



# Do soleus responses to transcutaneous spinal cord stimulation show similar changes to H-reflex in response to Achilles tendon vibration?

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## Abstract

**Introduction/purpose** Recently, the use of transcutaneous spinal cord stimulation (TSCS) has been proposed as a viable alternative to the H-reflex. The aim of the current study was to investigate to what extent the two modes of spinal cord excitability investigation would be similarly sensitive to the well-known vibration-induced depression.

**Methods** Fourteen healthy participants (8 men and 6 women; age:  $26.7 \pm 4.8$  years) were engaged in the study. The right soleus H-reflex and TSCS responses were recorded at baseline (PRE), during right Achilles tendon vibration (VIB) and following 20 min of vibration exposure (POST-VIB). Care was taken to match H-reflex and TSCS responses amplitude at PRE and to maintain effective stimulus intensities constant throughout time points.

**Results** The statistical analysis showed a significant effect of time for the H-reflex, with VIB ( $13 \pm 5\%$  of maximal M-wave ( $M_{\max}$ ) and POST-VIB ( $36 \pm 4\%$  of  $M_{\max}$ ) values being lower than PRE-values ( $48 \pm 6\%$  of  $M_{\max}$ ). Similarly, TSCS responses changed over time, VIB ( $9 \pm 5\%$  of  $M_{\max}$ ) and POST-VIB ( $27 \pm 5\%$  of  $M_{\max}$ ) values being lower than PRE-values ( $46 \pm 6\%$  of  $M_{\max}$ ). Pearson correlation analyses revealed positive correlation between H-reflex and TSCS responses PRE-to-VIB changes, but not for PRE- to POST-VIB changes.

**Conclusion** While the sensitivity of TSCS seems to be similar to the gold standard H-reflex to highlight the vibratory paradox, both responses showed different sensitivity to the effects of prolonged vibration, suggesting slightly different pathways may actually contribute to evoked responses of both stimulation modalities.

**Keywords** Electrical stimulation · Transcutaneous spinal cord stimulation · Vibration · Spinal loop excitability · Spinal reflex

## Abbreviations

CCC	Concordance correlation coefficient
H-reflex	Hoffmann reflex
$M_{\text{at H}}$	Associated M-wave amplitude when recording an H-reflex
$M_{\text{max}}$	Maximal M-wave

POST-VIB	Measurements performed after prolonged vibration
PRE	Measurements performed at baseline
SOL	Soleus
TA	Tibialis anterior
TSCS	Transcutaneous spinal cord stimulation
VIB	Measurements performed during vibration exposure
VL	Vastus Lateralis

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## Introduction

Percutaneous electrical stimulation is a well-known research tool in neuromuscular physiology (Millet et al. 2011). From a neurophysiological perspective, it can be used for eliciting the Hoffmann reflex (H-reflex), considered an analog of the stretch reflex that bypasses muscle spindles, and that can be recorded after electrical stimulation of Ia afferents

projecting monosynaptically their excitatory inputs onto alpha motoneurons (Zehr 2002). As such, the H-reflex is commonly used to investigate spinal cord excitability in the context of training (Aagaard et al. 2002), aging (Falco et al. 1994), pathology (Yamaguchi et al. 2018), fatigue (Espeit et al. 2021) or interventions like neuromuscular electrical stimulation (Vitry et al. 2021) or motor imagery (Grospretre et al. 2016) for some examples.

The H-reflex, despite its widespread utilization, has some limitations. Maybe one of the main limitations is the difficulty to evoke H-reflexes in a large variety of muscles. For instance, H-reflex can only be recorded in a few upper-limb muscles, i.e., flexor carpi radialis or extensor carpi radialis longus (Miller et al. 1995). Regarding lower-limb muscles, H-reflexes are commonly recorded on the soleus muscle and to a lesser extent on the tibialis anterior muscle (Palmieri et al. 2002). Its assessment on muscles as the quadriceps remains challenging (Doguet and Jubeau 2014). Moreover, peripheral nerve electrical stimulation is further limited by the very localized effect, necessitating several setups to investigate more than one muscle within the same experiment.

Recently, transcutaneous spinal cord stimulation (TSCS) has been proposed as an alternative to the H-reflex. By mainly activating spinal posterior roots (Danner et al. 2016), TSCS provides the advantage of evoking spinal reflexes with similar characteristics than the H-reflex in multiple lower limb muscles (Courtine et al. 2007), and with moderate to high levels of repeatability (Saito et al. 2019). Moreover, as suggested by Kitano and Koceja (2009), TSCS presents the advantage to evoke H-reflex-like responses without any associated motor response, avoiding underestimation of the connectivity between sensory and motor systems. As for the H-reflex, responses to TSCS can be depressed by prior stimulation (i.e., paired stimulation) through homosynaptic post-activation depression, suggesting it is actually a monosynaptic reflex (Andrews et al. 2015). Moreover, TSCS responses can be depressed during tendon vibration (Courtine et al. 2007). This mimics the well-known vibration paradox reported when considering H-reflex measurements, likely as a result of presynaptic inhibition of Ia afferents (Desmedt and Godaux 1978). Besides this decreased spinal cord excitability observed during ongoing tendon vibration, H-reflex is also known to be depressed after a prolonged (i.e., 20–60 min) exposure to local vibration (Souron et al. 2017), likely as a result of vibration-induced decreased motoneuron excitability (Souron et al. 2019). Yet this has never been demonstrated using TSCS responses. Moreover, if we assume that TSCS and H-reflex responses are two neurophysiological recordings investigating the same spinal cord pathways (Andrews et al. 2015), then both responses should not only behave the same but the magnitude of their

modulation in response to both ongoing and prolonged tendon vibration should be the same. In this study, we therefore aimed at comparing the effect of Achilles tendon vibration on soleus TSCS and H-reflex responses. It was hypothesized that the two modes of spinal cord excitability investigation would be similarly sensitive to the vibration-induced depression.

## Methods

### Participants

Fourteen healthy and recreationally active participants (8 men and 6 women; age:  $26.7 \pm 4.8$  years) were engaged in the study. Participants were asked to refrain from strenuous and unaccustomed physical activity for 24 h before testing. The study protocol was approved by the local ethical committee and adhered to the latest update of the Helsinki Declaration (except for registration in a database). All subjects gave their written informed consent before participation.

### Experimental design and procedures

Participants were invited to one 2-h session in which they were first introduced to the aim of the study and the methods. For one participant who was naïve to electrical stimulation, we further slowly introduced him to it, i.e., by progressively increasing stimulations intensity from sensory threshold to twitches. Following placement of EMG electrodes, participants were seated in a chair, with their right foot strapped in place, securing the ankle at  $90^\circ$  flexion and the knee at  $120^\circ$  flexion ( $180^\circ =$  full extension). The left foot was lightly strapped at  $90^\circ$  knee and ankle flexion. After careful determination of stimulations intensity (see below), right soleus (SOL) and tibialis anterior (TA) H-reflexes from sciatic nerve stimulation and SOL, TA and left vastus lateralis (VL) TSCS responses from transcutaneous spinal cord stimulation were recorded at baseline (PRE), during 100-Hz and 1-mm right Achilles tendon vibration (VIB) (VB115, Techno concept, Mane, France), and immediately after 20 min of tendon vibration (POST-VIB). For VIB, measurements began 5 min after the onset of the 20-min tendon vibration exposure, with measurements lasting approximately 6 min in total. At each time point, EMG responses from sciatic nerve (for right SOL and TA) and femoral nerve (for left VL) electrical stimulation were further recorded to obtain the maximal M-wave ( $M_{\max}$ ) needed for normalization of H-reflex and TSCS responses. Specifically, at each time point, the order of recordings was as follows: (1) 3  $M_{\max}$  on the right SOL and TA, (2) 3  $M_{\max}$  on the left VL, and lastly (3) 15 H-reflexes and 15 TSCS responses on the right SOL and TA. For those

H-reflex and TSCS measurements, we systematically alternated the two types of stimulation (i.e., one single H-reflex recording always followed one single TSCS response recording, and vice versa) with ~10 s in-between. This allowed us to compare the vibration-induced modulations of these responses without any potential influence of recording timing. Throughout the study, great care was taken to ascertain that participants did not change their seating or head position, as this is known to change the response to stimulation (Danner et al. 2016).

