methodological issue was rarely mentioned, suggesting that the investigators did not apply pressure on the stimulation electrode while eliciting the evoked potentials (Oya et al., 2008; Pinar et al., 2010; Tsuruike et al., 2012; Behrens et al., 2015; Mezzarane et al., 2015; Stutzig & Siebert, 2016a,b). In some studies, although pressure on the stimulation electrode was applied using adhesive tape and/or straps (Capaday & Stein, 1986; Duclay & Martin, 2005; Sefton et al., 2007; Querry et al., 2008; Cattagni et al., 2014; Baudry et al., 2015), the physiological rationale behind this manipulation was not specified.

The objective of this study was therefore to compare the effects of different types of pressure applied to the stimulation electrode placed at the level of the posterior tibial nerve, while assessing the efficiency of plantar flexors spinal transmission (i.e. H<sub>max</sub>/M<sub>max</sub> ratio) and the discomfort induced by electrical stimulation. Towards this aim, we recorded the  $H_{max}$  and  $M_{\rm max}$  from the SOL muscle with three types of pressure applied to the stimulation electrode: (i) no pressure (NP condition) applied to the stimulation electrode, (ii) pressure with manual application (manual pressure, MP condition) to the stimulation electrode, and (iii) pressure to the stimulation electrode using adhesive tape (tape pressure, TP condition) around the knee. Because varying knee angles can change the distance between the stimulation electrode and the tibial nerve or the cathode, we also proposed applying the three types of pressure applications at three different knee angles (i.e. 0°,  $30^{\circ}$  and  $90^{\circ}$  knee angle;  $0^{\circ}$  = full extension). The electrophysiological measurements were only collected on SOL muscle because this muscle presents (i) a high functional relevance (due to its involvement in balance control; Morasso & Schieppati, 1999; Loram et al., 2004; Cattagni et al., 2016), (ii) a physiological profile appropriate for investigating the Hreflex (Capaday & Stein, 1986; Palmieri et al., 2004; Tucker et al., 2005; Cattagni et al., 2014), and (iii) appears to be not affected to changes in knee angle (Alrowayeh et al., 2005). Because a lower stimulation intensity should be needed for evoking a given electrophysiological potential in MP and TP conditions compared with NP condition, we hypothesized that the self-reported discomfort would be higher in the NP condition than in the MP and TP conditions. We also expected that the type of pressure application would not change the SOL  $H_{max}/M_{max}$  ratio.

## Materials and methods

#### Subjects

Twelve healthy young adults (seven males and five females, age  $22.5 \pm 1.2$  years, height  $172.5 \pm 9.7$  cm, mass  $63.5 \pm 9.2$  kg, mean  $\pm$  SD) participated in this study. Before starting the experimentation, the minimum size of the sample needed for the study was calculated, using the G\*Power software 3.1.9.2 (Franz Faul, University of Kiel, Kiel, Germany; Faul et al., 2007). For an expected 'medium' effect size (f = 0.25), a significance level of 0.05, a power  $(1-\beta)$  of 0.9 and a correlation among repeated measures of 0.6, a required sample size of 10 subjects was obtained to compare stimulation intensity and selfreported discomfort changes among the experimental conditions. It was therefore decided to include 12 participants in the experiment, to account for potential dropouts. All of the participants were volunteered and informed about the nature, aims, risks and discomfort associated with the study before they gave their written consent prior to participating in the investigation. The protocol of the current investigation was approved by the French National Drugs and Health Administration and by the National Ethics Committee section Nantes Ouest IV (ID: 635/ 2015) and was in conformity with the Declaration of Helsinki (last modified in 2013).

#### Experimental protocol

The experiments were conducted by the same experimenter to insure a standardization of the procedure through the experimental sessions. For each subject, the SOL electrophysiological responses were recorded at three different knee angles (i.e. 0°, 30° and 90°) corresponding to three different experimental sessions (Fig. 1a). The three experimental sessions were separated by 2 weeks and were randomly administered to the subjects at the same time of day. Each session lasted ~2 h. In each experimental session, subjects were tested under three pressure applications for nerve stimulation (Fig 1b), randomly administered. In the first condition, the stimulation electrode was fixed without external pressure (NP condition). The second condition consisted of applied manual pressure on the stimulation electrode (MP condition), where the experimenter attempted to apply constant pressure. Finally, in the third condition, the pressure on the stimulation electrode was applied by fixing the stimulation electrode with tape (TP condition).

