RESEARCH REPORT

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Effect of posture and body weight loading on spinal posterior root reflex responses

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Abstract

Abstract

The posterior root muscle response (PRM) is a monosynaptic reflex that is evoked by single pulse transcutaneous spinal cord stimulation (tSCS). The main aim of this work was to analyse how body weight loading influences PRM reflex threshold measured from several lower limb muscles in healthy participants. PRM reflex responses were evoked with 1-ms rectangular monophasic pulses applied at an interval of 6 s via a self-adhesive electrode (9 × 5 cm) at the T11–T12 vertebral level. Surface electromyographic activity of lower limb muscles was recorded during four different conditions, one in decubitus supine (DS) and the other three involving standing at 100%, 50%, and 0% body weight loading (BW). PRM threshold intensity, peak-to-peak amplitude, and latency for each muscle were analysed in different conditions study. PRM reflex threshold increased with body weight unloading compared with DS, and the largest change was observed between DS and 0% BW for the proximal muscles and between DS and 50% BW for distal muscles. Peak-to-peak amplitude analysis showed only a significant mean decrease of 34.6% (SD 10.4, $p = 0.028$) in TA and 53.6% (SD 15.1, $p = 0.019$) in GM muscles between DS and 50% BW. No significant differences were observed for PRM latency. This study has shown that sensorimotor networks can be activated

Abbreviations: BF, biceps femoris; BW, body weight loading; DS, decubitus supine; EMG, electromyography; GM, gastrocnemius medialis; H-reflex, Hoffmann reflex; PRM, posterior root muscle response; RF, rectus femoris; SD, standard deviation; TA, tibialis anterior; tSCS, transcutaneous spinal cord stimulation.

with tSCS in various conditions of body weight unloading. Higher stimulus intensities are necessary to evoke reflex response during standing at 50% body weight loading. These results have practical implications for gait rehabilitation training programmes that include body weight support.

KEYWORDS

body weight support, neuromodulation, posterior root muscle reflex, spinal stimulation, stimulation threshold, transcutaneous spinal cord stimulation

1 | INTRODUCTION

Transcutaneous spinal cord stimulation (tSCS) selectively depolarises sensory fibres in the posterior roots at the level of stimulation (Krenn et al., 2013, 2015; Minassian et al., 2007). Single tSCS pulses also evoke posterior root-muscle (PRM) reflex responses within the target muscle. The PRM response is a monosynaptic reflex evoked by the direct stimulation of large-diameter afferent fibres (Ia, Ib, and II) within the spinal dorsal roots (Minassian et al., 2007). Many studies have demonstrated that the PRM reflex (Danner et al., 2016; Hofstoetter et al., 2008, 2018; Krenn et al., 2013, 2015; Minassian et al., 2007) can be generated by tSCS with surface electrodes applied to the middle of the back at thoracolumbar spinal level. Mathematical computational models to find the optimal neural stimulation site activated by tSCS have revealed that the posterior dorsal root entry is the first structure to be depolarised at low activation intensities. Evidence that tSCS may also modulate spinal reflex function has also been demonstrated during both passive and active muscle movement and also during experimental paradigms designed to evaluate reflex inhibition induced either by tendon vibration or paired pulse stimulation (Minassian et al., 2007).

The PRM reflex response has proposed to be similar to the Hoffmann reflex (H-reflex) (Krenn et al., 2013; Minassian et al., 2007). However, the advantage of evaluating PRM reflexes is that the tSCS-evoked muscle responses can be recorded simultaneously from several agonist and antagonist muscles innervated by the same spinal level of stimulation (Minassian et al., 2007; Sabbahi & Sengul, 2011; Saito et al., 2020). This reflex testing method provides a neurophysiological test to both confirm the activation of spinal motor control pathways of multiple lower limb muscles and also to measure changes in spinal excitability following tSCS intervention.

Hofstoetter et al. (2018) showed that tSCS-activated motor responses are identical to those evoked with epidural stimulation with respect to electromyographic characteristics, such as waveform and peak-to-peak amplitude. Both stimulation techniques evoke reflex responses from multiple lower limb muscles simultaneously (Krenn et al., 2015) and that the responses can be modulated by active muscle contraction (Hofstoetter et al., 2008) and posture (Danner et al., 2016). Several central modulatory mechanisms have been postulated to mediate changes in spinal excitability following tSCS (Knikou, 2014), through monosynaptic or oligosynaptic circuits (Capogrosso et al., 2013; Danner et al., 2011). In this regard, the PRM reflex is similar to the H-reflex, in that muscle response is depressed by tendon vibration, antagonist muscle contraction, and paired-pulse stimulation (which activates post-activation depression) (Minassian et al., 2007).

The therapeutic possibilities of tSCS for motor rehabilitation have also been explored in subjects with neurological impairments. Thus, tonic tSCS (biphasic square current, 30–50 Hz, 1 ms) has been shown to enhance motor evoked potential (Megía-García et al., 2020), voluntary motor activity (Hofstoetter et al., 2013, 2015), trunk stability (Rath et al., 2018), self-assisted standing (Sayenko et al., 2019), gait function (Gad et al., 2017; Hofstoetter et al., 2013, 2015), hand strength and dexterity (Freyvert et al., 2018; Inanici et al., 2018) and to reduce spasticity in people with spinal cord injury (Hofstoetter et al., 2014, 2020). However, the clinical effectiveness of this technique is still undetermined, and the stimulation parameters need to be optimized (Megía García et al., 2020).