## Instrumentation

### Electromyography (EMG)

Participants were prepared by shaving, gently abrading the skin, and cleaning it with isopropyl alcohol. EMG signals of the right SOL and TA, and left VL muscles were recorded with pairs of self-adhesive surface electrodes (Meditrace 100, Covidien, Mansfield, MA) in a bipolar configuration. TA and VL electrodes were placed following SENIAM's recommendations (Hermens et al. 2000). SOL electrodes were placed 2 cm below the muscle–tendon junction of the gastrocnemii. The reference was placed on the right patella. The signal was band pass-filtered (10–500 Hz), amplified by bio-amplifier (ML138, ADInstruments; common mode rejection ratio = 85 db, gain = 5000) and analog-to-digital converted at a sampling rate of 2000 Hz by a Powerlab system (16/30-ML880/P, ADInstruments, Bella Vista, Australia). All data were analyzed offline using Labchart 8 software (ADInstruments).

### Femoral nerve stimulation

The left femoral nerve was stimulated by a single rectangular electrical stimulus with a duration of 1-ms and a voltage of 400 V (DS7A, Digitimer, Welwyn Garden City, Hertfordshire, UK) delivered through an anode (35 × 35 mm, recording diameter: 10 mm, ECG Snap, McKesson Medical-Surgical Inc., Richmond, VA 23233 USA) placed on the femoral nerve in the hip crevice and a cathode (5 × 10 cm Complex Snap, MDSS GmbH, Hannover, Germany) and placed on the gluteus maximus. Electrical stimuli were first administered at 5 mA and then were increased by 10-mA steps until VL maximal M-wave amplitude ( $M_{\max}$ ) was obtained. The optimal intensity was then increased by 20% to ensure supramaximal stimulation (i.e.,  $98 \pm 23$  mA).

### Sciatic nerve stimulation

The right sciatic nerve was stimulated by a single rectangular electrical stimulus with a duration of 1-ms, and a voltage

of 400 V (DS7A, Digitimer, Welwyn Garden City, Hertfordshire, UK) delivered through a bipolar bar stimulating electrode with 30 mm anode–cathode spacing (Bipolar Felt Pad Stimulating Electrode Part Number E.SB020/4 mm, Digitimer) placed in the superior portion of the popliteal fossa, before the bifurcation of the nerve into the common fibular and tibial nerve. This stimulation setup allowed simultaneous recording of SOL and TA evoked responses (Espeit et al. 2017; Palmieri et al. 2002). Yet we adjusted the stimulus intensity by considering the SOL only (see below) and we made the choice not to present results from the TA muscle in this article. Results for TA recording can be found in Appendices 1. The optimal site of stimulation was determined as the location eliciting the greatest responses in tested muscles for a given stimulus intensity. Then, electrical stimuli were administered at 5 mA and then were increased by 5-mA steps until SOL and TA maximal M-wave amplitude ( $M_{\max}$ ) were obtained. The optimal intensity was then increased by 20% to ensure supramaximal stimulation when recording  $M_{\max}$  (i.e.,  $87 \pm 35$  mA). To evoke right SOL and TA H-reflexes, single electrical stimuli were first administered at 1 mA and then increased incrementally (i.e., by 2-mA steps). We initially wanted to set the stimulation intensity to the one needed to reach a target of approximately 7%  $M_{\max}$  (Knikou 2008) for the associated M-wave amplitude (i.e.,  $M_{\text{at H}}$ ) of the SOL of every participant. Yet we were unable to systematically reach this objective and we then prioritized setting an intensity (i.e.,  $27 \pm 11$  mA) allowing us to have a clear and consistent H-reflex concomitantly recorded with a stable  $M_{\text{at H}}$ , while remaining on the ascending part of the H-reflex recruitment curve.  $M_{\text{at H}}$  was systematically measured and we individually monitored the ratio between this associated M-wave and  $M_{\max}$  to keep it constant throughout the experiment (i.e., to ensure a constancy of effective stimulation) (Aagaard et al. 2002).

### Transcutaneous spinal cord stimulation

Single rectangular electrical pulses with a duration of 1-ms and a voltage of 400 V (DS7A, Digitimer, Welwyn Garden City, Hertfordshire, UK) were delivered through a 68 × 125 mm anode (axion GmbH, Leonberg Germany) placed over the midline of the abdomen between the xiphoid process of the sternum and the umbilicus, and a cathode (35 × 35 mm, delivering diameter: 10 mm, ECG Snap, McKesson) placed on the midline of the back between the spinous processes of L1 and L2 (Minassian et al. 2007; Courtine et al. 2007; Saito et al. 2019). Stimulation intensity was slowly increased until reaching a similar right SOL TSCS response amplitude as for the H-reflex (Hofstoetter et al. 2019). This was obtained at a mean intensity of  $91 \pm 27$  mA. To confirm that evoked responses were caused

by activation of the sensory fibers, a paired stimulation (inter-pulse interval: 50 ms) was delivered to test homosynaptic depression of the evoked responses (Minassian et al. 2007). This phenomenon was confirmed in the present study as the responses associated to the second transcutaneous spinal cord stimulation were noticeably smaller than those following the first stimulus at PRE ( $4.0 \pm 0.09$  and  $1.9 \pm 0.02$  mV, respectively). When recording SOL and TA TSCS responses at PRE, VIB or POST-VIB, we wanted to keep effective stimulation as constant as possible as for H-reflex recordings. Because there is no associated M-wave that can be monitored as for H-reflex recordings (Aagaard et al. 2002), we controlled effective stimulation by trying to maintain constant (in % of  $M_{\max}$ ) TSCS responses from the left VL. The left VL was chosen as a proxy of effective stimulation intensity as it was not expected to be influenced by vibration. We therefore measured left VL TSCS response mean amplitudes at PRE and targeted this amplitude at VIB and POST-VIB by adjusting electrical stimulation intensity if needed.

### Data analysis

The peak-to-peak amplitude of  $M_{\max}$ ,  $M_{\text{at H}}$ , H-reflex and TSCS responses was analyzed at each time point (i.e., PRE, VIB and POST-VIB). At each time point, the mean of the three  $M_{\max}$  was retained to normalize  $M_{\text{at H}}$  as well as H-reflex and TSCS responses. Because of some inter-trial variability for SOL H-reflex and TSCS responses, we chose to analyze the mean of the five most accurate responses out of the 15 recorded at each time point. For SOL H-reflex recordings, we first selected at PRE the five responses that presented the smallest coefficient of variation for  $M_{\text{at H}}/M_{\max}$  ratio. The mean of those five responses was retained for analysis and normalized to  $M_{\max}$  (i.e., PRE-value) and the mean  $M_{\text{at H}}/M_{\max}$  ratio was considered our target for VIB- and POST-VIB measurements. At both VIB and POST-VIB, for each participant, the five SOL H-reflex responses that presented  $M_{\text{at H}}/M_{\max}$  ratio the closest to the individual target ratio were averaged and normalized to  $M_{\max}$  (i.e., VIB and POST-VIB values). For TSCS responses, we applied the same procedures by considering left VL TSCS response amplitude instead of M-wave. To this aim, we first retained for analysis at PRE the five right SOL TSCS responses that were the closest to the mean SOL H-reflex previously determined. This allowed us to have comparable mean SOL TSCS response and mean SOL H-reflex at PRE. On those five selected responses, we measured left VL TSCS response mean amplitude before averaging it and normalizing it to  $M_{\max}$ . This ratio was then our target for selecting responses at VIB and POST-VIB. Specifically, at both VIB and POST-VIB, the five left VL TSCS responses that presented amplitude (in % of  $M_{\max}$ ), the closest to the target amplitude, were

