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RESEARCH PROTOCOL

The effects of transcutaneous spinal cord stimulation delivered with and without high-frequency modulation on spinal and corticospinal excitability

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Abstract

Transcutaneous spinal cord stimulation (TSCS) has been shown to improve motor recovery in people with spinal cord injury (SCI). Some groups deliver TSCS modulated with a kHz-frequency (TSCS–kHz); the intensity used for TSCS–kHz is usually set based on the motor threshold for TSCS, even though TSCS–kHz threshold is considerably higher than TSCS. As a result, TSCS–kHz interventions tend to be delivered at low intensities with respect to the motor threshold (~40%). In this study, we compared the effects of sub-threshold TSCS and TSCS–kHz, when delivered at similar intensity relative to their own motor threshold. Experiment I compared the after-effects of 20 min of sub-threshold (40% threshold) TSCS and TSCS–kHz on spinal and corticospinal excitability in able-bodied participants. Experiment II assessed the dose–response relationship of delivering short (10-pulse) trains of TSCS and TSCS–kHz at three different current intensities relative to the threshold (40%, 60%, and 80%). Experiment I found that 20 min of TSCS–kHz at a 40% threshold decreased posterior root reflex amplitude ($p < 0.05$), whereas TSCS did not. In experiment II, motor-evoked potential (MEP) amplitude increased following short trains of TSCS and TSCS–kHz of increasing intensity. MEP amplitude was significantly greater for TSCS–kHz compared with TSCS when delivered at 80% of the threshold ($p < 0.05$). These results suggest that TSCS and TSCS–kHz have different effects when delivered at similar intensity relative to their own threshold; both for immediate effects on corticospinal excitability and following prolonged stimulation on spinal excitability. These different effects may be utilized for optimal rehabilitation in people with SCI.

KEYWORDS

spinal cord stimulation, spinal cord injury, corticospinal excitability

1 | INTRODUCTION

In recent years, the application of spinal cord stimulation (SCS), both invasively and non-invasively, has been investigated as a therapeutic intervention to promote functional recovery[1–4] as well as to manage spasticity[5–10] in the spinal cord injury (SCI) population. SCS is thought to recruit large-to-medium diameter afferent nerve fibers

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within the posterior spinal roots.[11,12] Single pulses of SCS, delivered at the lumbosacral enlargement, transynaptically activate α-motoneurons, eliciting compound muscle action potentials in lower limb muscles, termed posterior root reflexes (PRRs).[13,14] SCS can be applied epidurally, involving the surgical implantation of electrodes, or transcutaneously (TSCS) via electrodes placed on the back, over the spinal cord. Studies suggest that TSCS activates the same neural structures as epidural SCS.[15]

Like studies using epidural SCS, TSCS has been applied with continuous biphasic pulses at 15–30 Hz,[1,16,17] however higher current amplitudes are required for TSCS, due to the layers of skin, bone, and fat between the electrode and nerve roots. To deliver high currents with less discomfort, some groups deliver TSCS modulated with high frequencies ranging from 5 to 10 kHz.[18–20] While higher current intensities could be tolerated when the TSCS was modulated with a kHz frequency (TSCS–kHz), much higher currents were also required to reach PRR threshold (~180–200 mA for TSCS–kHz, compared with ~50 mA for TSCS[21,22]), due to the TSCS–kHz waveform being active for half the duration of the TSCS waveform, and the apparently reduced neural recruitment efficiency with TSCS–kHz.[23] TSCS–kHz interventions are typically delivered in the range 60–120 mA[19,24]; therefore, they are delivered at considerably subthreshold intensities with respect to the PRR threshold (~40%). Despite the relatively low intensity being delivered, TSCS–kHz interventions are being used increasingly in clinical trials, and, in people with SCI, considerable increases in motor function have been reported.[19,24]

Previously, we investigated the effects of single pulses and trains of TSCS and TSCS–kHz on corticospinal excitability, when delivered at similar absolute current amplitudes.[22] We found that corticospinal excitability increased to a greater extent following bursts of TSCS, compared to TSCS–kHz, which was likely due to the higher intensity TSCS was delivered at, with respect to PRR threshold. We were surprised to find that TSCS–kHz did still significantly increase corticospinal excitability (albeit to a lesser extent than TSCS), even though it was delivered at ~30%–40% of the PRR threshold. This raised the question: would TSCS and TSCS–kHz have a similar effect on corticospinal excitability when delivered at comparable intensities with respect to their own PRR threshold? In the present study, we compared, in healthy, able-bodied human participants, the neurophysiological effects when TSCS and TSCS–kHz were delivered at similar intensities with respect to the PRR threshold.

The aims of our research were to determine (i) the effects of delivering 20 min of TSCS and TSCS–kHz at similar sub-threshold intensities (40% with respect to PRR threshold for each waveform) on post-stimulation spinal excitability and MEP amplitudes; and (ii) the immediate effects of delivering trains of TSCS and TSCS–kHz at various intensities relative to PRR threshold on corticospinal excitability.

