



Article

Transcutaneous Spinal Cord Stimulation Enhances Quadriceps Motor Evoked Potential in Healthy Participants: A Double-Blind Randomized Controlled Study

Álvaro Megía-García ^{1,2}, Diego Serrano-Muñoz ^{2,*}, Julian Taylor ^{3,4}, Juan Avendaño-Coy ² , Natalia Comino-Suárez ^{2,5} and Julio Gómez-Soriano ²

¹ Biomechanical and Technical Aids Unit, National Hospital for Paraplegia, SESCAM, 45071 Toledo, Spain; amegiag@externas.sescam.jccm.es

² Toledo Physiotherapy Research Group (GIFTO), Faculty of Physiotherapy and Nursing, Castilla La Mancha University, 45071 Toledo, Spain; juan.avendano@uclm.es (J.A.-C.); natalia.comino@cajal.csic.es (N.C.-S.); julio.soriano@uclm.es (J.G.-S.)

³ Sensorimotor Function Group, National Hospital for Paraplegia, SESCAM, 45071 Toledo, Spain; juliantaylorgreen2@gmail.com

⁴ Harris Manchester College, University of Oxford, Oxford OX1 3TD, UK

⁵ Neural Rehabilitation Group, Cajal Institute, Spanish National Research Council (CSIC), 28002 Madrid, Spain

* Correspondence: diego.serrano@uclm.es; Tel.: +34-925268800 (ext. 5830)

Received: 7 September 2020; Accepted: 12 October 2020; Published: 13 October 2020



Abstract: Transcutaneous electrical spinal cord stimulation (tSCS) is a non-invasive technique for neuromodulation and has therapeutic potential for motor rehabilitation following spinal cord injury. The main aim of the present study is to quantify the effect of a single session of tSCS on lower limb motor evoked potentials (MEPs) in healthy participants. A double-blind, sham-controlled, randomized, crossover, clinical trial was carried out in 15 participants. Two 10-min sessions of tSCS (active-tSCS and sham-tSCS) were applied at the T11-T12 vertebral level. Quadriceps (Q) and tibialis anterior (TA) muscle MEPs were recorded at baseline, during and after tSCS. Q and TA isometric maximal voluntary contraction was also recorded. A significant increase of the Q-MEP amplitude was observed during active-tSCS (1.96 ± 0.3 mV) when compared from baseline (1.40 ± 0.2 mV; $p = 0.01$) and when compared to sham-tSCS at the same time-point (1.13 ± 0.3 mV; $p = 0.03$). No significant modulation was identified for TA-MEP amplitude or for Q and TA isometric maximal voluntary contraction. In conclusion, tSCS applied over the T11-T12 vertebral level increased Q-MEP but not TA-MEP compared to sham stimulation. The specific neuromodulatory effect of tSCS on Q-MEP may reflect optimal excitation of this motor response at the interneuronal or motoneuronal level.

Keywords: transcutaneous spinal cord stimulation; evoked potentials motor; neuromodulation; motor activity

1. Introduction

Transcutaneous electrical spinal cord stimulation (tSCS) is a non-invasive technique designed to generate neuromodulation of the central nervous system at the spinal cord level. During the last ten years, studies have reported changes in excitability of neural networks organized at the spinal cord level by transcutaneous electrical stimulation applied via surface electrodes placed over the middle of back (T10-T11 spinal level) [1,2]. At the spinal level, tSCS modulates the excitability of sensorimotor circuits which are also affected by epidural stimulation [3]. Both techniques can evoke posterior root

muscle (PRM) reflex responses measured at the target muscle [3]. The low PRM reflex threshold response to stimulation, suggests that tSCS activates large-to-medium diameter afferent fibers (Ia, Ib, II) within the sensory dorsal roots [1,3]. The similarity between PRM and Hoffmann reflex responses also suggests that tSCS activates proprioceptive Ia afferent fibers [4,5].

Several studies have also revealed that tSCS mediates several therapeutic effects in subjects with neurological impairments [6]. Thus, tonic tSCS, applied as a biphasic rectangular current at a frequency of 30–50 Hz, has shown a therapeutic potential to enhance voluntary motor activity [7,8], trunk stability [9], standing [10], gait function [7,11,12] and as a method to reduce spasticity [13,14]. However, the clinical effectiveness of this technique is still undetermined, and the stimulation parameters need to be optimized [6].

The results of a recently published review by our group, Megía-García et al. [6], suggest that the optimal site for spinal stimulation to enhance voluntary motor control of the lower extremities is over the T11-T12 vertebrae, which corresponds to the L1-L2 spinal cord segments. The idea of the existence of the central pattern generation (CPG) at this level [15], together with a multisegmental muscle activity in response to stimulation applied at this level [16], suggests that activation of local or propriospinal motor control mechanisms potentiate descending corticospinal acting on spinal motoneurons, thereby mediating the neuromodulatory effect of tSCS leading to improve motor function following spinal cord injury [15]. However, studies performed on healthy subjects have shown that the optimal stimulation site differs according to which target muscle group is activated [17–19]. Hence, greater activation of the anterior rectus femoris and vastus lateralis has been observed with T10-T11 (L1-L2 spinal cord segments) stimulation when compared to the hamstring, triceps surae, and tibialis anterior muscles which are activated optimally with T12-L1 stimulation (L5-S1 spinal cord segments) [19,20]. In line with these observations, the phenomenon of spatial summation has also been reported, with greater motor responses recorded from the target muscle with simultaneous electrical stimulation of contiguous spinal levels [21].

Although tSCS has been shown to be a safe technique with therapeutic potential for motor function rehabilitation following neurological diseases [6], it is necessary to understand how the tSCS technique can be optimized in the clinical practice. The validation of neurophysiological measurements which reflect changes in both cortical and spinal excitability, such as motor evoked potentials (MEPs) evoked by transcranial magnetic stimulation (TMS), and closer attention to appropriate sham-protocols are important first steps for developing future tSCS studies related to better neuromodulation with optimized stimulation parameters and electrode locations.

Until now, tSCS-mediated neuromodulation of the motor response has been measured indirectly through electromyogram recordings of ongoing voluntary muscle activity in combination with dynamometry, manual muscle testing, kinematics and functional assessments [7,8,22,23]. However, these measurement tools have a low sensitivity to single session tSCS-induced neuromodulation and cannot detect small changes in neuromuscular activity. Neurophysiological methods aimed at measuring changes in spinal excitability, such as the PRM reflex and the Hoffman reflex, have been used to assess neuromodulation after tSCS [24–27]. However, the extent to which tSCS can lead to a change in cortical and spinal excitability has only been studied in the upper limb [27]. Therefore, the measurement of MEPs evoked by TMS has been used in this study as the standard and sensitive neurophysiological method to assess changes in excitability of the corticospinal pathway in response to tSCS.

The main aim of the present study is to quantify the effect of a single session of active T11-T12 tSCS on lower limb MEP amplitude measured from the quadriceps and tibialis anterior muscles of healthy participants. We hypothesized that a single session of tSCS would increase corticospinal excitability with a preferential effect on those motoneurons closer to the level of spinal stimulation. As a secondary aim the efficacy of blinding both the sham and active tSCS was carefully analyzed using specialized metrics appropriate to randomized control trials.

2. Experimental Section

The present study was approved by the local Toledo Ethical Committee (Ref. No. 158; 2/11/2017) and the clinical trial was registered in the ClinicalTrials.gov Protocol Registration System (NCT04241406). Participants were informed about the protocol and signed the informed consent approved by the local ethics committee.

2.1. Design

A double-blinded, randomized, controlled, crossover clinical trial was designed. All participants ($n = 15$) received two randomized sessions of tSCS stimulation (active-tSCS and sham-tSCS). Both, participants and the assessor (AMG) were blinded, and the order for each recruited participant to receive either the sham or active tSCS intervention was randomized, with the consecutive order concealed in individual sealed envelopes. Randomization was performed using the web software www.randomizer.org. Intervention sessions were separated with a minimal 48 h washout period, which was longer than that used in similar studies (Aarskog et al., 2007; Buonocore and Camuzzini, 2007; Chen and Johnson, 2009; Dean et al., 2006).