Both the location and intensity of the tSCS current are key parameters to evoke an effective stimulation of the spinal cord (Capogrosso et al., 2013; Danner et al., 2011). Most of the studies performing tSCS have defined the intensity of stimulation using subjective or ambiguous definitions based either on the perception of the current by participants (i.e., paraesthesia) or by visual observations of muscle contraction (Megía García et al., 2020). Recently, Serrano-Munoz et al. (2017) showed that, when therapists set the intensity of the tSCS current based on the perception of the stimulus, the actual stimulus intensity was highly variable, which in turn had a direct impact on the therapeutic effect. For

this reason, it is necessary to develop objective methods to find the optimal stimulus intensity to promote improved motor outcomes with tSCS intervention. One objective approach to define the tSCS intensity could be to use the PRM reflex threshold as a reference. The intensity at which a reflex response threshold is recorded has been defined in related tSCS studies as the lowest stimulus intensity required to evoke a muscle response (Danner et al., 2016; Hofstoetter et al., 2008; Saito et al., 2019). The PRM reflex response has a moderate to high interday reliability (Saito et al., 2019), which may also be subject to central and peripheral modulatory mechanisms. Importantly, Hofstoetter et al. (2008, 2014) showed that the threshold and amplitude of the PRM reflex response also depend on the position of the body. However, the influence of body weight loading, used in several gait training rehabilitation programmes, is unknown. The characterisation of body loading on PRM reflex threshold, and by extension standardisation of tSCS intensity, is relevant when spinal stimulation intervention is combined with gait training using body weight support systems.

The main objective of the present study is to analyse how body weight loading influences PRM reflex threshold from several lower limb muscles in healthy participants. Additionally, as a secondary objective, the study determines the spinal excitability changes induced by body weight loading by analysing PRM threshold, peak-to-peak amplitude, and latency. This study provides relevant information necessary to optimize tSCS parameters for its application for motor rehabilitation in future studies of neurological disorders.

2 | METHODS

2.1 | Population study

This population study was composed of 15 healthy volunteers without injury to the central or peripheral nervous system: eight women with a mean age of 24.5 years old (SD 2.3), a mean weight of 59.3 kg (SD 4.5), a mean height of 1.63 m (SD 0.1), and a mean body mass index of 22.4 (SD 2.1); seven men with a mean age of 26.1 years old (SD 5.2), a mean weight of 73.6 kg (SD 9.8), a mean height of 1.74 m (SD 0.05), and a mean body mass index of 24.3 (SD 2.5). Subjects were recruited after they met the exclusion criteria: nervous systems disorders, musculoskeletal pathology in lower limbs, metal or electronics implants, medications that influence neural excitability (antiepileptic, antipsychotics, or antidepressants), allergy to the electrode material, epilepsy, and pregnancy. All subjects signed informed consent and the study was approved by the Ethics Committee of Toledo, Spain (412-31/07/2019).

2.2 | Stimulation protocol

Posterior root muscle (PRM) reflex responses were evoked with 1-ms rectangular monophasic pulse applied at an interval of 6 s (Digitimer DS7A Current Stimulator). A self-adhesive electrode (9 × 5 cm) (ValuTrode, Axelgaard Manufacturing Co, LTD, Fallbrook, USA) was placed on the middle vertebral skin between the T11 and T12 spinous processes. Two interconnected electrodes (9 × 5 cm) were placed symmetrically on the abdomen. The spinal electrode acted as the cathode and abdominal electrodes as the anode. A rectangular foam was placed to add extra pressure on the spinal electrode, and this was held in place with a bandage to improve skin contact.