For each of the nine experimental conditions (3 knee angles  $\times$  3 pressure applications), a recruitment curve in which the intensity of stimulation was progressively increased by 2 mA from the SOL H-reflex threshold until the H<sub>max</sub> and by 5 mA from the H<sub>max</sub> until the M<sub>max</sub> was carried out. Four stimuli were delivered at each intensity and interspaced by a 10-s interval to avoid the confounding effect of homosynaptic postactivation depression (Hultborn *et al.*, 1996). During the recruitment curve, a horizontal visual analogue scale graduated from 0 to 10 was presented to the subjects after each level of stimulation intensity



**Figure 1** Illustration of the experimental protocol and data analysis. (a) represents the three tested positions corresponding to the three different knee angles. Each position was tested during one specific experimental session. (b) represents the three pressure applications. In the NP condition, no pressure was applied to the stimulation electrode. In the MP condition, manual pressure was applied by the experimenter to the stimulation electrode. In the TP condition, pressure was applied to the stimulation electrode using tape around the knee. (c) illustrates a typical trace of one representative subject at a stimulation intensity evoking maximal H-reflex amplitude on the soleus. (d) represents a typical H-reflex (continuous line) and M-wave (dashed line) recruitment curve of one representative subject associated with self-reported discomfort (grey circles). Each symbol of H-reflex or M-wave represents a mean of four peak-to-peak amplitudes of evoked potential. P-P: peak-to-peak.

to assess the discomfort associated with electrical nerve stimulation. The subjects were then asked to orally provide a value between 'no discomfort' (0) and 'worst possible discomfort' (10). When a subject reported a value of 10, that is worst possible discomfort, the recruitment curve was stopped.

#### **Experimental set-up**

#### Subjects' position

Participants were examined in the seated position with the trunk inclined at  $20^{\circ}$  with reference to the vertical, ankle joint angle at  $90^{\circ}$  and knee joint angle at  $0^{\circ}$ ,  $30^{\circ}$  or  $90^{\circ}$ . The right foot was secured by two straps to the footplate of a dynamometer (Biodex, Shirley, NY, USA). The centre of rotation of the dynamometer shaft was aligned with the flexion-extension axis of the anatomical ankle.

#### Electromyographic recordings

The subjects' skin was first carefully prepared by shaving, abrading and cleaning with alcohol. Bipolar silver chloride (Ag/ AgCL) surface electrodes (Kendall Medi-Trace<sup>TM</sup>, Mansfield, MA, USA) that were 1 cm in diameter with an interelectrode distance (centre to centre) of 2 cm were placed on the SOL muscle along the mid-dorsal line of the leg, ~5 cm below the insertion of the two heads of the gastrocnemii on the Achilles tendon. The reference electrode was placed on the patella. The placement of the electrodes was marked on the skin with an indelible pen to ensure that the same recording sites were used in the successive experimental sessions. The EMG signal was amplified with a bandwidth frequency ranging from 5 Hz to 1 kHz (Gain = 1000). The EMG signals were sampled at 2 kHz with the Biopac acquisition system (MP35; BIOPAC, Goleta, CA, USA) and stored with commercially available software (BIOPAC student Lab Pro; Biopac system Inc.) for analysis.

#### Electrical stimulation

The subjects were not familiarized with electrical nerve stimulation prior to the experiment. H-reflexes and M-waves were evoked by percutaneous stimulation of the posterior tibial nerve in the popliteal fossa with a single rectangular pulse (1 ms) automatically delivered by a constant current stimulator (Model DS7 AH; Digitimer, Hertfordshire, UK). The selfadhesive cathode (1-cm diameter, Ag/AgCl) was placed in the popliteal fossa and the anode (5  $\times$  10 cm, Medicompex SA, Ecublens, Switzerland) on the anterior surface of the knee. The optimum cathode position was located with a hand-held cathode ball (0.5-cm diameter). Once determined, the cathode electrode was fixed to this site without pressure (NP condition), with manual pressure (MP condition) or with tape pressure (TP condition). This procedure, consisting to find the optimal stimulation site, was repeated at the beginning of each experimental session.