In order to check the reliability of the neurophysiological outcomes, two measurements of quadriceps (Q) and tibialis anterior (TA) MEPs were recorded at baseline (PRE 1 and PRE 2) with a rest period of 2 min between them. Both Q and TA MEPs were recorded again during the intervention at 4 min from the onset of the tSCS intervention (DURING) and following the stimulus at 2 min (POST 1) and 4 min (POST 2). Postintervention measurements (POST 1 and POST 2) were recorded to determine the duration of the neuromodulatory effect following tSCS. Two measurements of isometric maximal voluntary contraction of the Q and TA muscles were recorded as secondary outcomes at PRE 1 and POST 2.

2.2. Procedures and Intervention

Fifteen healthy participants ≥ 18 years old, without injury to the central or peripheral nervous system, were recruited via non-probabilistic convenience sampling. The exclusion criteria for recruitment included musculoskeletal pathology of the lower limbs, metal or electronic implants, medications that influenced neural excitability (antiepileptic, antipsychotics, or antidepressants), allergy to the electrode material, epilepsy, and pregnancy.

Participants adopted a relaxed supine position without voluntary muscle contraction. Three self-adhesive surface electrodes (9×5 cm, ValuTrode, Axelgaard Manufacturing Co, Fallbrook, CA, USA) were used for tSCS. The anode was placed on the midline skin surface between the T11 and T12 spinous processes. Two interconnected cathodes were placed symmetrically on the abdomen, at both sides of the umbilicus. A rectangular foam ($12 \times 7 \times 2$ cm) was used to increase the pressure on the anode by strapping both the sponge and electrode around the torso, to improve skin contact. The electrical stimulator used for all interventions was an electrotherapeutical device (Enraf Nonius, Myomed 932, Rotterdam, The Netherlands).

2.2.1. Transcutaneous Spinal Cord Stimulation (Active-tSCS)

A symmetrical biphasic 30 Hz current of 1 ms pulse-width was applied at the threshold stimulation intensity required to evoke the PRM reflex. To calculate the PRM threshold the same device, electrode location, and waveform of current were used. The stimulus intensity was gradually increased in steps of 1 mA every 1 s until a minimally visible contraction was visually detected in the Q muscle. If subjects could not tolerate the stimulus intensity required to elicit the threshold PRM reflex, the stimulus was reduced to the intensity which was comfortable. The therapist informed the participant that the intensity could be set below the sensory threshold, with the possibility that the participant may or may not feel the stimulation current tSCS was applied for a total of 10 min with the subjects relaxed in the supine position.

2.2.2. Sham-tSCS

Sham stimulation consisted of the same stimulation parameters used for active tSCS except for the stimulus intensity, which was set at the sensory threshold, maintained for 30 s and then slowly decreased to zero, where the stimulus intensity was set for the remaining 10 min intervention. This method has been previously validated as an appropriate sham stimulus for controlled studies (Deyo et al., 1990; Petrie and Hazleman, 1985) [28,29]. The participants received the same instructions that given to the active tSCS session.

2.3. Blinding Assessment

Both, the intervention, and evaluation process of the protocol were carried out by two independent researchers (NCS and AMG, respectively).

The success of participant and assessor blinding were evaluated through a questionnaire [30] after each intervention (active-tSCS and sham-tSCS), by assessing whether they had received the sham or active tSCS with five questions: (1) "Strongly believe the applied intervention is new treatment"; (2) "Somewhat believe the applied intervention is new treatment"; (3) "Somewhat believe the applied intervention is a placebo", (4) "Strongly believe the applied intervention is a placebo", or (5) "Do not Know".

2.4. Primary and Secondary Outcome Measures

2.4.1. Motor Evoked Potential Recordings

Motor evoked potentials were elicited by transcranial magnetic stimulation (TMS, Magstim Rapid 2, Magstim Company Ltd., UK) using a double-cone coil. The optimal stimulation site (hot-spot, area where TMS elicited the largest MEP) was identified for both the Q and TA muscles individually with reference to the standard CZ point (approximately 1 cm lateral and 2 cm posterior to CZ). The coil was then fixed, and the rim of the coil was marked with a pen on the scalp, so that the stimulation site was maintained constant. MEP threshold was defined as the minimal TMS intensity required to evoke a motor response (>0.1 mV peak-to-peak amplitude) during slight tonic contraction of the target muscle (approximately 20% of the isometric MVC) [28,29]. Test MEPs during the protocol were recorded during 20% MVC as an average of in response to 10 single-pulse stimuli applied at 120% of the MEP threshold. A maximal voluntary contraction was performed individually for Q and TA to record 100% EMG activity for each muscle, so that the 20% EMG activity could be estimated and used as a visual feedback to instruct the subjects to maintain that level of muscle contraction. Once the subjects had learnt to contract either the Q or TA muscles at 20% maximal voluntary activation, TMS-evoked MEPs were performed. EMG was recorded using bipolar silver chloride electrodes ($\times 1000$ amplification) filtered with a built-in 20–450 Hz bandpass filter (Signal Conditioning Electrodes v2.3, Delsys Inc., USA). Electrodes were placed over the rectus femoris of the belly of Q muscle and the proximal third region TA following the SENIAM recommendations. Average MEP peak-to-peak amplitude and latency were analyzed as the primary outcome measure of tSCS.

2.4.2. Isometric Maximal Voluntary Contraction Strength

Isometric MVC strength was measured using the hand-held dynamometer Micro Fet 2TM (Hoggan Scientific, LLC, Utah, USA.), a method with intra and inter-rater reliability has been reported [31]. Assessment of isometric MVC strength was performed with participants in either the sitting or supine position to evaluate Q and TA muscles,, respectively. Participants were instructed to hold the side of the table for stabilization and contract the test muscle as hard and as fast as they could against the dynamometer and to maintain the contraction until the assessor requested them to "stop". Each test measured MVC strength during three to five seconds. Three test trials, with a 1 min interval resting period, were performed for each muscle group. Average peak force was used for statistical analysis.

2.5. Statistical Analysis

The sample size was calculated based on Q-MEP amplitude as the main variable. A mean difference of 0.5 mV (approx. 30%) between groups was expected, with a standard deviation of 0.065 was considered from a previous preliminary unpublished report, with a type I error (α) of 0.05 and a power of 80%. A sample size of 15 subjects was calculated.

Statistical analysis was performed using the commercial software package SPSS v22. Assessment of the relative reliability of baseline MEPs for PRE1-PRE2 was achieved with the intraclass correlation coefficient (ICC) that indicates the error in measurements as a proportion of the total variance in scores. An ICC of over 0.90 was defined as a high; from 0.80 to 0.90 as moderate; and below 0.80 as low reliability [32,33]. Moreover, the homogeneity between sessions for MEPs and PRM baseline threshold was analyzed with a t-student test. For statistical analysis of MEPs, the average of PRE1 and PRE 2 was considered as the unique baseline (PRE) measure. A two-way repeated-measures ANOVA, with “time” as one factor (PRE, DURING, POST1, POST2) and the “intervention” factor (tSCS and Sham) was performed to compare differences in MEP amplitude latency. Similarly, a two-way repeated-measures ANOVA was carried out for dynamometer data collected at PRE1 and POST2. A paired *t*-test comparison with a Bonferroni correction for multiple comparisons was used to highlight specific differences between time and interventions. To analyze the blinding outcome variable, James’ Blinding Index (BI) [34] and Bang’s BI [30] were obtained using Stata v15.0 (Stata Corp, TX, USA). James’ BI is used to infer the overall blinding success in RCTs. However, Bang’s BI is used to characterize and evaluate the blinding situation in each trial arm independently. James’ BI ranges from 0 to 1 (0 representing total lack of blinding, 1 representing complete blinding, and 0.5 representing completely random blinding). To interpret the results, this study considered a lack of blinding if the upper bound of the confidence interval (CI) was below 0.5. Bang’s BI can be directly interpreted as the proportion of the unblinding in each arm [30]. It ranges between -1 and 1 , with 0 as a null value indicating the most desirable situation representing random complete blinding. Therefore, when one-sided CI did not cover the 0 value, the study was regarded as lacking blinding.

3. Results

Fifteen participants were recruited and completed the study. Nine were female (60%) and 6 males, with a mean age of 25.2 years old (SD 3.8), weight of 60.9 kg (SD 12.62), height of 1.67 m (SD 0.11), and a mean body mass index of 21.6 (SD 2.47). None of the subjects withdrew from the study (Figure 1. CONSORT flow diagram). No sex significant differences were found when comparing the proportion of the subjects ($p > 0.05$) nor when comparing the effect of tSCS during the stimulation ($p > 0.1$) and after the stimulation ($p > 0.2$) for both Q-MEP and TA-MEP outcomes.