PRM reflex responses were evoked during four different conditions (Figure 1): (i) decubitus supine (DS), (ii) standing with total body weight loading (100% BW), (iii) standing with half body weight loading (50% BW), and (iv) standing without body weight loading (0% BW). The order of the conditions was randomized (www.randomizer.org) for each participant. Unloading of body weight in study participants was carried out using a suspension system constructed with a square metal structure, with three pulleys, a safety lock, and one commercial axial harness (Petzl F38920 Crolles). Body weight unloading was monitored using a weight balance scale. The same protocol was followed for all conditions. For supine condition, subjects were placed in a comfortable position, so that their legs were relaxed without voluntary muscle contraction. For standing condition, the participants were instructed to maintain a straight upright position, and they held onto and anterior support structure to control the centre of mass and stabilize the body during standing. Muscle activity was recorded as electromyograph (EMG) activity, and the upright standing position was monitored with feedback from the examiners. To identify the PRM reflex threshold response from a specific muscle, the intensity was increased in 5-mA steps, starting at 5 mA and increased until a specific contraction was evoked in the muscle. The stimulation intensity was then adjusted with 1-mA increments or decrements to identify the PMR reflex threshold (Saito et al., 2019). As with previous studies, the threshold intensity for each muscle was defined as the lowest stimulus intensity to evoke at least 5/10 muscle responses with a peak-to-peak amplitude ≥100 µV (Danner et al., 2016; Hofstoetter et al., 2008; Saito et al., 2019). The maximal stimulation intensity available was 100 mA.
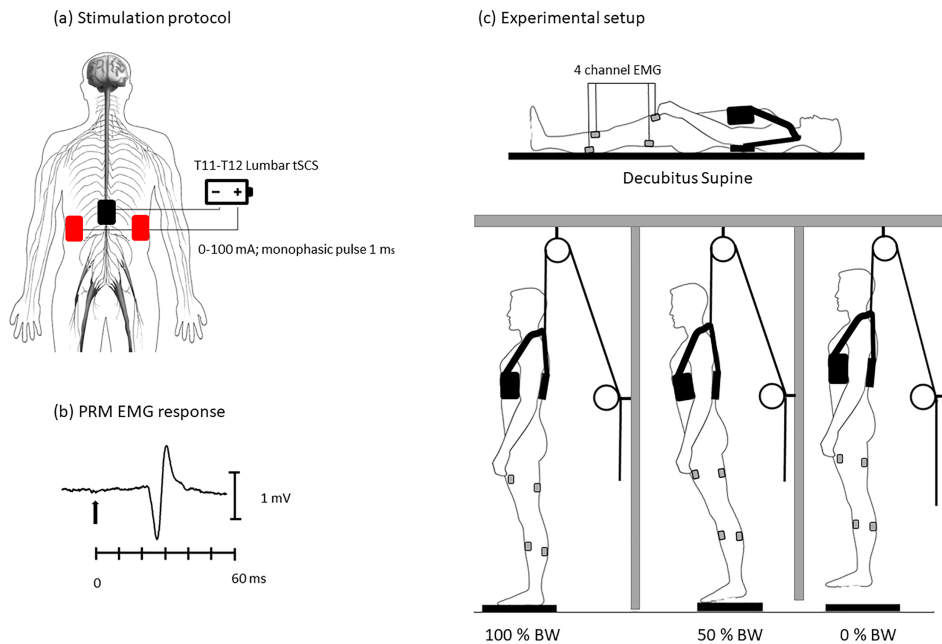
FIGURE 1 Schematic of transcutaneous spinal cord stimulation (tSCS), example posterior root muscle (PRM) response and setup for reflex responses in the supine and standing position at different body weights (loading). Schematic of the application of 1-ms tSCS pulses applied at the T11–T12 spinal level at a current intensity of 0–100 mA (a). An example of the PRM reflex electromyogram (EMG) responses evoked in the tibialis anterior (TA) muscle from participant 13# (b). PRM reflex responses were recorded in participants placed in the supine position, or standing with 100% body weight (100% BW), 50% body weight (50% BW), or 0% body weight (0% BW); surface electromyograph (EMG) electrodes were placed over the rectus femoris, biceps femoris, tibialis anterior, and gastrocnemius medialis (c)

Finally, to analyse PRM modulation, 10 single-pulse stimuli were applied at 110% of the PRM threshold, which was subsequently recorded for each muscle during the four studied conditions. The PRM reflex responses were recorded independently from each recorded muscle using different trials. The averaged PRM reflex peak-to-peak amplitude and latency were also analysed.

2.3 | EMG activity recording

Surface EMG activity was recorded using bipolar silver chloride electrodes (×1000 amplification) and filtered 20–450 Hz bandpass filter (Signal Conditioning Electrodes v2.3, Delsys Inc., USA). Electrodes were placed over the rectus femoris (RF), biceps femoris (BF), tibialis anterior (TA), and gastrocnemius medialis (GM) of the dominant lower limb, following the SENIAM guidelines (Surface Electromyography for Non-invasive Assessment of Muscles). Specifically, the EMG electrode locations were, for the RF muscle, halfway 1/3 on a line from the superior anterior iliac spine and the superior part of the patella; for BF, over the muscle identified with a voluntary contraction, between the greater trochanter and the lateral epicondyle of the femur; for TA, at the proximal 1/3 of the muscle belly on the line between the fibular

head and the medial malleolus; and for GM, over the medial heads of the gastrocnemius muscle which was identified with a voluntary contraction. Before fixture of the EMG electrodes, the skin was cleaned with alcohol and sandpaper (Trace Prep 2236. Red Dot. Ontario, Canada). The ground electrode was placed over the anterior iliac spine.

2.4 | Data analysis

Mean and standard deviation of the PRM threshold of the muscles (RF, BF, TA, and GM) were analysed during different conditions (DS, 100% BW, 50% BW, and 0% BW). The mean and standard deviation of the PRM peak-to-peak amplitude and latency were calculated as the average of 10 PRM reflex recordings, which was calculated for each muscle and condition. The peak-to-peak PRM reflex amplitude values were normalized with respect to the DS condition to better represent the percentage modulation. Statistical analysis was performed using the commercial software package IMB SPSS v25. The Kolmogorov–Smirnov test was used to study the normal distribution of the data. A repeated-measures ANOVA using the factor "condition" was performed to compare differences in threshold, peak-to-peak

amplitude, and latencies. The Bonferroni post hoc comparison test was used to reveal specific differences. Sphericity was assessed through Mauchly test and Greenhouse–Geisser and was applied for cases where sphericity was not assumed. A p value of ≤ 0.05 was considered statistically significant. Data are represented as mean and standard deviation.