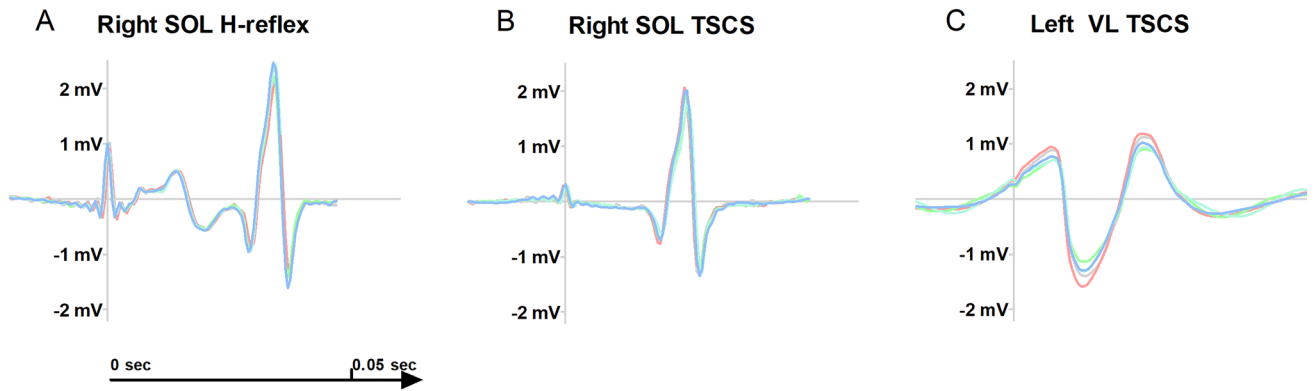
selected and the associated responses on the right SOL were averaged and normalized to  $M_{\max}$  to obtain our VIB and POST-VIB values. Mean results for the TA muscle were calculated on the same five responses as for the SOL at each time point and for each stimulation modality (i.e., H-reflex and TSCS).

### Statistics

Data were analyzed and presented using R (R Core Team 2022). Prior to analyses, data were tested for normality using packages *glmmTMB* and *DHARMA* (Brooks et al. 2017; Hartig 2022). Data were not normally distributed (all responses except  $M_{\max}$  responses from right SOL) and as such, for homogeneity, all data were analyzed using non-parametric approaches. The data analysis involved three steps. First, a Generalized Estimation Equation (i.e., a one-way ANOVA-like analysis) was conducted to investigate the effect of time between the three time points (PRE, VIB and POST-VIB) for each evoked response (i.e.,  $M_{\text{at H}}$ , H-reflex and TSCS responses) and each muscle separately (Hubbard et al. 2010). In case of significant differences, post-hoc testing was performed using Tukey correction. For this analysis, the *geepack* and *multcomp* packages were used (Yan 2002; Hothorn et al. 2008). Second, a Concordance Correlation Coefficient (CCC) was employed to investigate whether the soleus H-reflex and TSCS responses (expressed as a percentage of  $M_{\max}$ ) correlated over the three time points. Such analysis allowed us to test to what extent the evolution of responses over time was similar between stimulation methods. This was conducted using the *cccrm* package (Carrasco and Martinez 2023). Finally, Pearson correlations were further conducted between PRE-to-VIB changes as well as between PRE- to POST-VIB changes in H-reflex and TSCS responses amplitude (i.e., absolute changes of responses expressed in percentage of their respective  $M_{\max}$ ). This last analysis allowed us to test whether the two stimulation methods were similarly sensitive to the influence of ongoing and prolonged Achilles tendon vibration. Both Concordance Correlations and Pearson correlations were considered to be non-existent if  $r < 0.2$ , poor if  $0.2 < r < 0.4$ , moderate if  $0.4 < r < 0.6$ , strong if  $0.6 < r < 0.8$  and very strong above, similar to Pearson's correlations (Schober et al. 2018). Differences in all tests were considered significant when  $P < 0.05$ . Data are presented as individual means, median and interquartile ranges in figures and as group means in text.

### Results

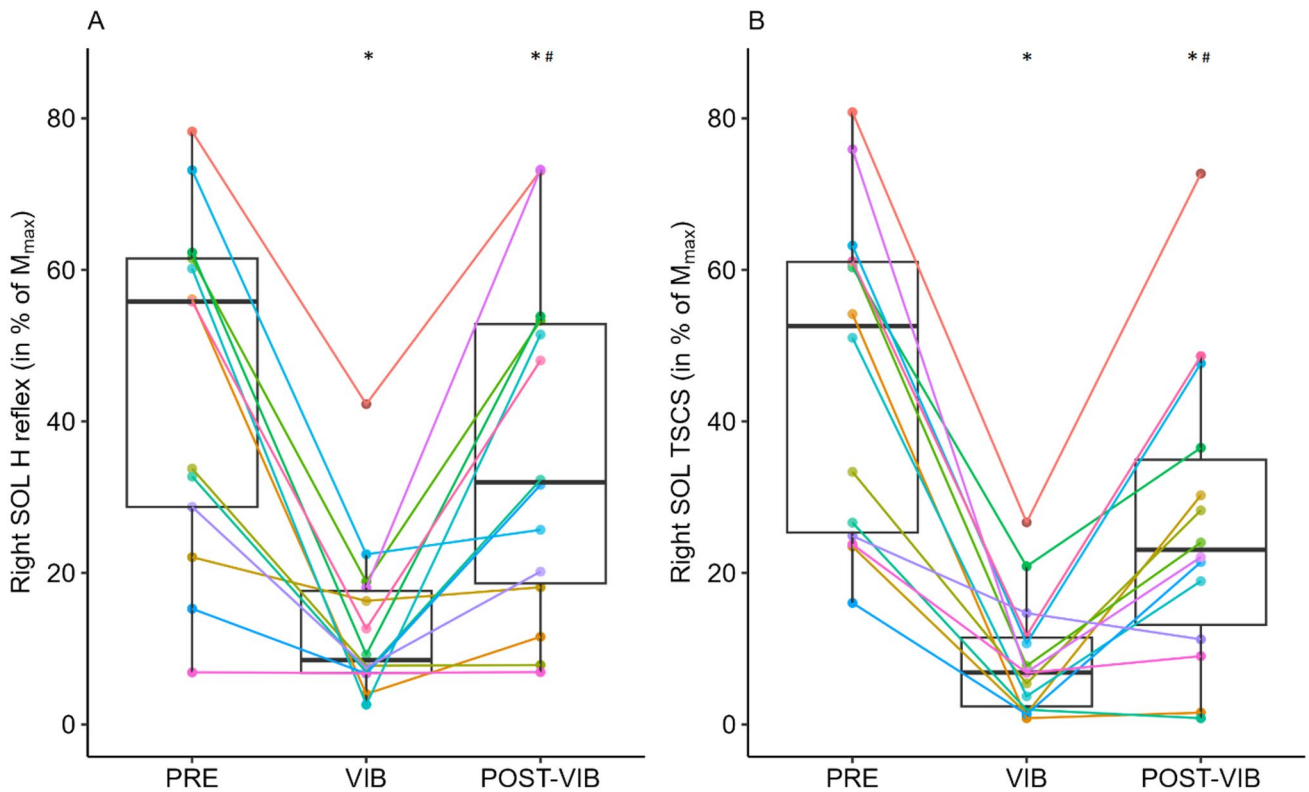
Only results from the SOL muscle are presented below. Data from the TA muscle can be found in Appendix 1. Typical traces from one subject are shown in Fig. 1.