With regard to baseline neurophysiological parameters, mean MEP thresholds for Q ($t = 0.00$, $p = 1.00$) and TA ($t = -0.187$, $p = 0.85$), and PRM reflex threshold ($t = -0.58$, $p = 0.56$) were similar before application of sham or active tSCS (Table 1). The averaged tSCS intensity during the active-tSCS session was 27.6 mA (8.9), that was 27.5% less than the PRM reflex threshold (Table 1). Basal peak-to-peak amplitude MEP measured at PRE1 and PRE2 were highly reliable for both active and sham interventions. During the active tSCS session, a 0.91 ICC was registered for Q-MEP amplitude and a 0.97 ICC for TA-MEP amplitude. During the sham intervention, a 0.89 ICC was registered for Q-MEP amplitude and a 0.97 ICC for TA-MEP amplitude were calculated. Regarding Q-MEP latency, a 0.97 ICC was calculated during the active tSCS session and an ICC of 0.97 during sham intervention. The latency of TA-MEPs showed an ICC of 0.95 during active tSCS intervention and 0.92 during the sham session.

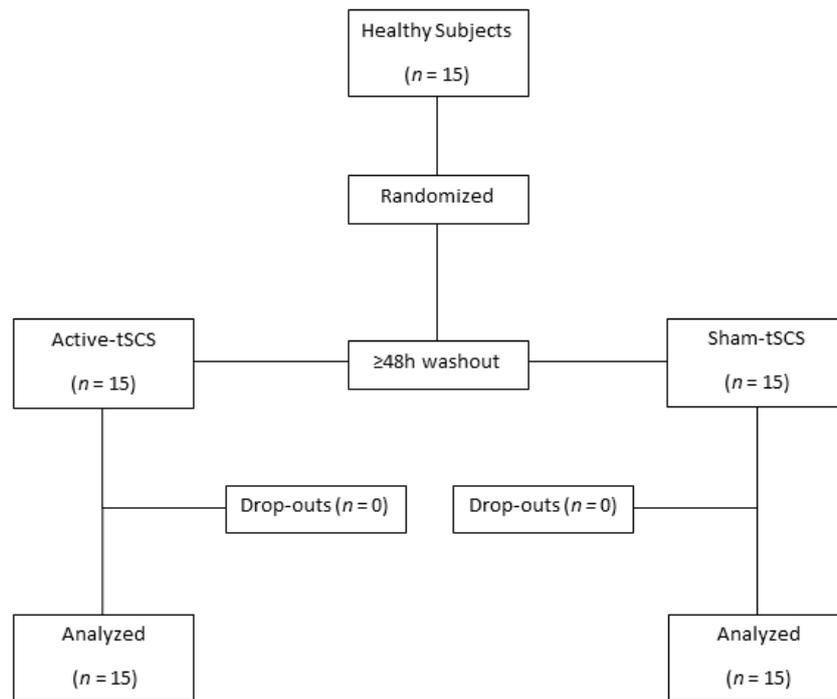


Figure 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram.

Table 1. Neurophysiological assessment at baseline time for each session.

Subject	Active-tSCS Session				Sham-tSCS Session		
	MEP-t Q (%)	MEP-t TA (%)	PRM-t (mA)	Intensity tSCS (mA)	MEP-t Q (%)	MEP-t TA (%)	PRM-t (mA)
01	55	50	45	45	55	55	41
02	31	31	40	25	32	32	40
03	42	35	29	29	35	35	29
04	45	40	33	33	55	44	32
05	50	50	33	33	55	55	33
06	42	42	35	26	40	40	30
07	32	32	50	37	30	30	40
08	32	32	40	21.5	45	35	43
09	47	42	35	14	37	35	29
10	35	35	35	27	33	38	35
11	42	37	41	39	38	33	45
12	35	35	40	17.5	34	34	40
13	38	35	38	22	38	35	38
14	35	35	36	13	35	35	26
15	54	40	44	32	53	42	46
Mean	41	38.1	38.3	27.6	41	38.5	36.5
(SD)	(7.9)	(5.9)	(5.2)	(8.9)	(9.1)	(7.6)	(6.3)

PRM = posterior root-muscle reflex; Q = quadriceps; SD = Standard deviation; -t = threshold; TA = tibialis anterior; tSCS = transcutaneous spinal cord stimulation.

3.1. Effect of tSCS on Peak-To-Peak MEP Amplitude

The effect of active or sham tSCS on MEPs recorded from subject (#7) (Figure 2) is illustrated on the Q and TA muscle (Figure 2), recorded at PRE 2, during the intervention (DURING), and after the intervention finished (POST 1). Mean peak-to-peak amplitude and the change in MEP amplitude DURING and POST tSCS are represented in Figure 3A–D, respectively. In the quantitative analysis, the two-way ANOVA showed no significant differences in Q-MEP amplitude for either the “intervention” factor ($F = 1.178, p = 0.28$) or the “time” factor ($F = 1.176, p = 0.18$). However, a significant “intervention-time” interaction was found for Q-MEP amplitude ($F = 3.88, p = 0.02$). A post-hoc

pairwise comparison of Q-MEP amplitude revealed a significant increase during active-tSCS (1.96 mV; SD: 0.3) when compared to the averaged PRE amplitude (1.40 mV; SD: 0.2; $p = 0.01$). Furthermore, a higher amplitude of Q-MEPs during active-tSCS (1.96 mV; SD: 0.3) was observed when compared to sham-tSCS at the same time-point (1.13 mV; SD: 0.3; $p = 0.03$; Figure 3A). Regarding TA-MEP amplitude, although a greater amplitude during the active tSCS intervention (Figure 3B) was identified (3.06 mV; SD: 2.0) with respect to the PRE baseline amplitude (2.67 mV; SD: 0.4) and higher than the equivalent MEP amplitude during sham tSCS (2.45 mV, SD: 1.1), no statistical significant differences were observed for the factors analyzed (“intervention” $F = 0.268$, $p = 0.609$; nor “time” TA $F = 0.339$, $p = 0.797$; nor “intervention-time” interaction $F = 1.137$, $p = 0.333$).

When the specific effect of each intervention was calculated subtracting the baseline activity in each time-point (Figure 3C,D), the pairwise comparison between groups with the Bonferroni correction revealed a mean difference of 0.73 mV (SD: 0.24; $p = 0.05$) during the stimulation for the Q-MEP, without significant differences for the POST-1 (0.001 mV; SD: 0.19; $p = 0.94$) and POST-2 (0.25 mV; SD: 0.16; $p = 0.13$) time-points (Figure 3C). For the specific effect of the intervention over TA-MEP, a non-significant difference between groups of 0.91 mV (SD: 0.62; $p = 0.15$) was evidenced during the stimulation. Neither significant differences were found for the POST-1 (0.69 mV; SD: 0.62; $p = 0.21$) and POST-2 (0.33 mV; SD: 0.53; $p = 0.53$) time-points (Figure 3D).