#### Data analysis

The mean peak-to-peak amplitudes of the SOL  $H_{\rm max}$  and  $M_{\rm max}$  were calculated over four recordings in each experimental

condition (Fig. 1c). The  $H_{max}/M_{max}$  ratio was calculated as a percentage of  $M_{max}$ . Among the 12 subjects, one subject reached a value of 10 in the self-reported discomfort scale in the NP condition at the 90° knee angle during the recruitment curve, excluding the  $M_{max}$  measurements. Therefore, this subject was not included in the statistical analysis when comparing the stimulation intensities or evoked potentials and was only included when comparing self-reported discomfort.

#### Statistical analysis

All data are presented as the mean  $\pm$  SD. The critical level for statistical significance was set at 5%. Statistical analysis was performed using Statistica 10 (Statsoft, Tulsa, OK, USA). Normality criteria were tested using the Kolmogorov-Smirnov test. Two-factor ANOVAs with repeated measures [knee angle (90°, 30° and 0°) x pressure application (NP, MP and TP)] were performed on the  $H_{max}$ ,  $M_{max}$ ,  $H_{max}/M_{max}$ . Three-factor ANOVA with repeated measures [evoked potential (Hmax and  $M_{max}$ ) x knee angle (90°, 30° and 0°) × pressure application (NP, MP and TP)] was performed for the stimulation intensity and the value of the self-reported discomfort scale. When a main effect or a significant interaction was found, a post hoc analysis was conducted using Tukey's test. The effect size for each ANOVA was also calculated as partial eta square  $(\eta_p^2)$ . For all experimental conditions [knee angle (90°, 30° and  $0^{\circ}$ ) × pressure application (NP, MP and TP)], the relationships between self-reported discomfort and stimulation intensity, expressed as a percentage of the stimulation intensity evoking  $M_{\rm max}$ , were assessed using Pearson's correlation coefficients (r).

#### Results

#### **Evoked potentials**

The  $H_{max}$ ,  $M_{max}$ ,  $H_{max}/M_{max}$ ,  $M_{at-}H_{max}$  and  $M_{at-}H_{max}/M_{max}$  for the SOL muscle across the nine experimental conditions are shown in Table 1.

For  $H_{max}$ ,  $M_{max}$ ,  $H_{max}/M_{max}$ ,  $M_{at}$ - $H_{max}$  and  $M_{at}$ - $H_{max}/M_{max}$ , statistical analysis revealed no knee angle ( $F_{2,20} = 0.19$ ; P = 0.82;  $\eta_p^2 = 0.01$ ,  $F_{2,20} = 0.5$ ; P = 0.64;  $\eta_p^2 = 0.04$ ,  $F_{2,20} = 1.0$ ; P = 0.39;  $\eta_p^2 = 0.08$ ,  $F_{2,20} = 0.6$ ; P = 0.54;  $\eta_p^2 = 0.06$  and  $F_{2,20} = 0.26$ ; P = 0.77;  $\eta_p^2 = 0.03$ , respectively) and pressure application ( $F_{2,20} = 0.85$ ; P = 0.44;  $\eta_p^2 = 0.07$ ,  $F_{2,20} = 1.1$ ; P = 0.36;  $\eta_p^2 = 0.09$ ,  $F_{2,20} = 1.30$ ; P = 0.30;  $\eta_p^2 = 0.11$ ,  $F_{2,20} = 0.8$ ; P = 0.46;  $\eta_p^2 = 0.08$  and  $F_{2,20} = 0.1$ ; P = 0.91;  $\eta_p^2 = 0.01$ , respectively) effects.

#### Stimulation intensity

The stimulation intensity levels at  $H_{\rm max}$  and  $M_{\rm max}$  in the nine experimental conditions are shown in Table 2.