2 | METHODS

This study was carried out at the Department of Medical Physics and Biomedical Engineering at University College London (UCL) and at the Aspire Centre for Rehabilitation Engineering and Assistive Technologies (CREATe) laboratories, at the Royal National Orthopedic Hospital. Ethical approval was provided by the UCL Research Ethics Committee (project ID number 14277/003) and all participants gave informed written consent prior to participating in the study. All experiments were carried out on healthy, able-bodied adults. Exclusion criteria were a history of epilepsy, metal implants in the head (other than dental) or close to the electrode sites, previous neurosurgery, or any neurological or musculoskeletal conditions involving the back or lower limbs.

Two experiments were carried out to determine the effects of delivering TSCS and TSCS–kHz on spinal and corticospinal excitability. To summarize: Experiment I compared 20 min of TSCS or TSCS–kHz delivered at 40% PRR threshold on corticospinal excitability, using MEPs, spinal excitability at the level of the spinal roots, using PRRs, and more distally in the lower limb, using the Hoffmann (H)-reflexes. Outcome measurements were assessed prior to the intervention, and for 30 min following the intervention.

As no effects on corticospinal excitability were noted, with either intervention, Experiment II assessed the dose–response relationship of delivering short (10-pulse) trains of TSCS or TSCS–kHz at three sub-PRR threshold intensities (40%, 60%, and 80%) on corticospinal excitability, assessed by MEPs. MEPs were evaluated at 3 different interstimulus intervals (ISIs) following the train to evaluate the time course of any changes.

2.1 | Experimental setup

The Experimental setup was similar for Experiments I and II. Participants were in a supine position on a physiotherapy plinth for the duration of each experiment. A wedge was positioned under the knees to maintain approximately 30° flexion at the hip and knee joints throughout the experiment. Electromyography (EMG) was recorded via adhesive surface electrodes (Ø 24 mm, Covidien, Medtronic, MI, USA), placed over tibialis anterior (TA) and soleus (SOL) muscles (see Figure 1). EMG signals

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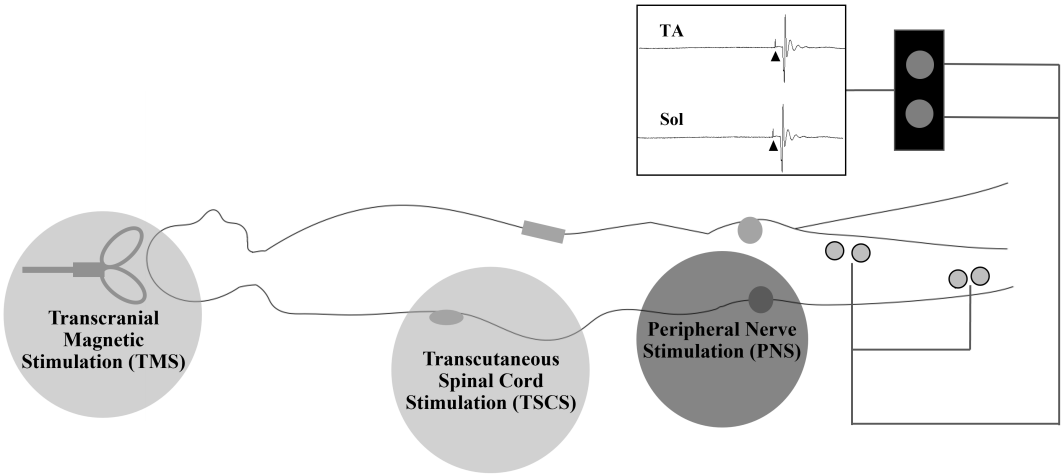

FIGURE 1 Schematic representation of the experimental setup of Experiment I and Experiment II. TSCS or TSCS–kHz delivered via a cathode placed over the T11–L1 vertebrae and anodes placed bilaterally over the iliac crests. PNS applied to the tibial nerve via a cathode placed within the popliteal fossa and anode over the patella (Experiment I only). TMS applied over the primary motor cortex. PNS, peripheral nerve stimulation; TSCS, transcutaneous spinal cord stimulation; TMS, transcranial magnetic stimulation.

were amplified (×1000) and filtered (2–10 000 Hz, with a 50 Hz notch) using a D360 patient preamplifier/amplifier system (Digitimer, Welwyn Garden City, Hertfordshire, UK), digitized at 5000 Hz (Digitimer 1401) and sampled into data acquisition software (Signal v7.07, Cambridge Electronic Design, Cambridge, UK).

2.2 | Experiment I

2.2.1 | Transcutaneous spinal cord stimulation intervention

The cathode (Ø 5 cm, PALS Neurostimulation Electrode, Nidd Valley Medical, Ltd., UK) was placed centrally between the T11—L1 vertebrae, and two interconnected 4 ×9 cm rectangular anodes (PALS Neurostimulation Electrode, Nidd Valley Medical, Ltd., UK) were placed bilaterally over the iliac crests.[13] TSCS was delivered using a constant current stimulator (DS8R, Digitimer, Welwyn Garden City, Hertfordshire, United Kingdom), which was triggered via Signal v7.07 software (Cambridge Electronic Design, Cambridge, UK). Initially, PRR threshold was determined, using single biphasic pulses of TSCS or TSCS–kHz, depending on the session. Stimulation current was increased progressively until peak-to-peak responses of >0.05 mV in the SOL muscle was recorded in at least 5 out of 10 consecutive stimuli. This current intensity was deemed to be threshold.