3 | RESULTS

3.1 | PRM reflex threshold

The PRM reflex threshold generally increased with body weight unloading in all muscles recorded compared with DS (Figure 2). In the DS position, the PRM threshold was 58.5 mA (12.3) for RF, 52.6 mA (11.7) for BF, 60.1 mA (12.8) for TA, and 59.1 mA (12.6) for GM. The largest change of threshold PRM was observed between DS and 0% BW conditions for the proximal muscles (RF and BF) and between DS and 50% BW for distal muscle (TA and GM) (see Figure 2).

Repeated measures ANOVA showed significant differences for the "condition" factor (RF $F = 16.08$, $p < 0.01$; BF $F = 10.43$, $p < 0.01$; TA $F = 12.89$, $p < 0.01$; GM $F = 8.87$, $p < 0.01$). The post hoc Bonferroni correction showed a significant increase in PRM threshold in

the standing position at 50% BW condition (RA $p < 0.01$, BF $p = 0.04$, TA $p = 0.02$, GM $p < 0.01$) and 0% BW condition (RA $p < 0.01$, BF $p < 0.01$, TA $p < 0.01$, GM $p = 0.01$) when compared with DS in all muscles recorded (see Figure 2). At the same time, there was a significant increase of PRM threshold during the 50% BW condition with respect to 100% BW condition in all muscles (RF $p < 0.01$, TA $p = 0.01$, GM $p = 0.01$), except for the BF muscle reflex ($p = 1.00$). Pairwise comparison among the conditions DS and 100% BW revealed significant differences only for the TA PRM threshold ($p = 0.03$) with an increment of 7.38 mA (2.24) during standing. Finally, no significant difference was observed between the 50% BW and 0% BW conditions in any muscle of the recorded lower limb muscles (Figure 2).

3.2 | PRM reflex amplitude and latency

Peak-to-peak PRM reflex amplitude recorded in the studied muscles are shown in Table 1 and Figure 3. In addition, a typical example of the PRM reflex for RF, BF, TA, and TS muscles is shown in Figure 4 from subject (#13).

The peak-to-peak PRM reflex amplitude was larger in the DS condition than the other conditions for all muscles, except for BF, which showed a no significant
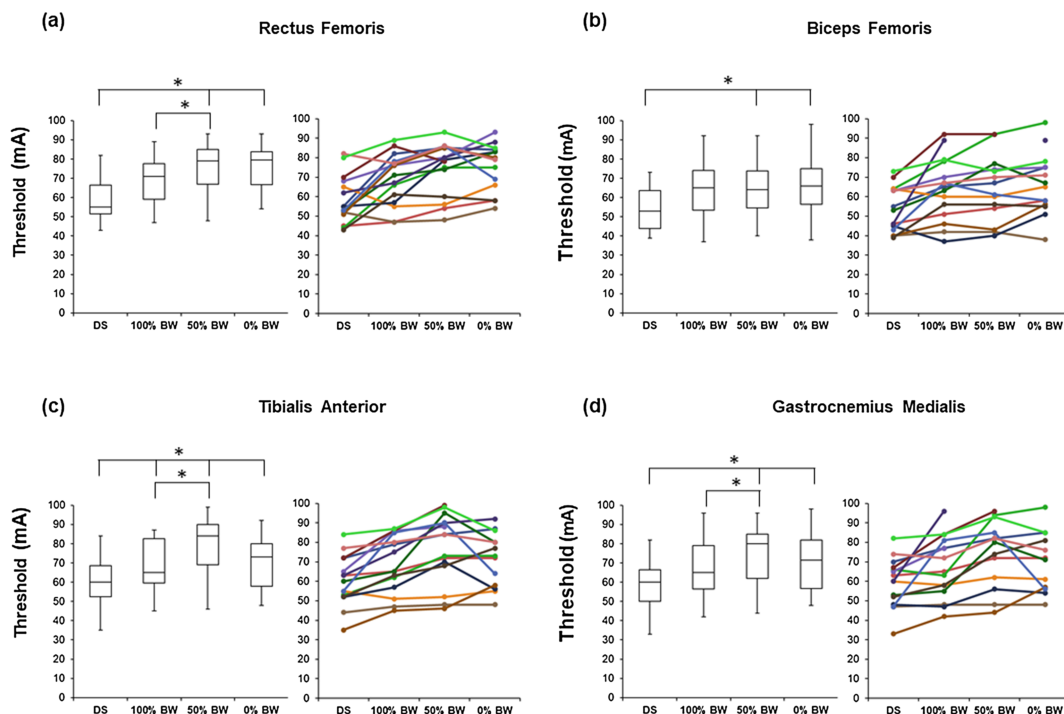