The statistical analysis performed on the stimulation intensity showed a significant interaction between the evoked

		°06			30°			00	
Knee angle Condition	NP	MP	ΤЪ	NP	MP	ΤЪ	NP	MP	ТР
H <sub>max</sub> , mV	$6.5 \pm 2.9$	$6.4 \pm 3.3$	$5\cdot 8 \pm 3\cdot 1$	$6 \cdot 1 \pm 4 \cdot 2$	5・3 ± 3・2	$6 \cdot 1 \pm 3 \cdot 0$	5.6 主 3.7	5.5 土 4.5	$6.1 \pm 4.4$
$M_{max}, mV$	$12.4 \pm 3.7$	$13.0 \pm 3.5$	$12.5 \pm 3.3$	$13.2 \pm 5.0$	$12.9 \pm 4.9$	$13.4 \pm 4.9$	$13.2 \pm 6.2$	$13.8 \pm 6.5$	$13.4 \pm 6.6$
$H_{max}/M_{max}$ , %	$51.7 \pm 20.6$	$49.1 \pm 25.5$	$46.4 \pm 24.5$	$45.8 \pm 26.6$	$39.4 \pm 17.9$	$46.7 \pm 23.9$	$43.5 \pm 17.4$	$39.2 \pm 21.0$	$43.8 \pm 18.8$
$M_{at-}H_{max}$	$1.6 \pm 1.0$	$1.2 \pm 1.0$	$1 \cdot 6 \pm 1 \cdot 1$	$1.9 \pm 2.3$	$2 \cdot 0 \pm 2 \cdot 0$	$1.4 \pm 0.7$	$1.9 \pm 1.9$	$1.4 \pm 1.2$	$1.3 \pm 1.1$
$M_{at}-H_{max}/M_{max}$ , %	$13.7 \pm 8.5$	$11\cdot 3 \pm 10\cdot 7$	$13.0 \pm 9.3$	$13.2 \pm 11.0$	$16.6 \pm 12.2$	$12.4 \pm 10.1$	$16.9 \pm 19.4$	$14.9 \pm 16.9$	$12 \cdot 0 \pm 13 \cdot 1$
NP. no pressure applied to	the stimulation ele	ectrode: MP. pressure	s with manual appli	ication to the stimul	ation electrode: TP.	pressure to the stir	nulation electrode u	sing adhesive tape a	cound the knee:

**Table 1** Effect of the knee angles and pressure applications on the amplitude of evoked potentials and the soleus H<sub>max</sub>/m<sub>max</sub> ratio

H<sub>max</sub>, maximal Hoffmann reflex; M<sub>max</sub>, maximal direct motor response. Evoked potentials were elicited by single pulses (duration 1 ms, voltage 400 mV), delivered using a constant current stimulator DS7 AH; Digitimer (Model ]

Values are means  $\pm$  SD, n = 11 subjects

		<b>00</b> °			<b>30</b> °			°o	
Knee angle Condition	NP	MP	đ	NP	MP	ЧL	NP	MP	Ъ
At H <sub>max</sub>									
3I, mA	$40.4 \pm 11.8$	$24.7 \pm 11.6$	$24.0 \pm 8.3$	$27.8 \pm 14.4$	$26.5 \pm 15.4$	$23.5 \pm 11.3$	$46.1 \pm 23.2$	$35.8 \pm 16.5$	$27.5 \pm 13.3$
Discomfort	$1.7 \pm 1.7$	$0.8 \pm 0.9$	$0.9 \pm 1.2$	$0.9 \pm 1.6$	$0.9 \pm 0.9$	$0.8 \pm 1.1$	$2.5 \pm 1.1$	$1 \cdot 7 \pm 1 \cdot 3$	$1 \cdot 1 \pm 1 \cdot 1$
At M <sub>max</sub>									
5I, mA	$80.1 \pm 16.5$	$60.8 \pm 21.8$	58・7 土 18・4	$60.7 \pm 21.5$	$61.6 \pm 24.3$	$54.3 \pm 21.2$	$100.0 \pm 42.2$	$84.9 \pm 29.5$	$70.0 \pm 35.4$
Discomfort	$5.0 \pm 2.6$	$3.1 \pm 2.6$	$3\cdot 3 \pm 2\cdot 2$	$3.7 \pm 1.4$	$3.2 \pm 2.2$	$2.6 \pm 1.6$	$6.1 \pm 0.9$	$4.8 \pm 1.3$	$4.1 \pm 1.4$