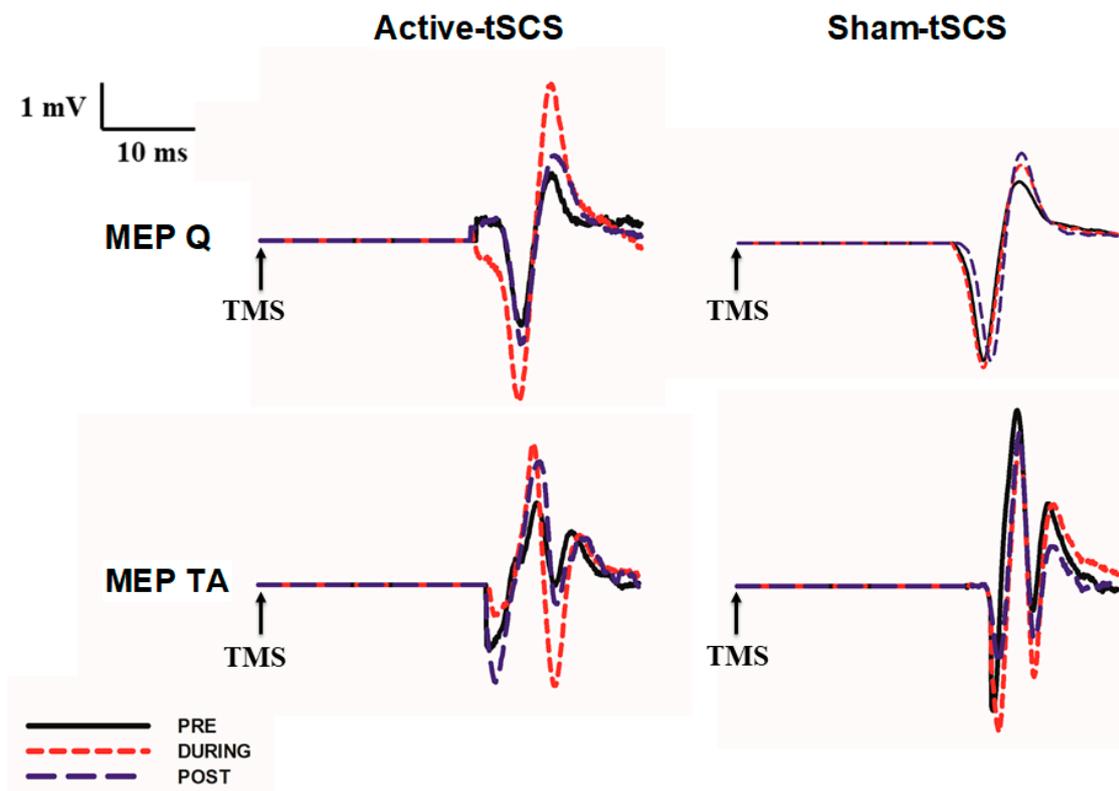


Figure 2. Representative motor-evoked potentials from participant #7 recorded from the Quadriceps and Tibialis Anterior muscles PRE, DURING and POST active or sham tSCS.

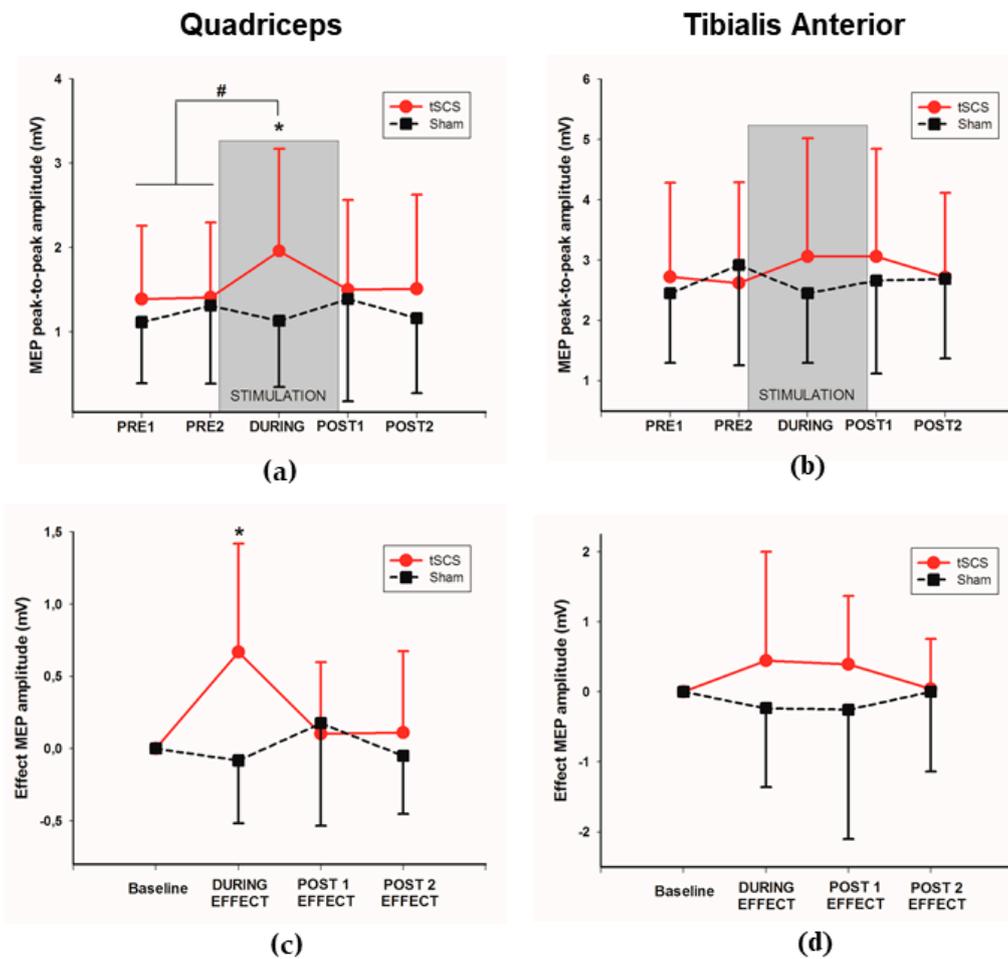


Figure 3. MEP peak-to-peak amplitude: (a) MEP of quadriceps muscle at each evaluation time; (b) MEP of tibialis anterior muscle at each evaluation time; (c) change obtained in MEP of quadriceps during and after tSCS respect to baseline; (d) change obtained in MEP of tibialis anterior during and after tSCS respect to baseline. * $p \leq 0.05$ when compared to sham-tSCS; # $p \leq 0.05$ when compared from baseline.

3.2. Effect on MEP Latency

Table 2 reveals that the application of tSCS intervention had no effect on MEP latencies for the Q and TA muscles. The two-way ANOVA revealed no significant differences neither for the “time” factor (Q muscle: $F = 1.952, p = 0.13$; TA muscle: $F = 0.89, p = 0.45$), “intervention” factor (Q muscle: $F = 0.549, p = 0.46$; TA muscle: $F = 2.621, p = 0.11$), nor “intervention-time” interaction (Q muscle $F = 0.937, p = 0.42$; TA muscle: $F = 0.254, p = 0.86$).

Table 2. Latencies MEPs following active-tSCS or sham-stimulation. Mean and standard deviation (SD).

		PRE 1	PRE 2	DURING	POST 1	POST 2
MEP Q (ms)	Active-tSCS	21.74 (1.98)	21.71 (2.07)	21.77 (2.11)	21.81 (2.01)	21.72 (2.04)
	Sham-tSCS	22.49 (2.78)	22.53 (3.1)	22.70 (2.77)	22.35 (3.04)	22.48 (3.07)
MEP TA (ms)	Active-tSCS	28.83 (1.81)	29.03 (1.7)	28.71 (1.75)	28.77 (1.79)	28.72 (1.67)
	Sham-tSCS	29.54 (2.66)	29.97 (1.97)	29.92 (2.27)	29.72 (2.00)	29.80 (1.86)

3.3. Effect on Isometric Maximal Voluntary Contraction Strength

Table 3 shows the effect of tSCS on mean peak isometric MVC force recorded from the Q and TA muscles before and after the active and sham interventions. No significant differences were observed in MVC strength when pre-post values were compared for active-tSCS and sham-tSCS (Q: $F = 0.583$, $p = 0.45$; TA: $F = 0.268$, $p = 0.60$). In the same way, no significant differences were observed for the “time” factor (Q: $F = 0.017$; $p = 0.89$; TA: $F = 0.339$; $p = 0.797$) nor the “intervention-time” interaction (Q: $F = 0.981$, $p = 0.33$; TA: $F = 0.808$, $p = 0.38$).

Table 3. Isometric maximal voluntary contraction strength following tSCS or Sham. Mean (SD).

	Active-tSCS			Sham-tSCS		
	PRE	POST	% of Change	PRE	POST	% of Change
Strength Q (Kgs)	76.18 (16.62)	77.91 (18.38)	3.10 (13.23) $p = 0.428$	82.84 (17.84)	81.30 (21.50)	-2.32 (11.47) $p = 0.562$
Strength TA (Kgs)	73.46 (11.96)	73.00 (14.69)	-1.09 (6.43) $p = 0.699$	82.84 (17.84)	81.30 (21.50)	1.55 (6.11) $p = 0.392$

Kgs = kilograms; POST = postintervention; PRE = preintervention; Q = quadriceps; SD = standard deviation, TA = tibialis anterior.

3.4. Adverse Effects

No moderate or severe adverse events were reported in any of the participants. Thus, the stimulation was determined as a safe procedure. However, 66.6% ($n = 10$) of participants could not tolerate the optimal intensity previously determined (PRM threshold), considering the intervention as “very uncomfortable”. Two participants reported paraesthesia in lower limb during stimulation. Moreover, two patients reported DOMS (delayed onset muscle soreness) in quadriceps muscle evaluated 24 h after stimulation which could be generated by the voluntary isometric muscle contractions performed during the strength evaluation.