FIGURE 2 Median and interquartile ranges of the PRM threshold and individual thresholds for each subject recorded from the (a) rectus femoris (RF), (b) biceps femoris (BF), (c) tibialis anterior (TA), (d) gastrocnemius medialis (GM), muscles in decubitus supine (DS) position, standing with total body weight loading (100% BW), standing with half body weight loading (50% BW) and standing without body weight loading (0% BW). *$p \leq 0.05$

TABLE 1 Mean and standard deviation of PRM peak-to-peak amplitude and latency for each muscle and condition

	Absolute peak-to-peak amplitude (mV)				Normalized value respect to DS (100%).			Absolute latency (ms)			
	DS	100% BW	50% BW	0% BW	100% BW	50% BW	0% BW	DS	100% BW	50% BW	0% BW
RF	0.89 (0.58)	0.65 (0.47)	0.81 (0.66)	0.68 (0.58)	74 (14)	92 (19)	71 (17)	15.9 (3.2)	15.4 (2.8)	15.7 (4.0)	15.8 (3.6)
BF	1.27 (1.05)	2.21 (2.07)	1.60 (1.54)	1.09 (1.14)	174 (42)	118 (31)	86 (23)	13.1 (2.2)	14.0 (13.2)	13.2 (1.3)	13.56 (1.6)
TA	0.86 (0.43)	0.68 (0.43)	0.56 (0.42)	0.94 (0.67)	79 (13)	65* (13)	94 (21)	20.3 (2.0)	19.8 (2.0)	19.9 (2.2)	20.2 (1.5)
GM	2.30 (1.31)	1.85 (1.23)	1.14 (0.74)	2.08 (1.17)	81 (14)	46* (9)	73 (15)	21.0 (1.9)	20.1 (1.3)	21.4 (1.9)	20.3 (1.6)

Abbreviations: DS, decubitus supine; 100% BW, standing with total body weight loading; 50% BW, standing with half body weight loading; 0% BW, standing without body weight loading. Significant differences compared with DS are represented in bold.

*$p < 0.05$.

increase of 17.5% (SD 33.7) during the 100% BW and 17.5% (SD 40.7) during the 50% BW. The repeated measures ANOVA showed significant differences for the "condition" factor in BF ($F = 3.58$, $p = 0.05$), TA ($F = 2.8$, $p = 0.05$), and GM ($F = 5.04$; $p < 0.01$). Pairwise comparison also showed a significant decrease in amplitude of 34.6% (10.4) in TA and 53.6% (15.1) in GM muscles, when the DS condition was compared with the 50% BW condition (TA $p = 0.028$, GM $p = 0.019$). No significant differences were observed in PRM reflex amplitude for either RF or BF in any of the conditions studied.

PMR reflex latencies are represented in Table 1 (right panel). The repeated measures ANOVA showed no significant differences between conditions in any of the muscles recorded. For example, in the DS condition, the latencies of the PRM reflex were 15.9 ms (3.2) in RF, 13.1 ms (2.2) in BF, 20.3 ms (2) in TA, and 21 ms (1.9) in GM.

4 | DISCUSSION

The present study has shown that lower limb PRM reflex responses can be elicited by transcutaneous spinal cord stimulation in conditions of body weight loading used in several gait rehabilitation programmes performed with standard overground or robotic-assisted treadmill gait training. The main finding of the present study is the demonstration that body weight unloading is associated with higher PRM thresholds and requiring a higher stimulation intensity to elicit PRM reflex responses in both proximal and distal leg muscles. At 100% BW the PRM threshold is significantly reduced to levels observed in the supine position. These results suggest that the stimulus intensities needed to elicit PRM reflex responses are higher during BW unloading, specifically for responses measured at the 50% BW standing position compared with the 100% BW standing position. These results should be taken into consideration when applying tSCS combined with gait training rehabilitation programmes which use body weight support. Both biophysical and neurophysiological factors may explain the differences in PRM threshold with posture and body weight loading.

4.1 | Biophysical influences on PRM reflex responses

The influence of body position has been reported previously by Danner et al. (2016), where PRM threshold intensities varied between the standing and supine positions (Danner et al., 2016; Hofstoetter et al., 2008).