The TSCS or TSCS–kHz intervention was delivered for 20 min at 40% PRR threshold (or at the participant's level of tolerance, whichever was lower). TSCS was delivered using 1 ms biphasic pulses at 30 Hz, and TSCS–kHz was delivered using 1 ms bursts containing 10 monophasic pulses, each with a 50 µs pulse width, delivered at 30 Hz, with a 9090 Hz carrier frequency. We decided to provide the intervention at 40% of the threshold because, at this intensity, the absolute current amplitudes for TSCS–kHz were comparable with those used in clinical trials applying TSCS–kHz to people living with SCI, in the range 60 to 120 mA.[19,24]

2.2.2 | Outcome measures

MEPs

MEPs were elicited in the lower limbs by transcranial magnetic stimulation (TMS) over the leg area of the primary motor cortex with a MagStim200^2 stimulator (Magstim Co., Ltd., United Kingdom) and a double cone coil (posterior–anterior current direction). Initially, the MEP threshold was determined as the minimum stimulation intensity required to elicit peak-to-peak responses of >0.05 mV in the SOL muscle in at least 5 out of 10 consecutive stimuli.[25] MEPs were evoked at ~1.2× motor threshold for the remainder of the experiment.

Peripheral nerve stimulation (PNS)

PNS was applied to the tibial nerve using a constant current stimulator (DS7A, Digitimer, Welwyn Garden City, Hertfordshire, UK) to elicit H-reflexes. Two stimulating electrodes (Ø 2.5 cm) were used, with the cathode placed in the popliteal fossa and the anode placed laterally on the patella. A recruitment curve was conducted by applying single monophasic pulses (1 ms pulse width) every ~7 seconds, while progressively increasing stimulation current. Three pulses were applied at

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each intensity starting at just below threshold for the H-reflex and increasing until the peak-to-peak amplitude of the M-wave plateaued.

M-wave and H-reflex amplitudes were determined automatically in Signal v7.07 (Cambridge Electronic Design, Cambridge, UK) software by measuring peak-to-peak amplitudes in two windows at 5–15 and 30–40 ms post-stimulus, respectively (these were also checked visually), M-wave and H-reflex amplitude data were then plotted against current intensity, from this, the stimulation intensity required to elicit an H-reflex on the rising edge of the recruitment curve, halfway between where it first emerged (amplitude >0.05 mV) and H_{max}, was determined, This intensity was then used to elicit 10 H-reflexes at each time-point, to enable H-reflexes to be both inhibited and facilitated after the intervention. The H–M recruitment curve was also repeated at each time-point.

Posterior root reflexes (PRRs)

Stimulation was applied using a constant current stimulator (DS8R, Digitimer, Welwyn Garden City, Hertfordshire, UK). The same TSCS electrodes were used for the intervention and outcome measures. PRR threshold was determined, using single monophasic pulses of TSCS. Stimulation current was increased progressively until peak-to-peak responses of >0.05 mV in the SOL muscle were recorded in at least 5 out of 10 consecutive stimuli. At each time-point, ten PRRs were elicited at ~1.2 × PRR threshold. PRRs were always elicited using monophasic square wave pulses at a 1 ms duration, regardless of the intervention being delivered.

2.2.3 | Experimental protocol

Baseline recordings of all outcome measurements were initially recorded, prior to the intervention, including 10 × PNS single pulses, 10 × PRRs, 10 × MEPs, and an H–-M recruitment curve. Biphasic TSCS or TSCS–kHz was delivered for 20 min, depending on the session. After the 20-min intervention, the outcome measures were repeated at 0-, 15-, and 30-min post-intervention. Sessions occurred on different days, with at least 24 h separating each session. The order of sessions was randomized, and participants were unaware of which intervention they were receiving.

2.3 | Experiment II

2.3.1 | Transcutaneous spinal cord stimulation intervention

TSCS and TSCS–kHz were both delivered using the same electrode placements, and PRR thresholds were determined as in Section 2.2.1 of Experiment I.

Trains of 10 stimuli (1 ms pulse width), were delivered at 30 Hz, lasting 300 ms in total. Stimuli were either single 1 ms monophasic pulses (TSCS) or 1 ms bursts containing 10 monophasic pulses, each with a 50 μs pulse width, delivered at 9090 Hz (TSCS–kHz, see Figure 2).

Bursts of TSCS, either with a kHz carrier frequency (TSCS–kHz), or without (TSCS), were delivered at 40%, 60%, or 80% of PRR threshold, which preceded a pulse of TMS by an ISI of 50, 100, or 200 ms (measured from the final pulse in the train, see Figure 2).