**Table 2** Effect of the knee angles and pressure applications on stimulation intensity and self-reported discomfort

H<sub>max</sub>, me produce of the summand section, my produce that manual approaches to the summand section, my produce of the summand section and another approaches the section section interactions was found between evoked potential versus knee angle, evoked potential versus sections versus and the section of th pressure application and knee angle versus pressure application. The discomfort scale is from 0 (no discomfort) to 10 (maximal discomfort). Single pulses (duration 1 ms, voltage 400 mV) were deliv-DS7 AH; Digitimer) ered using a constant current stimulator (Model

Values are means  $\pm$  SD, n = 11 subjects for SI; n = 12 subjects for discomfort

potential and knee angle ( $F_{2,20} = 3.9$ ; P<0.05;  $\eta_p^2 = 0.28$ ; Fig. 2a). At H<sub>max</sub>, no difference in stimulation intensity was observed among the three knee angles. At Mmax, the stimulation intensity was significantly (P<0.01) lower for knee angles at  $30^{\circ}$  and  $90^{\circ}$  than at  $0^{\circ}$ .

An interaction between the stimulation intensity and pressure application ( $F_{2,20} = 7.0$ , P<0.01;  $\eta_p^2 = 0.41$ ; Fig. 2b) was also found. At H<sub>max</sub>, the stimulation intensity was significantly (P<0.01) higher in the NP condition than in the TP and MP conditions. At M<sub>max</sub>, the stimulation intensity was significantly (P<0.001) higher in the NP condition than in both pressure conditions. The stimulation intensity was also significantly (P<0.001) higher in the MP condition compared with the TP condition.

The statistical analysis revealed an interaction between the pressure application and the knee angle ( $F_{4,40} = 6.9$ , P<0.001;  $\eta_p^2 = 0.40$ ; Fig. 2c). For 90° knee angle, the stimulation intensity was significantly (P<0.001) higher in the NP condition than in the MP and TP conditions. For 0° knee angle, the stimulation intensity was significantly (P<0.05) higher in the NP condition than in the pressure conditions and also higher in the MP condition compared with the TP condition. In the NP condition, the 30° knee angle showed the lower level of stimulation intensity compared with the  $0^\circ$  (P<0.001) and  $90^{\circ}$  (P<0.001) knee angles. The stimulation intensity was higher for the  $0^{\circ}$  knee angle compared with the  $30^{\circ}$  (P<0.01) and 90° (P<0.001) angles for the MP condition.

#### Discomfort

The self-reported discomfort at  $H_{max}$  and  $M_{max}$  in the nine experimental conditions is shown in Table 2.

The statistical analysis performed on the self-reported discomfort showed significant interactions between the evoked potential and knee angle ( $F_{2,22} = 4.2$ ; P<0.05;  $\eta_p^2 = 0.29$ ; Fig. 2d). At  $H_{max}$ , 30° appeared to be less painful than 0° (P<0.05). At Mmax, the self-reported discomfort was significantly (P<0.01) lower for  $30^{\circ}$  and  $90^{\circ}$  knee angles than  $0^{\circ}$ angle. Regardless of the knee angle, the electrical stimulation at  $M_{max}$  intensity induced a higher discomfort than at  $H_{max}$ (P<0·001).

The statistical analysis also showed an interaction between the evoked potential and the pressure application ( $F_{2,22} = 3.7$ , P<0.05;  $\eta_{\rm p}^2$  = 0.25; Fig. 2e). At the  $H_{\rm max}$  intensity, the selfreported discomfort was significantly (P<0.05) higher in the NP condition than in the TP condition, but was not different compared with the MP condition. At the M<sub>max</sub> intensity, the self-reported discomfort was significantly (P<0.001) higher in the NP condition than in the MP and TP conditions.