FIGURE 3 PRM reflex amplitudes for individual subjects presented for each muscle. Red line represents the mean and standard deviation. *$p \leq 0.05$
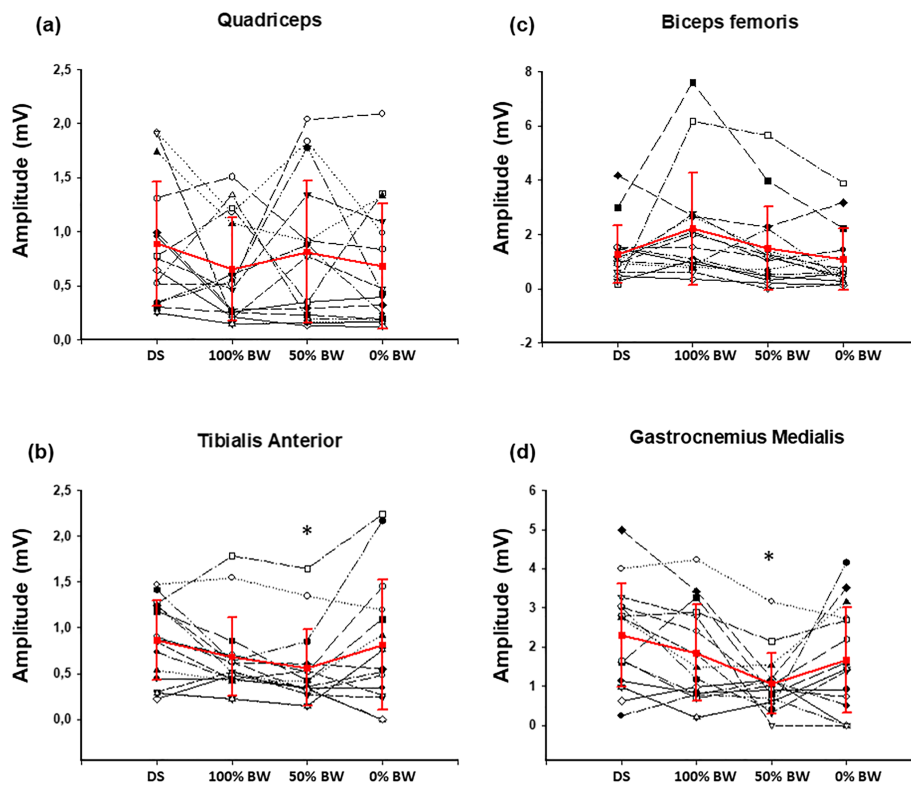

FIGURE 4 Representative PRM reflex responses recorded from participant #13 recorded from the rectus femoris (RF), biceps femoris (BF), tibialis anterior (TA), and gastrocnemius medialis (GM). The arrow is the time at which the single stimulation pulse was applied. The stimulation parameters for each muscle and condition were RF muscle, DS 58 mA, 100% BW 86 mA, 50% BW 95 mA, and 0% BW 76 mA. BF muscle, DS 47 mA, 100% BW 74 mA, 50% BW 67 mA, 0% BW 63 mA. TA muscle, DS 60 mA, 100% BW 94 mA, 50% BW 99 mA, 0% BW 70 mA. GM muscle, DS 52 mA, 100% BW 89 mA, 50% BW 94 mA, 0% BW 62 mA
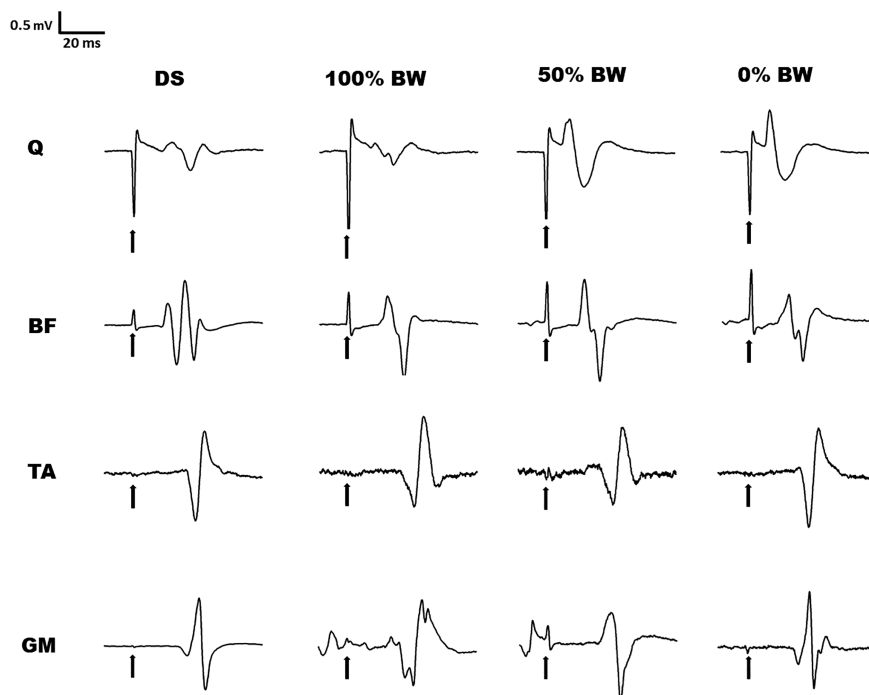
Computational models indicate that the PRM threshold is directly related to the distance between the structure and the electrode (Danner et al., 2011; Ladenbauer et al., 2010). These findings could explain why differences in efficient spinal cord stimulation are associated with a change in electrode position due to changes in the curvature of the spine and position of the lumbosacral spinal cord in the vertebral canal. For example, magnetic resonance imaging has shown that both the lumbosacral spinal cord and posterior nerve roots move anteriorly when the subject assumes the prone position or the lateral decubitus with lower limb flexion (Ranger et al., 2008). Thus, the position of the electrode and the curvature of the spinal column could be two essential biophysical

aspects that determine the PRM activation threshold. In the present study the threshold intensity was lower in the supine position, which would explain the low PRM threshold in this position. However, in general this study showed no significant differences in PRM threshold between the supine and standing position at 100% BW, which may be explained by methodological issues with electrode movement (see Section 4.4).