2.3.2 | Outcome measures

MEPs

MEPs were elicited as described in Section 2.2.2 of Experiment I.

2.3.3 | Experimental protocol

Trains of non-invasive SCS were delivered within six different conditions in separate blocks; (i) TSCS delivered

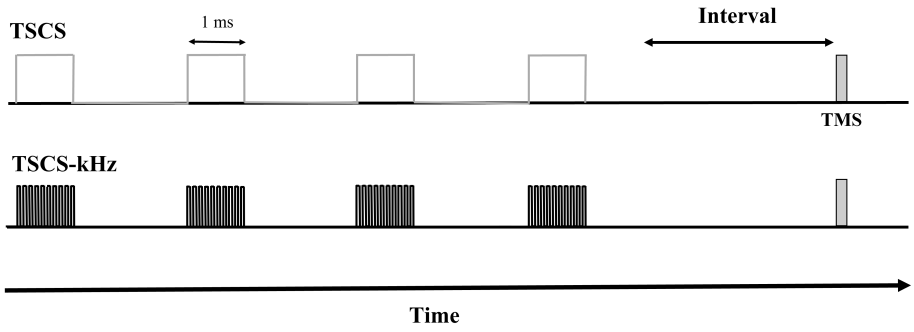

FIGURE 2 A burst of TSCS or TSCS–kHz delivered prior to a TMS pulse with a 50, 100 or 200 ms ISI. TSCS or TSCS–kHz was delivered at 40, 60, or 80% of PRR threshold. Not to scale. (PRR, posterior root reflex; TMS, transcranial magnetic stimulation; TSCS, transcutaneous spinal cord stimulation; TSCS–kHz, transcutaneous spinal cord stimulation with kHz frequency modulation.

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at 40%, 60%, or 80% of PRR threshold (TSCS40, TSCS60, TSCS80); (ii) TSCS–kHz delivered at 40%, 60%, or 80% of its corresponding PRR threshold (TSCS–kHz40, TSCS–kHz60, TSCS–kHz80), within a single session. Within each block, 10 trains of either TSCS or TSCS–kHz were delivered with an ISI of 50, 100, or 200 ms before the TMS pulse (see Figure 2, and 10 pulses of TMS alone were also delivered. The order of electrical stimulation conditions (i.e., TSCS or TSCS–kHz) was random, and within the type of electrical stimulation condition delivered, the three stimulation intensities (40%, 60%, or 80%) were also delivered randomly.

2.4 | Data analysis

Data analysis was carried out using Signal v7.07 software (Cambridge Electronic Design, Cambridge, UK) and Microsoft Excel 2016. All recorded PRRs, H-reflexes, and MEPs were initially visually inspected for pre-stim muscle activity; where muscle activity was present, it was measured, and the recording was discarded if pre-stim muscle activity was >0.05 mV peak-to-peak.

All the statistical analysis was carried out using SPSS statistics software (IBM Corporation, version 26, USA). All data were tested for normality using the Shapiro–Wilk's test. Where data were not normally distributed, log transformations were performed. Statistical significance was considered for $p < 0.05$.

2.4.1 | Experiment I

At each time-point, peak-to-peak amplitudes of PRRs, H-reflexes, and MEPs were measured within the following post-stimulus windows: 20–30, 30–40, and 30–50 ms, respectively (the data was also visually checked to ensure responses were within each window). H-reflex amplitudes were normalized to M_{max}, measured at each time-point. PRRs, H-reflexes, and MEPs elicited at each time-point post-stimulation were then averaged and normalized to the averaged responses at baseline.

Two-way repeated measures (RMs) ANOVAs were performed on MEP, PRR, and H-reflex amplitude data, to compare between intervention type (TSCS or TSCS–kHz) and time-point (Pre- and 0, 15, and 30 mins post-intervention). Post-hoc analysis was conducted with a Bonferroni correction.

To determine whether there was an effect of the order in which the interventions were given, paired t-tests were performed to compare MEP, PRR, and H-reflex amplitudes at baseline between each session.

2.4.2 | Experiment II

Peak-to-peak amplitude of MEPs was measured within a window of 30–50 ms after the TMS pulse (the data was also visually checked to ensure responses were within each window), and then averaged across each ISI within each block (TSCS or TSCS–kHz). The average MEP amplitude at each ISI was normalized to the TMS-only condition within each block.

A three-way ANOVA was performed on MEP amplitude data, normalized to the TMS-only condition, to compare between the type of non-invasive SCS (TSCS or TSCS–kHz), current intensity (40%, 60%, 80%) and ISI (50, 100, 200 ms). Post hoc analysis was carried out between current intensities and SCS type using two-way ANOVAs with a Bonferroni correction.

Regression analysis was carried out to investigate the dose–response relationship between the change in MEP amplitude (averaged across the three ISIs) and SCS intensity.