**Fig. 1** Typical traces from sciatic nerve stimulation (right SOL H-reflex, panel **A**) and TSCS (right SOL and left VL TSCS responses, panel **B** and **C**, respectively) obtained on a representative participant at PRE. The five responses used in the data analysis are overlaid

The Generalized Estimation Equation showed a significant effect of time for the right SOL H-reflex (Fig. 2A;  $P < 0.001$ ), with VIB and POST-VIB values being significantly lower than PRE-values ( $P < 0.001$  and  $P = 0.007$ , respectively). Mean values for PRE, VIB and POST-VIB were  $48 \pm 6$ ,  $13 \pm 5$ , and  $36 \pm 4\%$  of  $M_{max}$ , respectively. At the same time, right SOL  $M_{atH}$  did not change over time (Fig. 3A;  $P = 0.64$ ). Similarly, to the H-reflex,

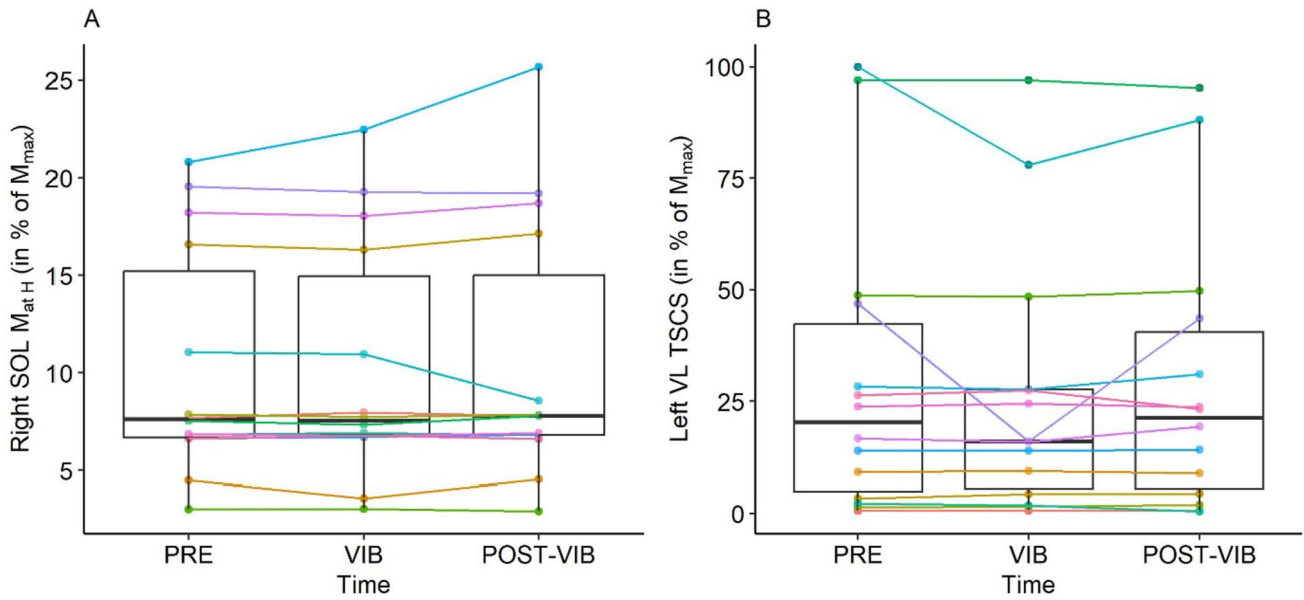
right SOL TSCS responses changed over time (Fig. 2B;  $P < 0.001$ ), VIB and POST-VIB values being significantly lower than PRE-values ( $P < 0.001$  and  $P = 0.006$ , respectively) and POST-VIB values being greater than VIB values ( $P < 0.001$ ). Mean values for PRE, VIB and POST-VIB were  $46 \pm 6$ ,  $9 \pm 5$ , and  $27 \pm 5\%$  of  $M_{max}$ , respectively. No effect of time was observed for the responses of the left VL (Fig. 3B;  $P = 0.23$ ).



**Fig. 2** Boxplots showing right soleus (SOL) H-reflex (**A**) and TSCS (**B**) responses at baseline (PRE), during right Achilles tendon vibration (VIB) and after prolonged exposure to vibration (POST-VIB).

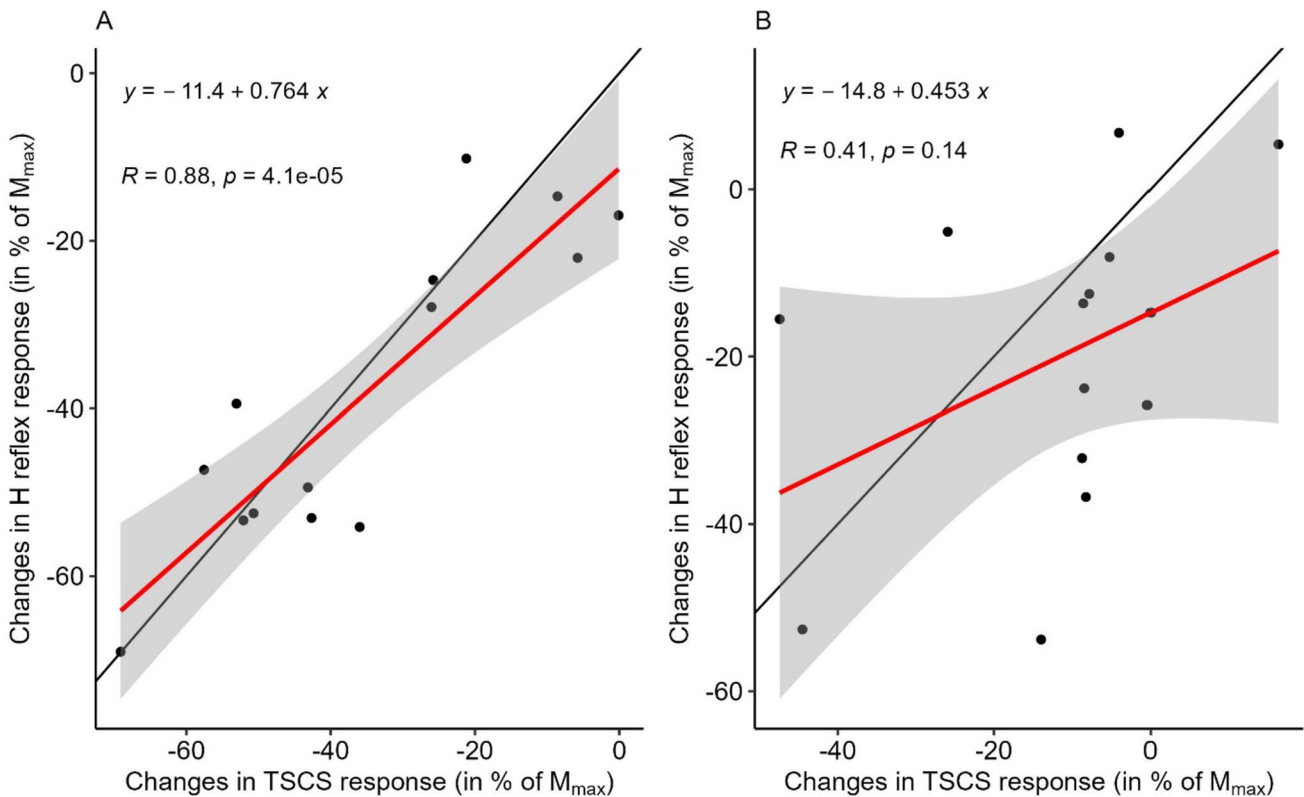
Responses are expressed as a percentage of right soleus  $M_{max}$ . \*Denotes significant differences when compared to PRE. #Denotes significant difference when compared to VIB





**Fig. 3** Boxplots showing right soleus (SOL) associated M-wave during H-reflex recordings (A) and left vastus lateralis (VL) TSCS (B) responses at baseline (PRE), during right Achilles tendon vibra-

tion (VIB) and after prolonged exposure to vibration (POST-VIB). Responses are expressed as a percentage of right SOL and left VL  $M_{max}$ , respectively



**Fig. 4** Pearson correlation plots between right SOL PRE-to-VIB changes (A) as well as between PRE- to POST-VIB changes (B) in H-reflex and TSCS responses amplitude (i.e., absolute changes

expressed in percentage of  $M_{max}$ ). The red line is the correlation, the black an identity slope (i.e., where  $y = x$ )

The CCC showed a strong correlation between the evolution of right SOL H-reflex and TSCS responses over time (CCC = 0.65 [0.58;0.71]). Yet, Pearson correlation analyses revealed significant correlation between right SOL H-reflex and TSCS responses PRE-to-VIB changes ( $r = 0.875$ ,  $P < 0.001$ ; Fig. 4A), but not for PRE- to POST-VIB changes ( $r = 0.41$ ,  $P = 0.14$ ; Fig. 4B).