3.5. Blinding and the Assessment of Its Success

The guesses of the participants and assessor are shown in Table 4 in a 2×5 table format (Table 4). Furthermore, Table 5 shows James’ BI and Bang’s BI values obtained for the study subjects and assessor. According to the interpretation of data established by James et al. [34] and Bang et al. [30], when blinding was analyzed globally by James’s BI a lack of blinding was observed in participants and assessor. However, when each treatment arm (Active tSCS and Sham tSCS) was analyzed independently by Bang’s BI a successful blinding was observed for the participants and assessor in the sham group and the active group, respectively.

Table 4. Assessment of the blinding. Absolutes values and percentages of answers selected by participants and assessor.

Assignment	Participants’ Guess, n (%)					Total
	Strongly Active tSCS	Somewhat Active tSCS	Strongly Sham tSCS	Somewhat Sham tSCS	Do not Know	
Active tSCS	9 (30.0)	4 (13.3)	0 (0)	0 (0)	2 (6.7)	15 (50.0)
Sham tSCS	4 (13.3)	4 (13.3)	2 (6.7)	4 (13.3)	1 (3.3)	15 (50.0)
Total	13 (43.3)	8 (26.7)	2 (6.7)	4 (13.3)	3 (10.0)	30 (100.0)
Assignment	Assessor’s Guess, n (%)					Total
	Strongly Active tSCS	Somewhat Active tSCS	Strongly Sham tSCS	Somewhat Sham tSCS	Do not Know	
Active tSCS	3 (10.0)	6 (20.0)	1 (3.3)	3 (10.0)	2 (6.7)	15 (50.0)
Sham tSCS	0 (0.0)	0 (0.0)	4 (13.3)	9 (30.0)	2 (6.7)	15 (50.0)
Total	3 (10.0)	6 (20.0)	5 (16.7)	12 (40.0)	4 (13.3)	30 (100.0)

Table 5. Statistical analysis of blinding assessment.

Participants Results				
Methods	Index	p-Value	95% Confidence Interval	Conclusion
James	0.36	0.031	0.24 to 0.48	Unblinded
Bang-Active/2 × 5	0.73	<0.001	0.58 to 0.89	Unblinded
Bang-Placebo/2 × 5	−0.07	0.63	−0.40 to 0.27	Blinded
Assessor Results				
Methods	Index	p-Value	95% Confidence Interval	Conclusion
James	0.27	0.001	0.14 to 0.40	Unblinded
Bang-Active/2 × 5	0.17	0.18	−0.13 to 0.46	Blinded
Bang-Placebo/2 × 5	0.73	<0.001	0.58 to 0.89	Unblinded

4. Discussion

The present study shows that a single session of 10 min of tSCS increases the peak-to-peak MEP amplitude of Quadriceps muscle, but without a change in muscle strength. Alpha-motoneurons located more distally below the level of tSCS, namely those innervating the TA muscle, did not show any change in MEP amplitude. This is the first study that describes an increase in corticospinal-evoked lower limb motor-evoked potentials after a single session of active-tSCS when compared to a sham tSCS. There is an urgent need to optimize the motor neuromodulatory effect of tSCS in subjects with SCI using neurophysiological measures that detect changes in both cortical and spinal excitability.

A number of studies in healthy individuals have observed that tSCS located over the T10-T11 vertebral level (L1-L2 spinal level) evokes multi-segmental responses from lower limb motoneurons [17,19–21]. However, both the excitation threshold and the motor response magnitude differ according to the specific positioning of the spinal stimulus [5,21,35]. Our study observed a significant increase of MEP amplitude in the Q muscle, which is innervated by the L1-L2 spinal level that corresponded to the level of tSCS. In contrast, the TA MEP amplitude, innervated by the L4-S1 spinal level, was not modulated by tSCS. Computational studies of the effect of tSCS suggest that afferent fibers with the lowest threshold are activated by spinal stimulation focused at the dorsal root entry zone, which is accessible via the intervertebral foramen [1,36–38]. In the case of TA, the afferents that optimally activate this muscle are located more distally to the site of the stimulating electrode. Another potential explanation for no change in TA MEP amplitude with tSCS is that the applied current intensity was insufficient to stimulate the afferents required to facilitate descending drive to the TA muscle, because the intensity was set with reference to the Q PRM reflex threshold. Previous studies have reported a higher PRM reflex threshold for the TA muscle [17,19,20,35]. In this study it is possible that the tSCS current intensity was not optimal to increase corticospinal drive to TA motoneurons.

Motor evoked potentials from the Q and TA muscles were recorded at 20% of MVC so as to reduce the variability due to changes in corticospinal excitability [39]. Knee extensor MEPs are reliably recorded in the active Q [40] in response to single pulse TMS, which permits measurement of MEP amplitude as a standard measure of corticospinal excitability [41]. This is the first study to identify a selective effect of tSCS on Q MEPs compared to distal lower limb muscles such as the TA. Recently, an effect of non-invasive spinal stimulation applied at 30 Hz pulses, with a 5 kHz carrier frequency, has been identified on MEPs recorded from the upper limb muscles following TMS in cases of cervical spinal cord injury [27]. Previously low frequency stimulation of afferents has been shown to increase corticospinal excitability in the upper limb [42]. In the current study tSCS was applied at 30 Hz at the spinal lumbar level, which led to an increase in CST excitability during spinal stimulation. The TA MEP also revealed a non-significant increase in amplitude both during and immediately after spinal stimulation.

Non-invasive spinal stimulation may activate several afferent systems which are known to modulate descending excitation of spinal systems involved in motor control. Activation of the skin of

the anterior aspect of the thigh activates both excitatory and inhibitory spinal interneurons-including transcortical pathways directed to biceps femoris (BF) and Q muscles [43]. Activation of cutaneous afferents within the sural nerve have been shown to facilitate TMS evoked TA muscle activity [44]. Cutaneous afferents are also known to activate transcortical pathways as suggested in studies of people with dorsal column injury or corticospinal tract abnormalities [45], although activation of subcortical pathways may also be possible [27,46]. Cutaneous volleys evoke muscle responses at a transcortical latency followed by a longer-latency excitatory response [47,48] and ongoing voluntary contraction of lower limb muscles can also potentiate this longer-latency excitatory cutaneomuscular response [48].

A role for group I proprioceptive afferents in tSCS-mediated Q muscle facilitation is also possible, especially as spatial facilitation of corticospinal evoked responses is increased with TMS and simultaneous afferent activation following common peroneal nerve activation, mediated at an interneuronal site [49]. Furthermore, during weak voluntary contraction of the Q muscle, common peroneal nerve [50] or femoral nerve [51] group I mediated facilitation of the Q H-reflex was increased. These studies suggest that the main role of descending facilitation of feedback inhibitory interneurons to lumbar propriospinal neurons during weak Quadriceps contraction is to focus corticospinal control of a limited number of motoneurons required for muscle activation.

The increase of the Q MEP amplitude was first detected 4 min after the onset of the 10 min stimulation, and supports previous studies that have observed an immediate increase of voluntary EMG activity, voluntary movement, and gait function in subjects with spinal cord injury as soon as the current is switched on [8,52]. In our study, the increase in peak-to-peak Q and TA MEP amplitude was not maintained up to 2 and 4 min after tSCS. In contrast in a recent study a 20-min single session of tSCS increased the amplitude of subcortical motor evoked responses in arm muscles of people with a high level of SCI for up to 75 min [27]. Moreover, other studies have identified prolonged post intervention effects of tSCS based on improvement of spasticity [13] or the subjective perception of voluntary movement [8]. Other studies have identified improved upper arm function at 3–6 months after 20–30 repeated sessions of tSCS measured with handgrip strength, upper limb EMG activity and an improvement in general functionality (Upper Extremity Motor scores and Action Research Arm Test) [23,53]. These studies applied tSCS for greater than 20 min, which in addition to our study, supports the hypothesis that repeated tSCS interventions of longer than 20 min is required to elicit immediate postintervention effects that lead to an effective therapeutic option.