3 | RESULTS

3.1 | Experiment I

In total, twelve healthy participants (8 females, 4 males) took part in Experiment I. Mean (standard deviation (SD)) age, height, and weight were 24 (7.3) years, 170 (7.8) cm, and 66 (12.9) kg, respectively. Mean (SD) PRR thresholds for TSCS and TSCS–kHz were 52 (9.47) mA and 205 (42.0) mA, respectively. The current intensity and charge per pulse delivered during the intervention for each participant are provided in Table 1. Example TA PRR responses at each time point in one participant are shown in Figure 3.

A two-way RM ANOVA showed statistically significant effects of timepoint for SOL ($p = 0.013$) and TA ($p = 0.006$) muscles. Post-hoc analysis revealed a significant reduction in SOL PRR amplitude at 0-, 15-, and 30-min post-intervention compared to pre-intervention following TSCS–kHz only ($p < 0.05$; Figure 4C). A statistically significant reduction in TA PRR amplitude was also noted at 15- and 30-min after the TSCS–kHz intervention only ($p < 0.05$; Figure 4D). No significant differences were found in the PRR data following TSCS. No significant changes were found in the MEP (Figures 4A,B) and H-reflex (Figure 5) data, although H-reflex amplitude tended to reduce after the intervention for both TSCS and TSCS–kHz. Paired *t*-tests revealed no effect of the order in which the interventions were given.

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TABLE 1 PRR threshold, current intensity and charge per pulse for both TSCS and TSCS–kHz interventions delivered during Experiment I for each participant.

	TSCS intervention			TSCS–kHz intervention		
	PRR threshold (mA)	Current intensity (mA)	Charge per pulse (μC)	PRR threshold (mA)	Current intensity (mA)	Charge per pulse (μC)
01	65.0	26.0	26.0	190.0	76.0	38.0
02	39.0	15.6	15.6	170.0	68.0	34.0
03	51.0	20.4	20.4	280.0	112.0	56.0
04	58.0	23.2	23.2	240.0	96.0	48.0
05	46.5	18.6	18.6	155.0	62.0	31.0
06	43.0	17.2	17.2	180.0	57.2	28.6
07	41.0	16.4	16.4	214.5	85.8	42.9
08	56.0	22.4	22.4	170.8	68.3	34.2
09	60.0	24.0	24.0	242.0	96.8	48.4
10[a]	63.0	18.9	18.9	225.0	67.5	33.8
11	42.0	16.8	16.8	200.8	80.0	40.0
12	38.0	15.2	15.2	210.0	60.0	30.0

Abbreviations: PRR, posterior-root reflex; TSCS, transcutaneous spinal cord stimulation; TSCS–kHz, transcutaneous spinal cord stimulation with kHz frequency modulation.

[a]TSCS and TSCS–kHz interventions were delivered at 30% of PRR threshold for this participant, which was their maximum tolerated intensity.

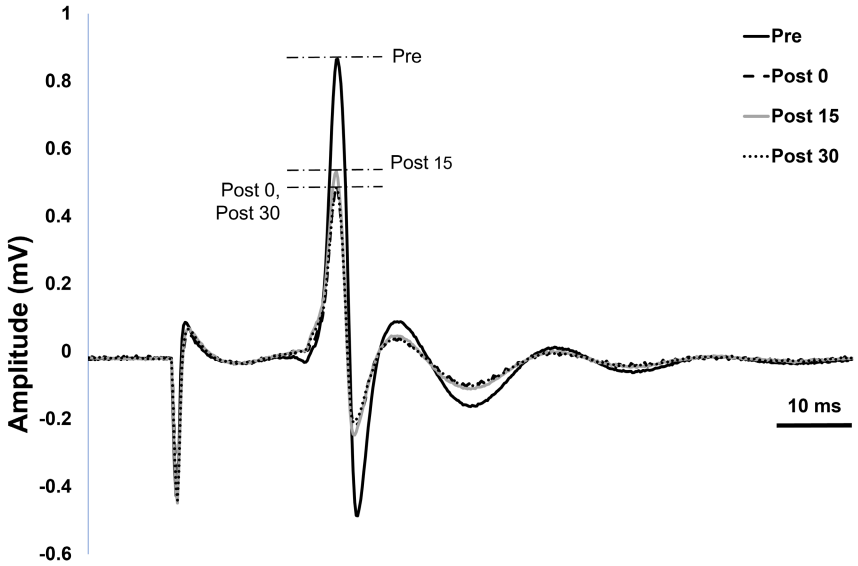

FIGURE 3 EMG traces of TA PRRs in one participant before TSCS–kHz (Pre), immediately following TSCS–kHz (Post 0), 15-min following TSCS–kHz (Post 15) and 30-min following TSCS–kHz (Post 30) in Experiment I. EMG, electromyography; PRR, posteriorroot reflex; TA, tibialis anterior.

3.2 | Experiment II

Ten healthy participants (4 females, 6 males) took part in Experiment II. Mean (SD) age, height, and weight were 22 (6.6) years, 172 (12.5) cm, and 63 (16.7) kg respectively. The mean (SD) PRR threshold for the TSCS and TSCS–kHz interventions were 40.7 (10.8) mA and 170 (44.6) mA respectively. The current intensity and charge per pulse delivered for each intensity and intervention type for each participant are provided in Table 2.