## Discussion

The present study aimed to compare Achilles tendon vibration-induced changes in soleus spinal loop excitability between assessments through either classical H-reflex recordings or transcutaneous spinal cord stimulation. The main finding was that both H-reflex and TSCS responses decreased during Achilles tendon vibration, as well as immediately after prolonged vibration exposure. While responses to both modes of stimulation correlated throughout the study (i.e., strong concordance correlation coefficient), the Pearson correlations only showed a very strong correlation between H-reflex and TSCS responses changes observed from PRE to VIB, but not from PRE to POST-VIB. Such results suggest that those two neurophysiological recordings do not allow investigation of the exact same spinal cord pathways.

To compare H-reflex and TSCS responses throughout the study, we initially matched their size at baseline (Hofstoetter et al. 2019). To minimize the influence, that the inherent variability of the evoked responses could have had on our results, we first adjusted the stimulation intensity throughout the subsequent time points (i.e., VIB and POST-VIB) to ensure that effective stimulation intensity remained constant. While this can easily be done for H-reflex recordings by monitoring and keeping the size of the associated M-wave ( $M_{at\ H}$ ) constant (Aagaard et al. 2002), no such solution exists for TSCS responses. Therefore, we opted to monitor the size of TSCS responses of a remote muscle that was not targeted by our vibration intervention (i.e., the left VL), as also suggested by previous findings using corticospinal tract electrical stimulation (Vitry et al. 2021). While we chose the left VL as our control muscle in the present study, it is likely that one can choose one or another muscle for such control, or even a combination of muscles, as suggested by results of Hofstoetter et al. (2019). Such procedure is only useful in setups investigating the effects of unilateral interventions with no crossed effects. Second, while 15 H-reflex and TSCS responses were recorded at the three time points, we only kept the five responses from each stimulation mode that were the closest to our targets for the subsequent analysis. This method, with more stimulations administered than analyzed, can be viewed as time-consuming and cumbersome but allows us to reduce inter-trial variability of the

measurements. For instance, at baseline, the coefficients of variation of the 15 associated soleus M-wave, H-reflex and TSCS recordings were 15, 16, and 32%, respectively. After keeping the five closest responses to our targets, coefficients of variation fell to 3, 12, and 15%. This clearly highlights that controlling constancy of the effective stimulation intensity is crucial to reduce response variability. Of note, averaged results of the present study were nonetheless somewhat similar when considering the 15 responses within the analysis, yet with some dissimilarities because of greater variability of the data (see Appendix 2).

We used tendon vibration as a model to modulate spinal cord excitability (Souron et al. 2017), but other models could have been considered for such a study comparing H-reflex and TSCS responses sensitivity to changes in spinal loop excitability, e.g., fatigue (Espeit et al. 2021), neuromuscular electrical stimulation (Vitry et al. 2021), motor imagery (Grospretre et al. 2016) or postural modulation (Hayashi et al. 1992). In the present study, both soleus H-reflex and TSCS responses were depressed during Achilles tendon vibration, as already observed Courtine et al. (2007). Such decrease in spinal loop excitability during ongoing tendon vibration is known as the so-called vibration paradox (Desmedt and Godaux 1978). This phenomenon is considered to be mainly attributed to an increased presynaptic inhibition of the discharging Ia afferents through GABAergic interneurons (Hultborn et al. 1987; Gillies et al. 1969). In the same time, we observed similar results on the tibialis anterior muscle (Appendix 1) where both H-reflex and TSCS responses were decreased during ongoing vibration, likely as a result of reciprocal inhibition (DeForest et al. 2020). While decreases in both responses from the tibialis anterior muscle did not correlate, likely because size of responses were not matched at baseline (mean amplitude at PRE was 10.0 and 19.3% of  $M_{max}$  for tibialis anterior H-reflex and TSCS responses, respectively), there was a very strong correlation between H-reflex and TSCS responses vibration-induced depressions of the soleus muscle (Fig. 4A). This suggests that the two stimulation methods were similarly sensitive to changes in these pathways. Yet, this may not be the case when considering pathways involved in the acute spinal loop excitability depression commonly reported after prolonged exposure to vibration (Souron et al. 2017). For instance, while both soleus H-reflex and TSCS responses were depressed after 20 min of Achilles tendon vibration, the magnitudes of their depression did not correlate (Fig. 4B). This would suggest that changes in pathways mediating post-vibration spinal loop excitability depression cannot be captured with the same sensitivity through the two stimulation methods. Because TSCS mainly activates spinal posterior roots (Danner et al. 2016), it can have wide-spread effects. We used paired stimulation to test homosynaptic depression

of the evoked TSCS responses (Minassian et al. 2007), and while results were within the range of what was previously reported (Hofstoetter et al. 2019, 2008; Andrews et al. 2015), contribution of motor neurons activation to evoked responses cannot be fully ruled out. Moreover, while TSCS can evoke spinal monosynaptic reflexes with similar characteristics to the H-reflex in multiple lower limb muscles from both sides (Courtine et al. 2007), it can provide the disadvantage when compared to the H-reflex to be somehow contaminated by heteronymous influences (Hofstoetter et al. 2019). Minassian et al. (2007) therefore warned about a possible influence between each evoked response (i.e., synergist facilitation; antagonist inhibition; contralateral influence). In a recent paper, different recovery cycles for post-activation depression were accordingly reported for soleus H-reflex and TSCS responses, suggesting that the site of stimulation may actually influence spinal loop excitability measurements (Hofstoetter et al. 2019). This can be the result of heteronymous monosynaptic facilitation of soleus Ia afferents by femoral nerve stimulation that can happen during TSCS. Why such heteronymous influences could have affected post-vibration measurements but not measurements during ongoing vibration in the present study remains however to be determined. Finally, it should also be acknowledged that while our H-reflex recording procedure, stimulating the superior portion of the popliteal fossa (i.e., before the bifurcation of the nerve into the common fibular and tibial nerve), allowed us to concomitantly record soleus and tibialis anterior responses, it may have provided the disadvantage to involve potential influence of reciprocal

inhibition on those responses (Crone et al. 1987). We cannot therefore rule out the possibility that this experimental choice might have increased the similarity between H-reflex and TSCS responses.