The statistical analysis revealed an interaction between the pressure application and the knee angle ( $F_{4,44} = 2.9$ , P<0.05;  $\eta_p^2 = 0.21$ ; Fig. 2f). For 90° knee angle, the self-reported discomfort was significantly (P<0.01) higher in the NP condition than in the TP and MP conditions. For  $0^{\circ}$  knee angle, the selfreported discomfort was significantly (P<0.05) higher in the



**Figure 2** Effect of the evoked potentials, knee angles and pressure applications on the stimulation intensity and self-reported discomfort. (a) and (d) represent the results of the statistical interaction between knee angle and evoked potential on the stimulation intensity and self-reported discomfort, respectively. (b) and (e) represent the results of the statistical interaction between pressure application and evoked potential on the stimulation intensity and self-reported discomfort, respectively. (c) and (f) represent the results of the statistical interaction between the knee angle and pressure application on the stimulation intensity and self-reported discomfort, respectively. (c) and (f) represent the results of the statistical interaction between the knee angle and pressure application on the stimulation intensity and self-reported discomfort, respectively. \* Significant difference from 90°, P<0.05; <sup>#</sup>significant difference from NP, P<0.05; <sup>§</sup> significant difference from MP, P<0.05; <sup>1</sup> significant difference between evoked potential, P<0.05; 90°, 30° and 0° represent the three tested knee angles. NP, no pressure; MP, manual pressure; TP, tape pressure.

NP condition than TP and MP. In the NP condition, the 30° knee angle induced less discomfort compared with the 0° (P<0.001) and 90° (P<0.05) knee angles. In this condition, the self-reported discomfort was higher for 0° than 90° knee angle (P<0.05). In the MP condition, the 0° of knee angle appeared to induce higher discomfort compared with the 30° (P<0.01) and 90° (P<0.01) angles. In the TP condition, the self-reported discomfort was significantly (P<0.05) higher for 0° knee angle than for 30° and 90° angles.

For all the experimental conditions, significant positive correlations (P<0.001) were found between self-reported discomfort and stimulation intensity (% of the maximal stimulation intensity). Pearson's correlation coefficients (r) were ranged between 0.50 and 0.81.

#### Discussion

The main finding of this study was that the application of pressure on the stimulation electrode decreases subjects' discomfort by reducing the stimulation intensity needed to evoke electrophysiological responses, while the efficiency of the spinal transmission from Ia afferent inputs to  $\alpha$ -motoneurons remains constant.

# Effect of the electrical nerve stimulation intensity on the self-reported discomfort

Electrical nerve stimulation is widely known to induce discomfort (Motl et al., 2002, 2003, 2004; Button & Behm, 2008; Place et al., 2010). We confirmed in the present study that the stimulation intensity needed to evoke  $M_{max}$  induced a higher discomfort compared with that required to evoke  $H_{max}$ , indicating that pain perception is related to the current intensity (Motl et al., 2003). Furthermore we showed for all the experimental conditions a discomfort-stimulation intensity relationship, showing that the discomfort induced by the electrical nerve stimulation is related to the current intensity. The noxious evoked stimuli imply a recruitment of A $\delta$  and C nociceptors. The increase in self-reported discomfort by increasing the electrical stimulation intensity therefore reflects a larger recruitment of nociceptive afferents.

#### Effect of pressure application

In the present investigation, we recorded the M-wave evoked concomitantly with the H-reflex and calculated the  $M_{at}$ - $H_{max}/M_{max}$  ratio, which is used to control the stability of recording conditions (Schieppati, 1987). The SOL  $M_{at}$ - $H_{max}$  represented 11–17% of the SOL  $M_{max}$  in all the experimental conditions, in agreement with earlier values reported in the literature (Maffiuletti et al., 2001; Scaglioni et al., 2003; Grospretre & Martin, 2012; Cattagni et al., 2014). The lack of change in  $M_{at}$ - $H_{max}/M_{max}$  ratio through the three types of pressure application demonstrated the stability of conditions during H-reflex recordings. The present results showed that the  $H_{max}$ ,  $M_{max}$  and  $H_{max}/$ 

 $M_{\rm max}$  ratio recorded from the SOL muscle were not influenced by the three pressure applications (Table 1). This indicates that the examination of the efficiency of spinal transmission is independent of the pressure applied to the stimulation electrode.