Three-way ANOVAs (intervention type × ISI × intensity) revealed a statistically significant main effect for current intensity, in both the SOL and TA muscles ($p < 0.01$; Figure 6). Post-hoc analysis revealed significant differences between 40% and 80% PRR threshold both for SOL ($p = 0.002$) and TA ($p = 0.005$). No main effect or interaction was found for ISI; however, two-way ANOVAs (intervention type × intensity) revealed an interaction in the SOL muscle ($p = 0.05$), and this approached significance in the TA muscle ($p = 0.07$). Within TSCS–kHz, MEP amplitude was significantly greater with 60% ($p = 0.02$) and 80% ($p < 0.001$) PRR threshold compared to 40%. No significant effects of intensity were found for TSCS. Finally, at the 80% PRR threshold, MEP amplitude was significantly greater with TSCS–kHz compared to TSCS ($p = 0.03$).

Regression analysis revealed a strong dose–response relationship between change in MEP amplitude and SCS intensity (Figure 7A,B) both for TSCS (SOL $R^2 = 0.97$

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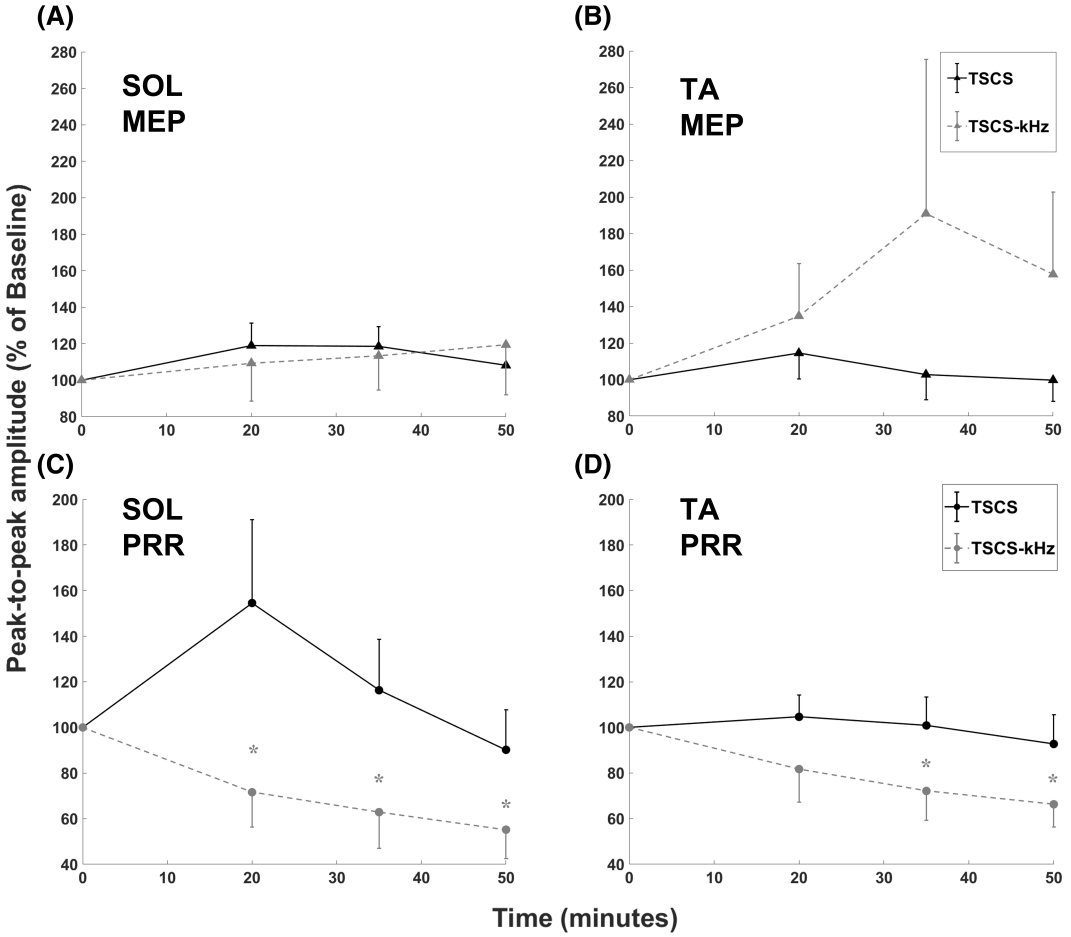

FIGURE 4 Normalized peak-to-peak change from baseline following 20 min of TSCS (black, solid line) or TSCS–kHz (gray, dashed line) of (A) SOL MEPs, (B) TA MEPs, (C) soleus PRRs and (D) tibialis anterior PRRs. Mean ± SEM. MEP, motor-evoked potential; PRR, posterior-root reflex; SOL, soleus; TA, tibialis anterior; TMS, transcranial magnetic stimulation; TSCS, transcutaneous spinal cord stimulation; TSCS–kHz, transcutaneous spinal cord stimulation with kHz frequency modulation. *represents results for which $p<0.05$-t-hoc analysis.

and TA $R^2=0.91$) and TSCS–kHz (SOL $R^2=1.0$ and TA $R^2=0.89$).