4.2 | Neural influences on PRM reflex responses

Several studies have implicated the role of trunk, hip, knee, and ankle muscle antagonists in the control of postural tasks. Muscle synergies measured from several muscles, including the trunk, and proximal and distal lower limb muscles, recorded during a postural task in healthy individuals, suggest that different subsets of these muscles are activated together to maintain balance during controlled load bearing (Krishnamoorthy et al., 2003). The role of several muscles in controlling posture and balance during standing has also been observed in a case series of people with motor complete spinal cord injury (Audu et al., 2018). Indeed in neuropathological cases of total loss of proprioception, intact neuromuscular mechanisms that mediate lower leg balance have been shown to be triggered by the activation of hip and, possibly, trunk afferent input (Bloem et al., 2002). Finally, an examination of motor patterns during posture in healthy individuals suggests that distal lower limb muscles, such as the ankle extensors (soleus/gastrocnemius), are activated before the onset of activity in the proximal muscles such as the hip extensors/knee flexors (hamstrings) including the trunk extensor (erector spinae) muscles. Postural adjustments after early muscle activation were detected as ankle flexion with accompanying tibialis anterior activity and often also in the ankle extensors. Taken together these studies suggest that postural motor control mechanisms mediated by proprioceptive input, such that activated with tSCS, is more evident in distal lower limb muscles, although an important role of trunk and proximal lower limb muscles cannot be excluded (Oddsson, 1989).

Previous studies have shown that the H-reflex is modulated by vestibular, visual, and also by peripheral sensory inputs from both cutaneous and muscular receptors (Alrowayeh et al., 2005). Thus, soleus H-reflex amplitudes are suppressed during standing when compared with sitting (Chen & Zhou, 2011). In the same way, studies have reported a reciprocal relationship between the amplitude of the H-reflex and the magnitude of displacement from the centre of pressure during standing

(Alrowayeh et al., 2005; Chen & Zhou, 2011). Thus, Hofstoetter et al. (2008) showed that postural tasks involving leaning forward could produce a facilitation of the PRM reflex of the triceps surae, which may be related to non-voluntary motor control mechanisms. In the same way, spinal processing of body position during changes in the centre of mass is also critical (Stein & Thompson, 2006). In our study, participants were permitted to control their centre of mass and stabilize the body during standing by holding onto a frame support structure. Previous studies (Morita et al., 2000; Nielsen, 2016; Saito et al., 2020) have also demonstrated modulation of spinal reflex excitability during voluntary contraction of agonist and antagonist muscle, but that the magnitude of this modulation depended on the lower limb muscle. A recent study (Saito et al., 2020) showed an increase of PRM amplitudes of lower limb muscle (quadriceps, hamstring, and triceps surae) during agonist contraction, except for the TA reflex where no change in amplitude was detected. Therefore, modification of PRM threshold in our study may be related to spinal motor control mechanisms activated during standing or by body weight unloading. In general, our study showed no significant modulation of PRM amplitude, except for a decrease in TA and GM activity with body weight loading at 50% (Table 1). This observation may reflect the activation of optimal inhibitory modulation of afferents activated by body load on the distal muscle activity within the mid-range of the length-tension curve. Although an earlier study has shown that distal muscle reflex activity in response to cutaneous afferent activation is facilitated during the late stance to swing phase of gait (37), further characterisation of reflex excitation of distal muscle reflexes during 100% of body weight loading, compared with 50% and 0% of body weight loading, is now required.

With regard to the motor control of body weight loading and posture, both segmental and transcortical motor control mechanisms are known to control proprioceptive reflexes (Evarts & Fromm, 1981), such as those reflexes studied here. Stimulation of plantar cutaneous receptors and proprioceptive receptors at the ankle joint are known to modulate H-reflex activity (Chen & Zhou, 2011). In line with our results, phasic changes in cutaneous-evoked tibialis anterior muscle activity was detected during a loading task, but only during walking rather than standing (Nakajima et al., 2008). Increased lower limb loading during standing also leads to Soleus H-reflex inhibition (Nakazawa et al., 2004). In our study, a higher PRM reflex threshold intensity was recorded at 0% BW for proximal muscle (RF and BF) and at 50% BW for distal muscles (TA and GM). This effect could be mediated by a decrease in plantar cutaneous input experienced during

body weight unloading. Similarly, PRM amplitude of distal muscles was also decreased during unloading at 50% of body weight. The contribution of visual and vestibular input to the changes in PRM threshold observed in this study was assumed to be negligible, especially as the participants maintained the same visual reference and the same cephalic position during the different study conditions (Chen & Zhou, 2011). Thus, the change in PRM reflex response presented in this study with body position and body weight loading are thought to reflect changes in spinal motor control excitability. Future studies should focus on the influence tSCS on proprioceptive feedback from Group Ia/II muscle spindle afferents and Group Ib Golgi tendon afferents.