To date, the vast majority of studies have fixed the intensity of tSCS by using the subjective perception of sensations made by participants or more directly by detecting muscle contraction in response to the stimulus [6]. Recently, Serrano-Munoz et al. [54] demonstrated the practical limitations of adjusting the intensity of stimulation current based on subjective perception and highlighted the importance of defining objective parameters to adjust current intensity. In this study, PRM reflex threshold was used as an objective measure to adjust stimulation intensity. However, only 44% of the participants could tolerate this objectively defined intensity when tSCS was applied at this intensity at 30 Hz (see Table 1). The other participants received a more tolerable stimulus intensity based on their subjective tolerance threshold. Further protocols are necessary to explore a wide range of tSCS frequencies and intensities to improve the tolerance threshold of subjects in order to optimize the effect of neuromodulation on motor function.

The study was performed with the subject in a relaxed supine position, a posture which has been shown to be associated with the most effective tSCS neuromodulatory effect [55]. Indeed, currently the most optimal translation of the tSCS technique to clinical practice is to adopt supine-position stimulation strategies in combination with other activity-based neurorehabilitation tasks. However future studies should examine position-dependent tSCS efficacy using the MEP technique with the subject performing tasks such as sitting or standing, which are more compatible with actual rehabilitation techniques.

Although MEPs evoked by TMS have been demonstrated to be a reliable tool to quantify the excitability of the corticospinal pathway [40,56], it is questionable if MEPs are sensitive enough to reflect functionally relevant changes evoked by the tSCS. In the present study, both TA and Q MEPs

were stable at baseline, and were reproducible when measured at the two pre-evaluation test periods. Stable Q MEPs recorded before tSCS together with the absence of changes during the sham intervention support the significant neuromodulatory effect of active tSCS on Q muscle activity. Furthermore, this finding supports the use of MEPs in future studies designed to optimize tSCS parameters including the specific location of the electrodes.

Sham-controlled interventions have been applied previously to control the effect of peripheral electrical stimulation [28,29] and electrical stimulation of the CNS [57,58]. To our knowledge this is the first study of tSCS where the success of the blinding procedure has been formally assessed using the specialized blinding questionnaire [30], which was completed by both the assessor and the participants. The sham protocol was evaluated as a good method to blind participants who were to receive the sham tSCS, but not when they received active intervention, because 73% of participants correctly guess the assignment. This issue may have arisen due to fact that the previous experience of participants with electrical stimulation was not controlled appropriately, possibly because of the perception of the high stimulus intensity. Previous studies of transcranial direct current stimulation (tDCS) have shown that the application of high intensity electrical stimulation hinders the blinding of participants [59,60]. In contrast, the current study has shown that the blinding procedure was evaluated as good when used to mask the assessor using the active tSCS intervention, but not with sham stimulus application, because the assignment was correctly guessed by the assessor in 73% of participants. Although James's Blinding Index indicated that blinding was not generally effective as assessed by participants and the assessor, the upper 95% CI limit was 0.48 for participants and 0.40 for the assessor, close to the value of 0.5 which is defined as successful. Finally, it is necessary to highlight that crossover studies are inherently difficult to blind compared to more effective parallel design studies. Although further improvement in intervention blinding techniques are still required for the design of future randomized controlled trials, the current sham protocol based on applying only 30 s of active tSCS intensity appears to be a good option to blind both subjects and assessors to the intervention.

No serious side effects were observed after the single 10-min tSCS session in this study. In a recent systematic analysis of the effect of tSCS on motor function after spinal cord injury, this technique has been shown to be painless, well tolerated and safe [6]. However, in our study, eight participants reported discomfort around the electrodes when tSCS commenced. In most cases, this discomfort was related to a strong contraction of abdominal muscle generated by the electrical stimulation. For this reason, 10 participants could not tolerate the intensity of current previously set according to the PRM reflex threshold. Considering that the participants of this study received a single session of tSCS, it is possible that stimulus training sessions are necessary so that participants become accustomed to the tSCS and to improve tolerance. Thus, in a recent study which involved the self-administration of the tSCS applied over 6 week in subjects with spinal cord injury has been shown to be safe and well tolerated in a home-based setting [13]. Future studies should consider employing repeated sessions of tSCS where the intensity is increased incrementally to improve subject tolerance and therefore allowing higher current density and more effective neuromodulation.

5. Conclusions

A single 10 min session of active tSCS applied over the T11-T12 vertebral level increased the quadriceps muscle MEP but not for the TA muscle, when compared to sham stimulation. The neuromodulatory effect of the motor response was specific to the stimulation of the metameric level innervating the Q muscle. In this study we have shown that MEP amplitude is a reliable and sensitive technique to demonstrate changes in the excitability of the corticospinal tract after active tSCS. The used method for sham stimulation could be a feasible option for future tSCS interventions. More randomized sham-controlled clinical trials will be needed to optimize the efficacy of active tSCS to promote motor recovery in subjects with spinal cord injury.

Author Contributions: Conceptualization, J.G.-S., D.S.-M. and J.T.; methodology, Á.M.-G., J.G.-S., D.S.-M. and J.T.; formal analysis, Á.M.-G. and J.A.-C.; investigation, Á.M.-G. and N.C.-S.; writing—original draft preparation, Á.M.-G., D.S.-M. and J.G.-S.; writing—review and editing, Á.M.-G., D.S.-M., J.G.-S., J.T. and J.A.-C.; visualization, D.S.-M., N.C.-S. and J.A.-C.; supervision, J.G.-S. and J.T.; project administration, J.G.-S. and J.T.; funding acquisition, J.G.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the “Instituto de Salud Carlos III” and “Fondos FEDER” (NEUROTRAIN project, grant number PI17/00581). DSM has received funding by the European Regional Development Fund (2019/7375).

Acknowledgments: We are grateful to all volunteers for their altruism to participate in this study.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Danner, S.M.; Hofstoetter, U.S.; Ladenbauer, J.; Rattay, F.; Minassian, K. Can the human lumbar posterior columns be stimulated by transcutaneous spinal cord stimulation? A modeling study. *Artif. Organs* **2011**, *35*, 257–262. [[CrossRef](#)] [[PubMed](#)]
2. Gorodnichev, R.M.; Pivovarova, E.A.; Puhov, A.; Moiseev, S.A.; Savochin, A.A.; Moshonkina, T.R.; Chsherbakova, N.A.; Kilimnik, V.A.; Selionov, V.A.; Kozlovskaya, I.B. Transcutaneous electrical stimulation of the spinal cord: A noninvasive tool for the activation of stepping pattern generators in humans. *Hum. Physiol.* **2012**, *38*, 158–167. [[CrossRef](#)]
3. Hofstoetter, U.S.; Freundl, B.; Binder, H.; Minassian, K. Common neural structures activated by epidural and transcutaneous lumbar spinal cord stimulation: Elicitation of posterior root-muscle reflexes. *PLoS ONE* **2018**, *13*, e0192013. [[CrossRef](#)] [[PubMed](#)]
4. Minassian, K.; Persy, I.; Rattay, F.; Dimitrijevic, M.R.; Hofer, C.; Kern, H. Posterior root-muscle preflexes elicited by transcutaneous stimulation of the human lumbosacral cord. *Muscle Nerve* **2007**, *35*, 327–336. [[CrossRef](#)] [[PubMed](#)]
5. Kitano, K.; Koceja, D.M. Spinal reflex in human lower leg muscles evoked by transcutaneous spinal cord stimulation. *J. Neurosci. Methods* **2009**, *180*, 111–115. [[CrossRef](#)]
6. Megía García, A.; Serrano-Muñoz, D.; Taylor, J.; Avendaño-Coy, J.; Gómez-Soriano, J. Transcutaneous Spinal Cord Stimulation and Motor Rehabilitation in Spinal Cord Injury: A Systematic Review. *Neurorehabil. Neural Repair* **2020**, *34*, 3–12. [[CrossRef](#)]
7. Hofstoetter, U.S.; Hofer, C.; Kern, H.; Danner, S.M.; Mayr, W.; Dimitrijevic, M.R.; Minassian, K. Effects of transcutaneous spinal cord stimulation on voluntary locomotor activity in an incomplete spinal cord injured individual. *Biomed. Tech.* **2013**. [[CrossRef](#)]
8. Hofstoetter, U.S.; Krenn, M.; Danner, S.M.; Hofer, C.; Kern, H.; McKay, W.B.; Mayr, W.; Minassian, K. Augmentation of Voluntary Locomotor Activity by Transcutaneous Spinal Cord Stimulation in Motor-Incomplete Spinal Cord-Injured Individuals. *Artif. Organs* **2015**, *39*, 176–186. [[CrossRef](#)]
9. Rath, M.; Vette, A.H.; Ramasubramaniam, S.; Li, K.; Burdick, J.; Edgerton, V.R.; Gerasimenko, Y.P.; Sayenko, D.G. Trunk stability enabled by noninvasive spinal electrical stimulation after spinal cord injury. *J. Neurotrauma* **2018**, *35*, 2540–2553. [[CrossRef](#)]
10. Sayenko, D.G.; Rath, M.; Ferguson, A.R.; Burdick, J.W.; Havton, L.A.; Edgerton, V.R.; Gerasimenko, Y.P. Self-assisted standing enabled by non-invasive spinal stimulation after spinal cord injury. *J. Neurotrauma* **2019**, *36*, 1435–1450. [[CrossRef](#)]
11. Minassian, K.; Hofstoetter, U.S.; Danner, S.M.; Mayr, W.; Bruce, J.A.; McKay, W.B.; Tansey, K.E. Spinal rhythm generation by step-induced feedback and transcutaneous posterior root stimulation in complete spinal cord-injured individuals. *Neurorehabil. Neural Repair* **2016**. [[CrossRef](#)] [[PubMed](#)]
12. Gad, P.; Gerasimenko, Y.; Zdunowski, S.; Turner, A.; Sayenko, D.; Lu, D.C.; Edgerton, V.R. Weight bearing over-ground stepping in an exoskeleton with non-invasive spinal cord neuromodulation after motor complete paraplegia. *Front. Neurosci.* **2017**, *11*, 333. [[CrossRef](#)] [[PubMed](#)]
13. Hofstoetter, U.S.; Freundl, B.; Danner, S.M.; Krenn, M.J.; Mayr, W.; Binder, H.; Minassian, K. Transcutaneous Spinal Cord Stimulation Induces Temporary Attenuation of Spasticity in Individuals with Spinal Cord Injury. *J. Neurotrauma* **2020**, *37*, 481–493. [[CrossRef](#)]