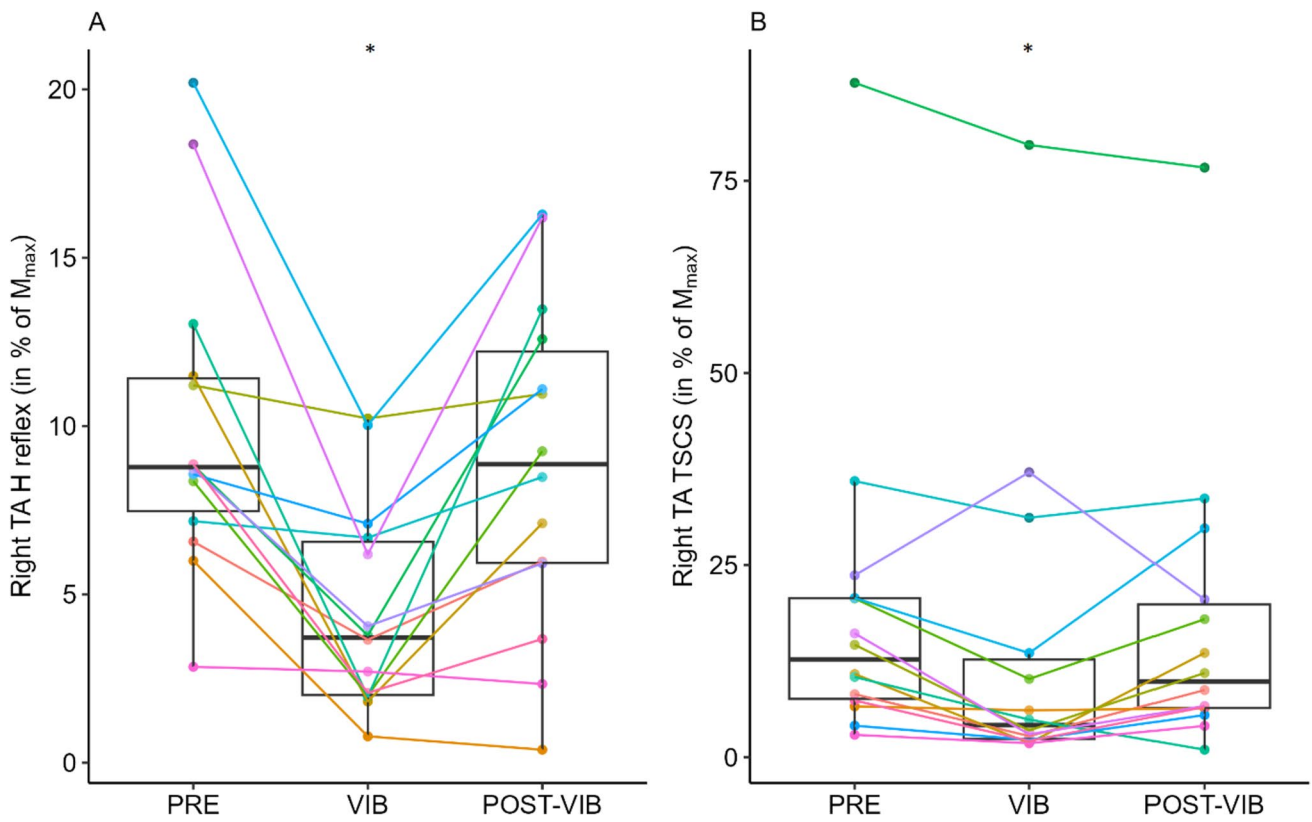
In conclusion, this study demonstrated that both soleus H-reflex and TSCS reflex responses can be depressed during ongoing Achilles tendon vibration as well as after prolonged vibratory exposure, confirming tendon vibration is a relevant model to modulate spinal loop excitability. Yet, while the sensitivity of TSCS seems to be similar to the gold standard H-reflex to highlight the vibratory paradox, both responses showed different sensitivity to the effects of prolonged vibration, suggesting slightly different pathways may actually contribute to evoked responses of both stimulation modalities.

## Appendix 1

Here we present data concerning the right Tibialis Anterior muscle.

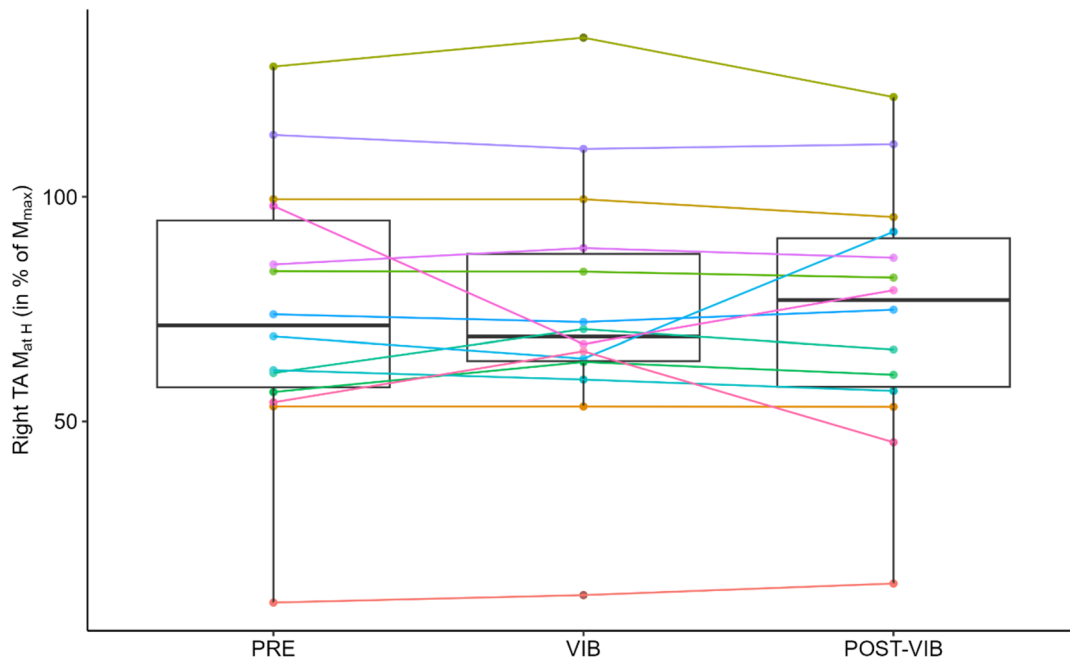
For the five chosen responses, the Generalized Estimation Equation showed a significant effect of time for the right TA H-reflex (Figs. 2 and 5A;  $P < 0.001$ ), with VIB but not POST-VIB values being significantly lower than PRE-values ( $P < 0.001$  and  $P = 0.18$ , respectively). Mean values for PRE, VIB and POST-VIB were  $10 \pm 1$ ,  $4 \pm 1$ , and  $9 \pm 1\%$  of  $M_{\max}$ , respectively. At the same time, right TA  $M_{\text{at H}}$  did not change over time (Fig. 6;  $P = 0.87$ ). Similarly, to the H-reflex, right TA TSCS responses changed over





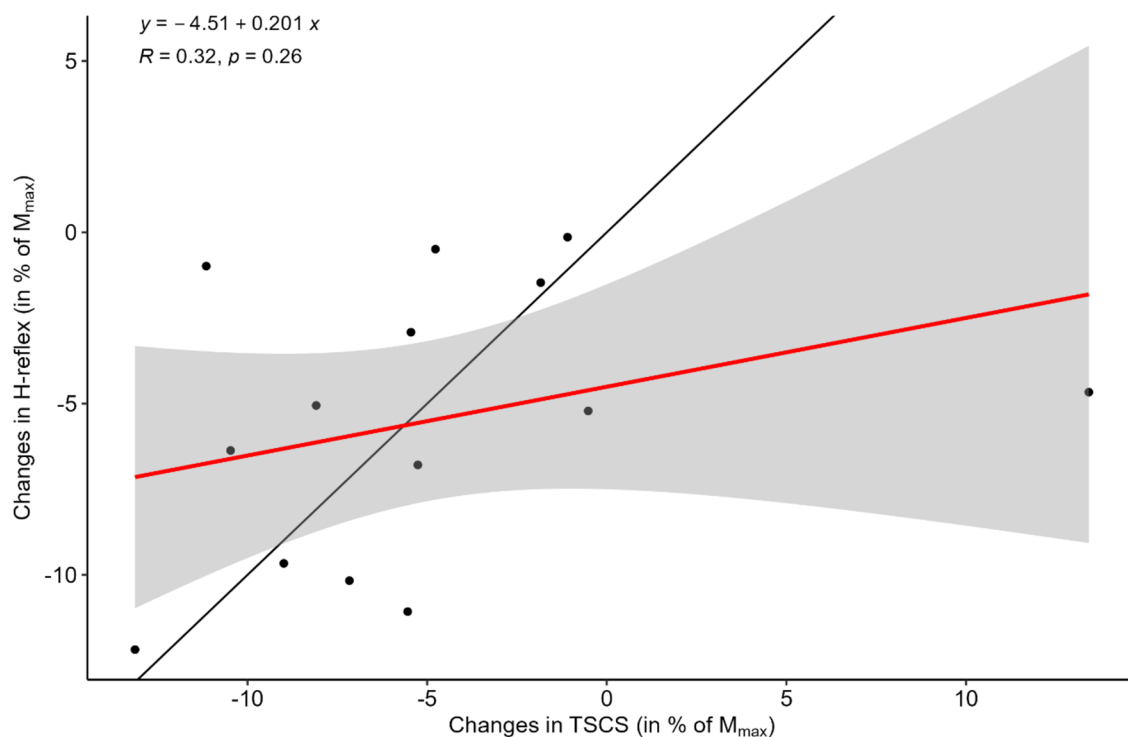
**Fig. 5** Boxplots showing right tibialis anterior (TA) H-reflex (A) and TSCS (B) responses at baseline (PRE), during right Achilles tendon vibration (VIB) and after prolonged exposure to vibration (POST-