4.3 | Mechanisms mediating tSCS modulation of PRM reflex responses

The effect of activation of cutaneous afferents with single pulse tSCS on the proprioceptive feedback from Group Ia/II muscle spindle afferents or from Group Ib Golgi tendon afferents has been evaluated previously in clinical research and in animal model experimentation. Although a role for cutaneous afferent modulation of Group Ia and Group II excitation has been largely excluded (Marque et al., 2005), it is possible that cutaneous inputs converge onto group II interneurons (Jankowska, 1992). However, at rest, similar to the conditions tested in this study, homonymous Group II-mediated excitation of quadriceps is suppressed (such as when the subject holds a standing frame) (Nardone et al., 1990). This mechanism may explain the higher PRM reflex threshold at 50% body weight loading when compared with 100% loading. In contrast during postural and locomotor tasks, group II pathways are mainly responsible for excitation of the quadriceps motoneurons (Marchand-Pauvert et al., 2005). If the distal leg muscle are tonically activated during 100% body weight loading, then it is also possible that the reduced PRM reflex threshold may also be mediated by activation of Group II afferents during this condition in our study. A role for tSCS-activated cutaneous activation of PRM reflex mediated via spinal motor control mechanisms mediated via Group II afferents need to be fully explored during different conditions of body weight loading.

The possibility that cutaneous afferents modulate Group Ib-mediated inhibition has also been largely excluded (Cavallari et al., 1992; Pierrot-Deseilligny et al., 1981), although either a suppression or facilitation of Group1b-mediated inhibition may exist during specific conditions (Brink et al., 1983), suggesting that the effect is task-dependent. Cutaneous facilitation of Group Ib

inhibition may be focused to limit further (exploratory) movement (Lundberg et al., 1977), with a significant role of suppression of Group Ib inhibition of the quadriceps muscle following contact of the foot sole during bipedal walking.

However, in clinical studies, the most frequent effect of low-threshold cutaneous volleys is a facilitation of Group Ib inhibition to motoneurons; at rest cutaneous input facilitates heteronymous Group Ib inhibition from the gastrocnemius medialis to biceps and also facilitates Group Ib inhibition for quadriceps motoneurones (Pierrot-Deseilligny et al., 1981). Taken together these mechanisms do not explain why a significant reduction in PRM threshold was observed for most distal and proximal muscle in body weight loading at 100%.

4.4 | Limitations

The methodological limitations associated with reliable and repeatable PRM evaluation during different body postures, particularly associated with body weight support should be considered. Although the principal investigator ensured that reflex testing was performed at the major body positions examined in this study, it is possible that more subtle changes in the position of the spine or lower limbs could also influence PRM excitability, unrelated to body weight loading. The spinal electrode was fixed with adhesive tape and the lumbar area was stabilized in a neutral position using an elastic bandage. It is possible therefore that there was a minor modification in electrode position due to differences in lumbar curvature in the supine and standing. Furthermore specific PRM reflex responses may also be modulated indirectly by unintended coactivation of other agonist or antagonist reflex responses (Edgerton et al., 2008).

In this study, PRM reflexes were recorded from multiple muscles in response to stimulation at one spinal level. It is possible that specific PRM reflex responses can be elicited by applying the tSCS to more specific optimal sites of spinal levels. The use of more muscle-specific stimulus protocols in the future may reveal clearer relationships with PRM reflex threshold, latency and amplitude. Furthermore, changes in biophysical factors related to the electrode stimulation site, such as the position of the electrode and the pressure that it applies during each body posture and body loading, may also influence the PRM reflex recordings.

Finally, the spinal components of the PRM reflex responses with single pulse tSCS were not confirmed with the application of the paired pulse protocol to identify post-activation depression as previous studies (Danner et al., 2016; Hofstoetter et al., 2008; Minassian

et al., 2007; Saito et al., 2020) with similar stimulation protocols performed. The main objective of this study was to analyse the feasibility of recording PRM reflex responses and threshold at different body weight loading during standing because several gait training rehabilitation programmes use body weight support systems. The obvious limitation of this study was that the influence of unloading body weight was only evaluated during standing (static) and not during a gait task (dynamic). Future studies should address the influence of proprioceptive and cutaneous input on PRM reflex responses, especially when the efficacy of tSCS intervention is to be evaluated.

5 | CONCLUSIONS

This study has shown that spinal sensorimotor networks can be activated with the application of transcutaneous spinal cord stimulation in various conditions of body weight unloading. Higher stimulus intensities are necessary to evoke spinally-mediated reflex responses, and by extension spinal excitability, during standing at 50% body weight loading for proximal muscle and during 0% body weight loading for distal muscle. These results have practical implications for futures studies of tSCS, especially when combined with gait rehabilitation training programmes that include body weight support.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS
A.M.G. performed the study, analysed data, and drafted the paper. D.S.M. supervised experiment and drafted paper. N.C.S. ran experiment, drafted paper, and analysed data. A.J.A.E. designed study and drafted paper. J.C.M. designed study, drafted paper, and analysed data. A.G.A. designed study and drafted paper. J.T. designed study and drafted paper. J.G.S. designed study, drafted paper, and analysed data.

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