14. Hofstoetter, U.S.; McKay, W.B.; Tansey, K.E.; Mayr, W.; Kern, H.; Minassian, K. Modification of spasticity by transcutaneous spinal cord stimulation in individuals with incomplete spinal cord injury. *J. Spinal Cord Med.* **2014**, *37*, 202–211. [[CrossRef](#)]
15. MacKay-Lyons, M. Central pattern generation of locomotion: A review of the evidence. *Phys. Ther.* **2002**, *82*, 69–83. [[CrossRef](#)]
16. Sabbahi, M.A.; Sengul, Y.S. Thoracolumbar multisegmental motor responses in the upper and lower limbs in healthy subjects. *Spinal Cord* **2011**. [[CrossRef](#)] [[PubMed](#)]
17. Krenn, M.; Hofstoetter, U.S.; Danner, S.M.; Minassian, K.; Mayr, W. Multi-Electrode Array for Transcutaneous Lumbar Posterior Root Stimulation. *Artif. Organs* **2015**, *39*, 834–840. [[CrossRef](#)] [[PubMed](#)]
18. Bocci, T.; Caleo, M.; Tognazzi, S.; Francini, N.; Briscese, L.; Maffei, L.; Rossi, S.; Priori, A.; Sartucci, F. Evidence for metaplasticity in the human visual cortex. *J. Neural Transm.* **2014**, *121*, 221–231. [[CrossRef](#)]
19. Sayenko, D.G.; Atkinson, D.A.; Dy, C.J.; Gurley, K.M.; Smith, V.L.; Angeli, C.; Harkema, S.J.; Edgerton, V.R.; Gerasimenko, Y.P. Spinal segment-specific transcutaneous stimulation differentially shapes activation pattern among motor pools in humans. *J. Appl. Physiol.* **2015**. [[CrossRef](#)]
20. Krenn, M.; Toth, A.; Danner, S.M.; Hofstoetter, U.S.; Minassian, K.; Mayr, W. Selectivity of transcutaneous stimulation of lumbar posterior roots at different spinal levels in humans. *Biomed. Tech.* **2013**. [[CrossRef](#)]
21. Gerasimenko, Y.; Gorodnichev, R.; Puhov, A.; Moshonkina, T.; Savochin, A.; Selionov, V.; Roy, R.R.; Lu, D.C.; Edgerton, V.R. Initiation and modulation of locomotor circuitry output with multisite transcutaneous electrical stimulation of the spinal cord in noninjured humans. *J. Neurophysiol.* **2015**, *113*, 834–842. [[CrossRef](#)] [[PubMed](#)]
22. Gad, P.; Lee, S.; Terrafranca, N.; Zhong, H.; Turner, A.; Gerasimenko, Y.; Edgerton, V.R. Non-invasive activation of cervical spinal networks after severe paralysis. *J. Neurotrauma* **2018**, *35*, 2145–2158. [[CrossRef](#)] [[PubMed](#)]
23. Freyvert, Y.; Yong, N.A.; Morikawa, E.; Zdunowski, S.; Sarino, M.E.; Gerasimenko, Y.; Edgerton, V.R.; Lu, D.C. Engaging cervical spinal circuitry with non-invasive spinal stimulation and buspirone to restore hand function in chronic motor complete patients. *Sci. Rep.* **2018**, *8*, 1–10. [[CrossRef](#)] [[PubMed](#)]
24. Hofstoetter, U.S.; Freundl, B.; Binder, H.; Minassian, K. Recovery cycles of posterior root-muscle reflexes evoked by transcutaneous spinal cord stimulation and of the H reflex in individuals with intact and injured spinal cord. *PLoS ONE* **2019**, *14*, 1–20. [[CrossRef](#)] [[PubMed](#)]
25. Hofstoetter, U.S.; Minassian, K.; Hofer, C.; Mayr, W.; Rattay, F.; Dimitrijevic, M.R. Modification of reflex responses to lumbar posterior root stimulation by motor tasks in healthy subjects. *Artif. Organs* **2008**, *32*, 644–648. [[CrossRef](#)] [[PubMed](#)]
26. Andrews, J.C.; Stein, R.B.; Roy, F.D. Post-activation depression in the human soleus muscle using peripheral nerve and transcutaneous spinal stimulation. *Neurosci. Lett.* **2015**, *589*, 144–149. [[CrossRef](#)]
27. Benavides, F.D.; Jo, H.J.; Lundell, H.; Edgerton, V.R.; Gerasimenko, Y.; Perez, M.A. Cortical and Subcortical Effects of Transcutaneous Spinal Cord Stimulation in Humans with Tetraplegia. *J. Neurosci.* **2020**, *40*, 2633–2643. [[CrossRef](#)]
28. Petrie, J.; Hazleman, B. Credibility of placebo transcutaneous nerve stimulation and acupuncture. *Clin. Exp. Rheumatol.* **1985**, *3*, 151–153.
29. Deyo, R.A.; Walsh, N.E.; Schoenfeld, L.S.; Ramamurthy, S. Can trials of physical treatments be blinded? The example of transcutaneous electrical nerve stimulation for chronic pain. *Am. J. Phys. Med. Rehabil.* **1990**, *69*, 6–10. [[CrossRef](#)]
30. Bang, H.; Flaherty, S.P.; Kolahi, J.; Park, J. Blinding assessment in clinical trials: A review of statistical methods and a proposal of blinding assessment protocol. *Clin. Res. Regul. Aff.* **2010**, *27*, 42–51. [[CrossRef](#)]
31. Mentiplay, B.F.; Perraton, L.G.; Bower, K.J.; Adair, B.; Pua, Y.-H.; Williams, G.P.; McGaw, R.; Clark, R.A. Assessment of lower limb muscle strength and power using hand-held and fixed dynamometry: A reliability and validity study. *PLoS ONE* **2015**, *10*, e0140822. [[CrossRef](#)]
32. Hopkins, W.G. Sports Medicine 30: 1–15 July 2000. *Sport. Med.* **2000**, *30*, 1–15. [[CrossRef](#)]
33. Abián-Vicén, J.; Aparicio-García, C.; Ruiz-Lázaro, P.; Simón-Martínez, C.; Bravo-Esteban, E.; Gómez-Soriano, J. Test-retest reliability and responsiveness of a comprehensive protocol for the assessment of muscle tone of the ankle plantar flexors in healthy subjects. *Isokinet. Exerc. Sci.* **2019**, *27*, 107–115. [[CrossRef](#)]