4 | DISCUSSION

In this study, we compared the effects of TSCS and TSCS–kHz, when applied at comparable sub-threshold intensities with respect to the PRR threshold. In both experiments, the PRR threshold was found to be approximately four times higher when TSCS–kHz was used, compared with TSCS. This agrees with previous studies,[21–23] and may reflect less efficient activation of afferent fibers with TSCS–kHz, as proposed by Dalrymple et al.[23] We found that TSCS and TSCS–kHz, delivered at 40% PRR threshold, either over 20 min or in a short (10-pulse train), had no effect of corticospinal excitability. After a 20-min intervention of TSCS–kHz only, a reduction in PRR amplitude was found in TA and SOL, which lasted for 30 min after the intervention.

4.1 | Experiment I

4.1.1 | Corticospinal excitability

This experiment has shown, in healthy human volunteers, that a 20-min intervention of TSCS or TSCS–kHz, delivered at 40% of PRR threshold, had no effect on corticospinal excitability (MEP amplitude). Previous studies applying an intervention of TSCS in healthy participants have delivered TSCS at or close to the PRR threshold and found either increased[26] or unchanged[27] corticospinal excitability after the intervention. The inconsistency in findings is likely related to the differences in the TSCS intensity used. Higher intensity TSCS appears more likely to result in lasting increases in corticospinal excitability, however many healthy participants cannot tolerate TSCS delivered continuously at an intensity close to PRR threshold, and the highest tolerable threshold is often used, which can vary substantially from person to person. Indeed, in our experiment, there was one

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participant who could not tolerate TSCS at the required intensity, even though it was delivered well below PRR threshold.

In agreement with our findings, a previous study that delivered an intervention of TSCS–kHz in healthy participants, found no significant effect on MEP amplitude,[27] which is likely due to the fact that TSCS–kHz was delivered at a low intensity with respect to PRR threshold in both studies (~30%–40%). Overall, our data indicate that non-invasive SCS delivered at a low intensity with respect to the PRR threshold does not have a lasting effect on corticospinal excitability, regardless of whether or not kHz modulation is applied. Due to impairments in sensation to varying degrees in people with SCI, it is possible that stimulation can be tolerated at a higher intensity.

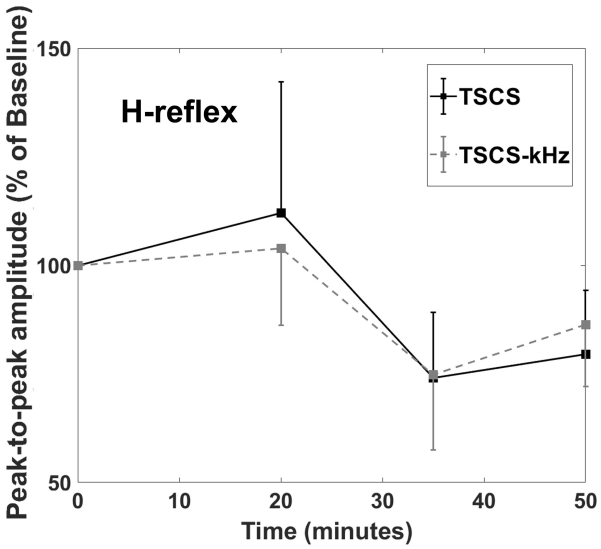

FIGURE 5 Normalized peak-to-peak change of H-reflexes from baseline following 20 min of TSCS (black, solid line) or TSCS–kHz (gray, dashed line). Mean ± SEM. TSCS, transcutaneous spinal cord stimulation; TSCS–kHz, transcutaneous spinal cord stimulation with kHz frequency modulation.

4.1.2 | Spinal excitability

TSCS has previously been reported to attenuate hyperexcitability and recover spinal inhibitory control in people with SCI when delivered at an intensity that induces paraesthesia, just below motor threshold[7,14,28] and has been successfully used in the treatment of spasticity.[29,30] In the present study, we found that H-reflex amplitude tended to reduce after TSCS, although this was not statistically significant, and PRR amplitudes were also unaffected after 20 min of TSCS. Our data likely contradicts previous studies because we applied TSCS at a low intensity with respect to threshold (to directly compare it to TSCS–kHz). In agreement, a recent study in healthy participants using cervical TSCS found no effect of 10 min of sub-threshold TSCS on PRR amplitude.[31] They did not report the precise sub-threshold intensity used, but average PRR threshold was reported to occur at 57 mA, and the intervention was given at 20 mA on average, similar to the 40% PRR threshold used here. We may have found greater effects on spinal inhibition if we had delivered TSCS at an intensity closer to the threshold.

In contrast, TSCS–kHz, delivered for 20 min at a 40% PRR threshold, had an inhibitory effect on PRR amplitude in both SOL and TA for at least 30 min post-intervention.