The stimulation intensities needed to evoke  $H_{max}$  and  $M_{max}$ were higher without pressure applied to the stimulation electrode than with pressure regardless of the knee angle. This finding can, in part, be accounted for by two main explanations that are not mutually exclusive. First, the pressure applied to the stimulation electrode reduces the distance between the anode and the cathode causing a lower current diffusion in tissue. Second, the distance between the stimulation electrode and the posterior tibial nerve was also reduced with pressure. Because the current varies with the inverse of the square of the distance (Coulomb's law), a lower electrical stimulation intensity is required to evoke a similar potential (Wu et al., 2011) in conditions with pressure compared with NP condition. This finding is in line with previous studies in which the increased distance between the stimulation site and the nerve or muscle was associated with reduced stimulation efficacy. Using neuromuscular electrical stimulation, Maffiuletti et al. (2011) found that the current intensity needed at the motor threshold was higher in obese patients (i.e. in which the distance between stimulation electrodes and muscle was the highest) compared with non-obese subjects. Using magnetic nerve stimulation and adding fat tissues between the coil and the femoral nerve, Tomazin et al. (2011) observed that for an identical stimulus, the motor response was altered, indicating a reduced efficacy of the stimulation. The reduced stimulation intensity with the application of pressure to evoke potentials was associated with a low level of self-reported discomfort, confirming that the perception of pain is related to the intensity of nerve stimulation.

The effect of the pressure application on the stimulation intensity appeared to be specific to the knee angle. Indeed, for  $30^{\circ}$  knee angle, no difference was reported among the pressure applications. A higher stimulation intensity was required at  $90^{\circ}$  and  $0^{\circ}$  in the NP condition compared with pressure conditions, leading to an increased self-reported discomfort (Table 2). For  $0^{\circ}$  knee angle, we also observed a lower stimulation intensity and self-reported discomfort in the TP condition compared with the MP condition. Overall, it can be concluded that it is better to apply a pressure (preferentially with tape) to the stimulation electrode to reduce the stimulation intensity and the subjects' discomfort.

#### Effect of knee angle

In agreement with previous findings (Alrowayeh et al., 2005), the present results demonstrated that the peak-to-peak amplitudes of the  $H_{max}$ ,  $M_{max}$  and  $H_{max}/M_{max}$  ratios recorded from the SOL muscle were not influenced by the knee angles while  $M_{at}$ - $H_{max}/M_{max}$  remained constant.

When comparing the three knee angles, we observed that for the maximal stimulation intensity (evoking  $M_{max}$ ), the 0°

knee angle was the position for which the stimulation intensity was the highest and induced therefore the greatest selfreported discomfort (Table 2). The protocol of the present study was not initially designed to investigate the potential mechanisms that cause a rise in electrical stimulation intensity required to elicit a given amplitude of evoked potential. These mechanisms remain therefore to be clarified in future research. We suggest that the difference in stimulation intensity needed to evoked potentials with varying knee angles could be related to a difference in nerve depth, adipose tissue width and/or distance between the anode and cathode.

#### **Study limitations**

A limitation of this study was the lack of control of the pressure applied to the stimulation electrode while eliciting myoelectric responses. This experimental precaution, that is pressure application, is rarely mentioned in studies in which EMG potentials were evoked. Although this can be viewed as a limitation, it should be acknowledged that the pressure to the stimulation electrode was applied by the same experienced experimenter. This latter applied a constant pressure to the stimulation electrode until it is constrained by the force feedback effect of the skin and the other tissues under the skin.

#### Recommendations

In clinical practice and research protocols, it is important to obtain the most relevant measurements while simultaneously optimizing patient/subject comfort. In this study, we showed that all types of pressure applications are comparable in terms of the quality of the collected data. However, the lack of

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pressure applied against the cathode significantly increases the discomfort for subjects, particularly at 0° and 90° knee angles. Therefore, we strongly recommend applying pressure against the cathode, especially using tape pressure, when electrically stimulating the posterior tibial nerve in future studies, to optimize the participants/patients comfort. Concerning the optimum knee angle during the elicitation of SOL evoked potentials, we observed the highest stimulation intensities and discomforts when the leg was extended and no difference between 90° and 30° knee flexion. With the objective to reduce the subject's discomfort associated with electrical nerve stimulation of the SOL, we therefore recommend for future investigations to perform the electrical nerve stimulation with a knee flexion.

#### Conclusion

The results from this study legitimize the application of pressure against the stimulation electrode, especially using tape pressure, when eliciting evoked potentials, regardless of the knee angle. Indeed, this allows for collecting good quality data and also optimizes the comfort of participants during the electrical stimulation of the posterior tibial nerve.

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## **Conflict of interest**

The authors have no conflicts of interest.

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