34. James, K.E.; Bloch, D.A.; Lee, K.K.; Kraemer, H.C.; Fuller, R.K. An index for assessing blindness in a multi-centre clinical trial: Disulfiram for alcohol cessation—A VA cooperative study. *Stat. Med.* **1996**, *15*, 1421–1434. [[CrossRef](#)]
35. Knikou, M. Neurophysiological characterization of transspinal evoked potentials in human leg muscles. *Bioelectromagnetics* **2013**. [[CrossRef](#)] [[PubMed](#)]
36. Hofstoetter, U.S.; Danner, S.M.; Minassian, K. Paraspinal Magnetic and Transcutaneous Electrical Stimulation. *Encycl. Comput. Neuroscience*. **2014**. [[CrossRef](#)]
37. Capogrosso, M.; Wenger, N.; Raspopovic, S.; Musienko, P.; Beuparlant, J.; Luciani, L.B.; Courtine, G.; Micera, S. A computational model for epidural electrical stimulation of spinal sensorimotor circuits. *J. Neurosci.* **2013**, *33*, 19326–19340. [[CrossRef](#)]
38. Ladenbauer, J.; Minassian, K.; Hofstoetter, U.S.; Dimitrijevic, M.R.; Rattay, F. Stimulation of the human lumbar spinal cord with implanted and surface electrodes: A computer simulation study. *IEEE Trans. Neural Syst. Rehabil. Eng.* **2010**, *18*, 637–645. [[CrossRef](#)]
39. Darling, W.G.; Wolf, S.L.; Butler, A.J. Variability of motor potentials evoked by transcranial magnetic stimulation depends on muscle activation. *Exp. Brain Res.* **2006**, *174*, 376–385. [[CrossRef](#)]
40. O’leary, T.J.; Morris, M.G.; Collett, J.; Howells, K. Reliability of single and paired-pulse transcranial magnetic stimulation in the vastus lateralis muscle. *Muscle Nerve* **2015**, *52*, 605–615. [[CrossRef](#)]
41. Burke, M.J.; Fried, P.J.; Pascual-Leone, A. Transcranial magnetic stimulation: Neurophysiological and clinical applications. In *Handbook of Clinical Neurology*; Elsevier: Amsterdam, The Netherlands, 2019; Volume 163, pp. 73–92. ISBN 0072-9752.
42. Kaelin-Lang, A.; Luft, A.R.; Sawaki, L.; Burstein, A.H.; Sohn, Y.H.; Cohen, L.G. Modulation of human corticomotor excitability by somatosensory input. *J. Physiol.* **2002**, *540*, 623–633. [[CrossRef](#)] [[PubMed](#)]
43. Hagbarth, K.E.; Finer, B.L. The plasticity of human withdrawal reflexes to noxious skin stimuli in lower limbs. In *Progress in Brain Research*; Elsevier: Amsterdam, The Netherlands, 1963; Volume 1, pp. 65–81. ISBN 0079-6123.
44. Nielsen, J.; Petersen, N.; Fedirchuk, B. Evidence suggesting a transcortical pathway from cutaneous foot afferents to tibialis anterior motoneurons in man. *J. Physiol.* **1997**, *501*, 473–484. [[CrossRef](#)] [[PubMed](#)]
45. Jenner, J.R.; Stephens, J.A. Cutaneous reflex responses and their central nervous pathways studied in man. *J. Physiol.* **1982**, *333*, 405–419. [[CrossRef](#)] [[PubMed](#)]
46. Shimamura, M.; Mori, S.; Yamauchi, T. Effects of spino-bulbo-spinal reflex volleys on extensor motoneurons of hindlimb in cats. *J. Neurophysiol.* **1967**, *30*, 319–332. [[CrossRef](#)] [[PubMed](#)]
47. Aniss, A.M.; Gandevia, S.C.; Burke, D. Reflex responses in active muscles elicited by stimulation of low-threshold afferents from the human foot. *J. Neurophysiol.* **1992**, *67*, 1375–1384. [[CrossRef](#)]
48. Gibbs, J.; Harrison, L.M.; Stephens, J.A. Cutaneomuscular reflexes recorded from the lower limb in man during different tasks. *J. Physiol.* **1995**, *487*, 237–242. [[CrossRef](#)]
49. Marchand-Pauvert, V.; Simonetta-Moreau, M.; Pierrot-Deseilligny, E. Cortical control of spinal pathways mediating group II excitation to human thigh motoneurons. *J. Physiol.* **1999**, *517*, 301–313. [[CrossRef](#)]
50. Forget, R.; Hultborn, H.; Meunier, S.; Pantieri, R.; Pierrot-Deseilligny, E. Facilitation of quadriceps motoneurons by group I afferents from pretibial flexors in man. 2. Changes occurring during voluntary contraction. *Exp. Brain Res.* **1989**, *78*, 21–27. [[CrossRef](#)]
51. Hultborn, H.; Meunier, S.; Pierrot-Deseilligny, E.; Shindo, M. Changes in polysynaptic Ia excitation to quadriceps motoneurons during voluntary contraction in man. *Exp. Brain Res.* **1986**, *63*, 436–438. [[CrossRef](#)]
52. Gerasimenko, Y.P.; Lu, D.C.; Modaber, M.; Zdunowski, S.; Gad, P.; Sayenko, D.G.; Morikawa, E.; Haakana, P.; Ferguson, A.R.; Roy, R.R.; et al. Noninvasive Reactivation of Motor Descending Control after Paralysis. *J. Neurotrauma* **2015**, *32*, 1968–1980. [[CrossRef](#)]
53. Inanici, F.; Samejima, S.; Gad, P.; Edgerton, V.R.; Hofstetter, C.P.; Moritz, C.T. Transcutaneous electrical spinal stimulation promotes long-term recovery of upper extremity function in chronic tetraplegia. *IEEE Trans. Neural Syst. Rehabil. Eng.* **2018**, *26*, 1272–1278. [[CrossRef](#)] [[PubMed](#)]
54. Serrano-Munoz, D.; Gómez-Soriano, J.; Bravo-Esteban, E.; Vazquez-Farinas, M.; Taylor, J.; Avendano-Coy, J. Intensity matters: Therapist-dependent dose of spinal transcutaneous electrical nerve stimulation. *PLoS ONE* **2017**, *12*, e0189734. [[CrossRef](#)] [[PubMed](#)]

55. Danner, S.M.; Krenn, M.; Hofstoetter, U.S.; Toth, A.; Mayr, W.; Minassian, K. Body position influences which neural structures are recruited by lumbar transcutaneous spinal cord stimulation. *PLoS ONE* **2016**. [[CrossRef](#)] [[PubMed](#)]
56. Groppa, S.; Oliviero, A.; Eisen, A.; Quartarone, A.; Cohen, L.G.; Mall, V.; Kaelin-Lang, A.; Mima, T.; Rossi, S.; Thickbroom, G.W. A practical guide to diagnostic transcranial magnetic stimulation: Report of an IFCN committee. *Clin. Neurophysiol.* **2012**, *123*, 858–882. [[CrossRef](#)]
57. Geiger, M.; Supiot, A.; Zory, R.; Aegerter, P.; Pradon, D.; Roche, N. The effect of transcranial direct current stimulation (tDCS) on locomotion and balance in patients with chronic stroke: Study protocol for a randomised controlled trial. *Trials* **2017**, *18*, 492. [[CrossRef](#)]
58. Straudi, S.; Buja, S.; Baroni, A.; Pavarelli, C.; Pranovi, G.; Fregni, F.; Basaglia, N. The effects of transcranial direct current stimulation (tDCS) combined with group exercise treatment in subjects with chronic low back pain: A pilot randomized control trial. *Clin. Rehabil.* **2018**, *32*, 1348–1356. [[CrossRef](#)]
59. O’connell, N.E.; Cossar, J.; Marston, L.; Wand, B.M.; Bunce, D.; Moseley, G.L.; De Souza, L.H. Rethinking clinical trials of transcranial direct current stimulation: Participant and assessor blinding is inadequate at intensities of 2mA. *PLoS ONE* **2012**, *7*, e47514. [[CrossRef](#)]
60. Palm, U.; Reisinger, E.; Keeser, D.; Kuo, M.-F.; Pogarell, O.; Leicht, G.; Mulert, C.; Nitsche, M.A.; Padberg, F. Evaluation of sham transcranial direct current stimulation for randomized, placebo-controlled clinical trials. *Brain Stimul.* **2013**, *6*, 690–695. [[CrossRef](#)]



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).