VIB). Responses are expressed as a percentage of right TA  $M_{max}$ . \*Denotes significant differences when compared to PRE



**Fig. 6** Boxplots showing right tibialis anterior (TA) associated M-wave ( $M_{at.H}$ ) during H-reflex responses at baseline (PRE), during right Achilles tendon vibration (VIB) and after prolonged exposure

to vibration (POST-VIB). Responses are expressed as a percentage of right TA  $M_{max}$ , respectively



**Fig. 7** Pearson correlation plots between right TA PRE-to-VIB changes in H-reflex and TSCS responses amplitude (i.e., absolute changes expressed in percentage of  $M_{\max}$ ). The red line is the correlation, the black an identity slope (i.e., where  $y = x$ )

time (Fig. 5B;  $P < 0.001$ ), VIB but not POST-VIB values being significantly lower than PRE-values ( $P < 0.001$  and  $P = 0.25$ , respectively). Mean values for PRE, VIB and POST-VIB were  $19 \pm 6$ ,  $14 \pm 2$ , and  $17 \pm 1\%$  of  $M_{\max}$ , respectively.

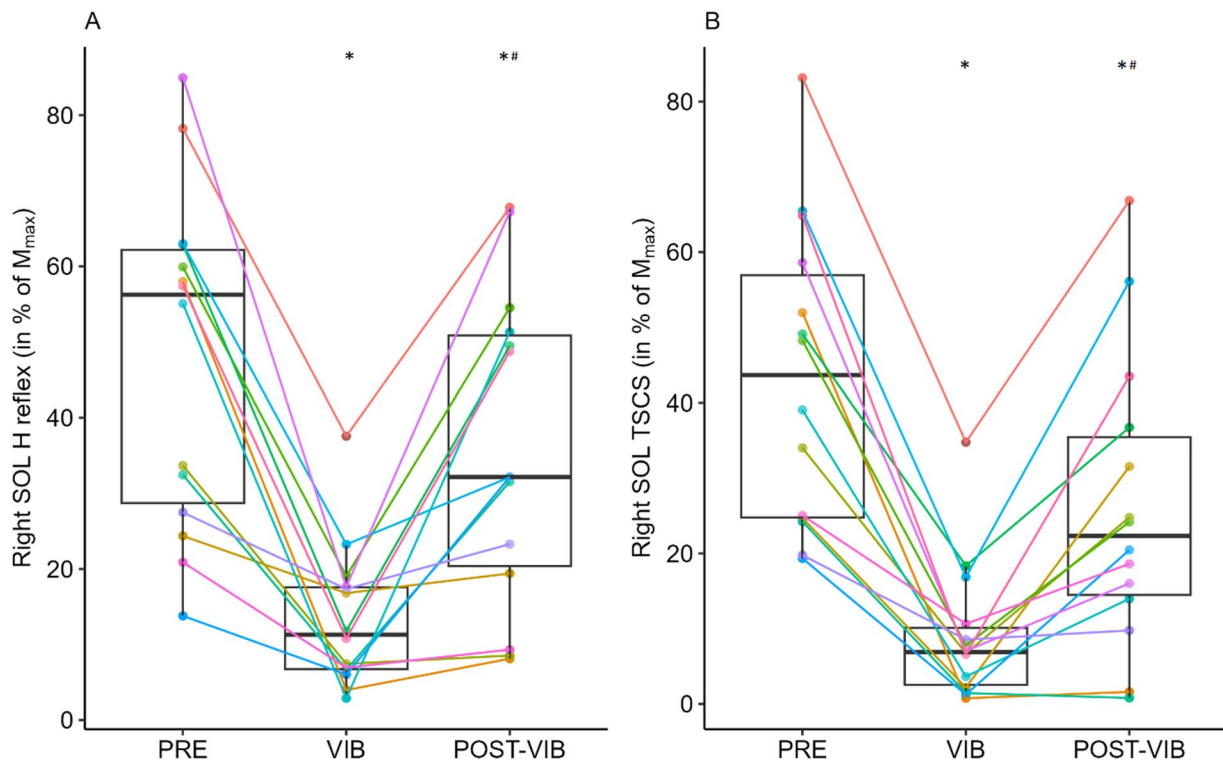
The CCC showed no correlation between the evolution of right TA H-reflex and TSCS responses over time (CCC = 0.003 [−0.09;0.09]). Pearson correlation analyses revealed no correlation between right TA H-reflex and TSCS responses PRE-to-VIB changes (Fig. 7;  $R = 0.32$ ,  $P = 0.26$ ).

## Appendix 2

Here we present the results section if one were to include every stimulation utilized in the present study. Notice how the CCC changes dramatically, while all other tests remain somewhat similar to the other analysis. The CCC is, coincidentally also the only statistical test that concerns the full dataset and includes the within-subject variation, not a mean value of  $x$  stimulations. One should be aware of the greater variation when interpreting the results in both result sections.

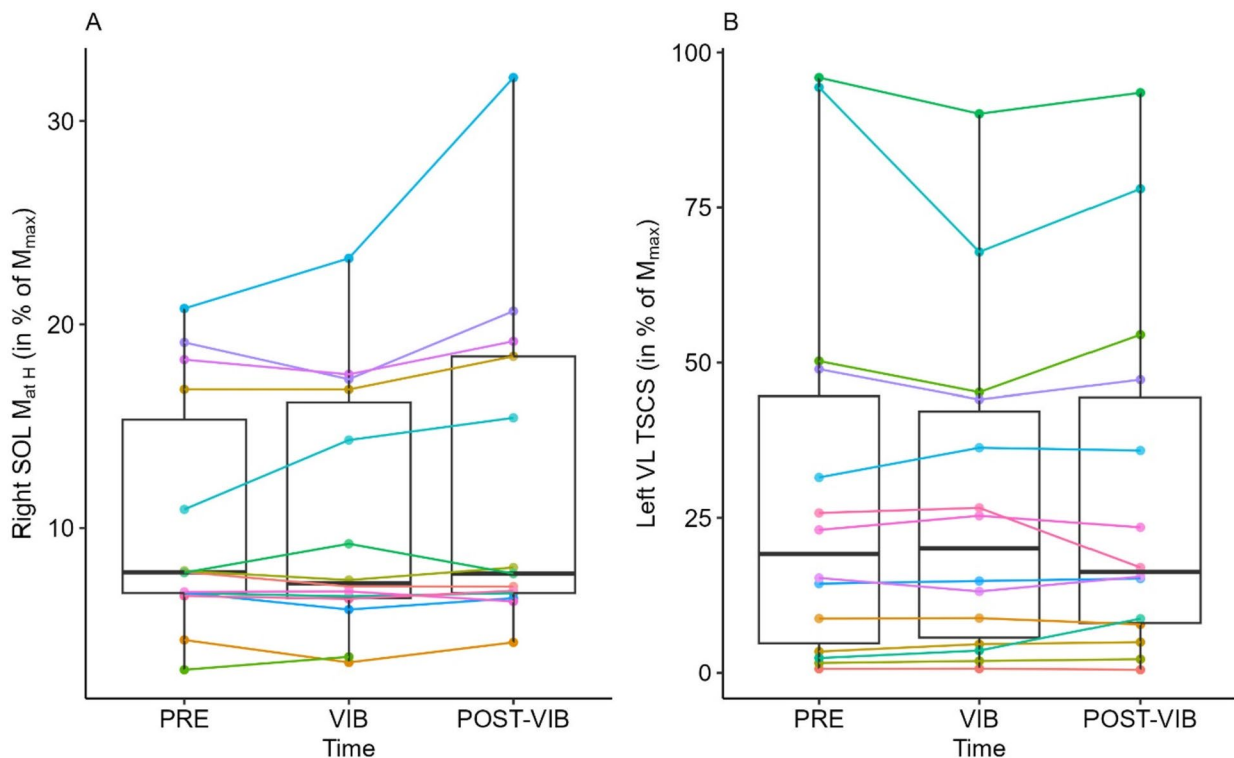
The Generalized Estimation Equation showed a significant effect of time for the right SOL H-reflex (Fig. 8A;  $P < 0.001$ ), with VIB and POST-VIB values being significantly lower than PRE-values ( $P < 0.001$  and  $P = 0.003$ , respectively). Mean values for PRE, VIB and POST-VIB were  $48 \pm 5$ ,  $13 \pm 5$ , and  $36 \pm 4\%$  of  $M_{\max}$ , respectively. At the same time, right SOL  $M_{\text{at H}}$  did not change over time (Fig. 9A;  $P = 0.69$ ). Similarly, to the H-reflex, right SOL TSCS responses changed over time (Fig. 8B;  $P < 0.001$ ), VIB and POST-VIB values being significantly lower than PRE-values ( $P < 0.001$ ) and POST-VIB values being greater than VIB values ( $P < 0.001$ ). Mean values for PRE, VIB and POST-VIB were  $43 \pm 5$ ,  $10 \pm 4$ , and  $25 \pm 4\%$  of  $M_{\max}$ , respectively. No effect of time was observed for the responses of the left VL (Fig. 9B;  $P = 0.13$ ).

The CCC showed a non-existent correlation between the evolution of right SOL H-reflex and TSCS responses over time (CCC = 0.10 [0.05;0.16]). Yet, Pearson correlation analyses revealed significant correlation between right SOL H-reflex and TSCS responses PRE-to-VIB changes ( $r = 0.82$ ,  $P < 0.001$ ; Fig. 10A), and for PRE- to POST-VIB changes ( $r = 0.54$ ,  $P = 0.047$ ; Fig. 10B).



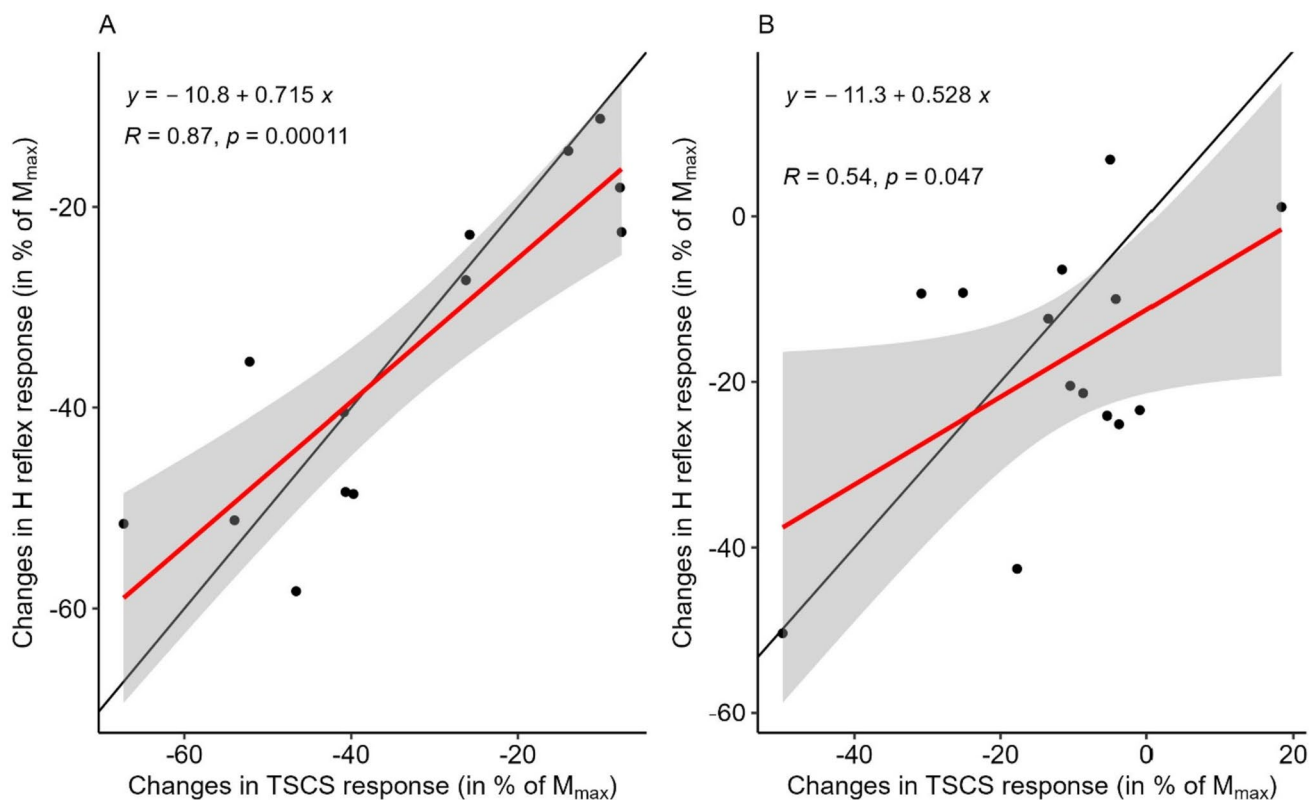
**Fig. 8** Boxplots showing right soleus (SOL) H-reflex (A) and TSCS (B) responses at baseline (PRE), during right Achilles tendon vibration (VIB) and after prolonged exposure to vibration (POST-VIB).

Responses are expressed as a percentage of right soleus M<sub>max</sub>. \*Denotes significant differences when compared to PRE. #Denotes significant difference when compared to VIB



**Fig. 9** Boxplots showing right soleus (SOL) associated M-wave during H-reflex recordings (A) and left vastus lateralis (VL) TSCS (B) responses at baseline (PRE), during right Achilles tendon vibra-

tion (VIB) and after prolonged exposure to vibration (POST-VIB). Responses are expressed as a percentage of right SOL and left VL M<sub>max</sub>, respectively



**Fig. 10** Pearson correlation plots between right SOL PRE-to-VIB changes (A) as well as between PRE- to POST-VIB changes (B) in H-reflex and TSCS responses amplitude (i.e., absolute changes

expressed in percentage of  $M_{\max}$ ). The red line is the correlation, the black an identity slope (i.e., where  $y = x$ )

**Author contributions** In accordance with CRediT taxonomy: Conceptualization: AG, CP, SG, AM, TL; Methodology: AG, CP, SG, AM, TL; Formal analysis and investigation: AG, CP, TL; Writing—original draft preparation: AG, TL; Writing—review and editing: AG, CP, SG, AM, TL; Funding acquisition: TL; Resources: AG, CP, TL; Supervision: TL. In accordance with submission guidelines: AG, CP, SG, AM, TL conceived and designed the study, AG and CP conducted experiments. AG and TL conducted initial analysis of data. AG and TL wrote first draft of manuscript. All authors contributed to secondary and final draft(s) of manuscript. All authors read and approved of the submitted manuscript.

**Data availability** The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Conflict of interest** None of the authors declare any conflict of interest—No funding was receiving for this study.

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