TABLE 2 PRR threshold and charge per pulse for bursts of TSCS and TSCS–kHz delivered at 40%, 60%, and 80% of PRR threshold during Experiment II for each participant.

	TSCS				TSCS–kHz			
	PRR threshold (mA)	Charge per pulse (μC)			PRR threshold (mA)	Charge per pulse (μC)		
		40%	60%	80%		40%	60%	80%
01	44.0	17.6	26.4	35.2	96	19.2	28.8	38.4
02	28.0	11.2	16.8	22.4	112	22.4	33.6	44.8
03	39.0	15.6	23.4	31.2	172	34.4	51.6	68.8
04	52.0	20.8	31.2	41.6	188	37.6	56.4	75.2
05	44.0	17.6	26.4	35.2	226	45.2	67.8	90.4
06	25.0	10.0	15.0	20.0	138	27.6	41.4	55.2
07	50.0	20.0	30.0	40.0	182	36.4	54.6	72.8
08	43.0	17.2	25.8	34.4	194	38.8	58.2	77.6
09	27.0	10.8	16.2	21.6	160	32.0	48.0	64.0
10	55.0	22.0	33.0	44.0	231	46.2	69.3	92.4

Abbreviations: PRR, posterior root reflex; TSCS, transcutaneous spinal cord stimulation; TSCS–kHz, transcutaneous spinal cord stimulation with kHz frequency modulation.

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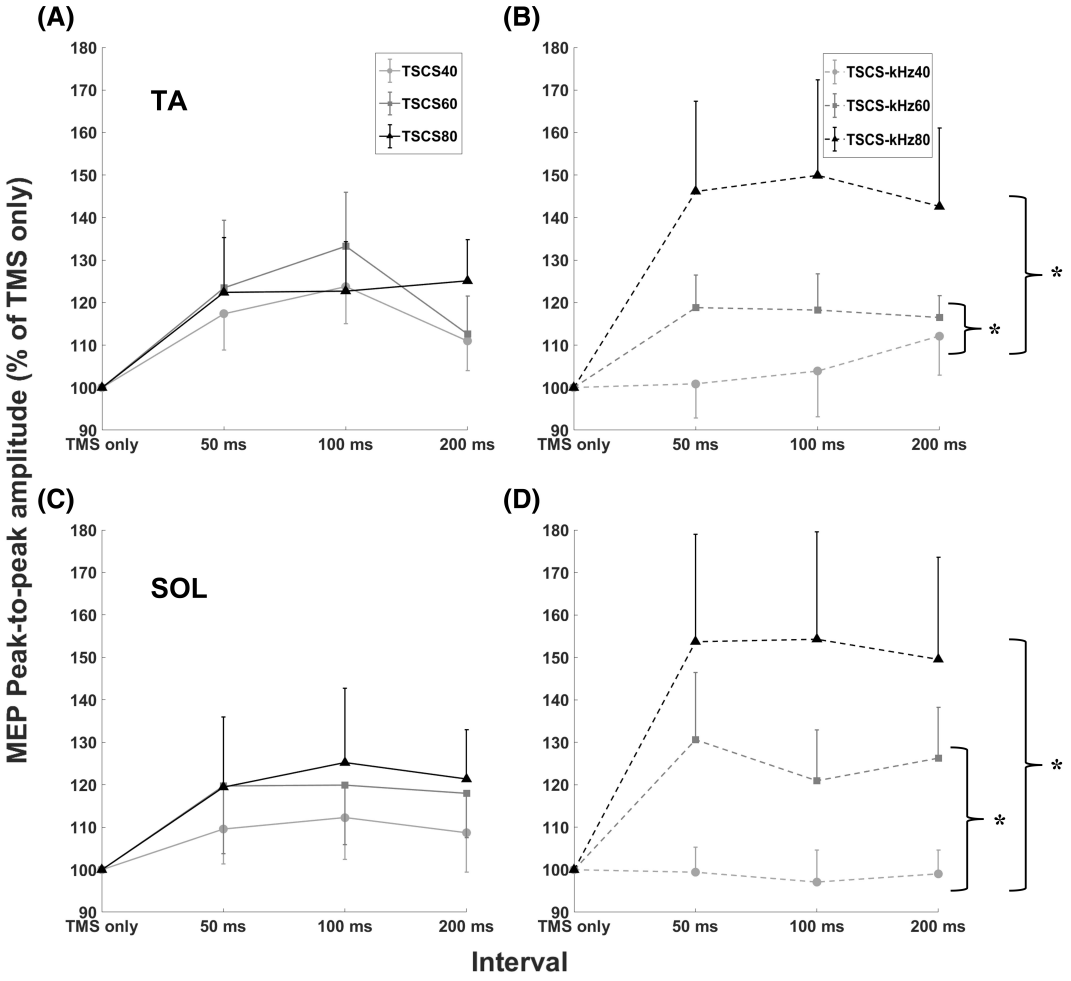

FIGURE 6 Peak-to-peak MEP changes normalized to TMS-only state MEPs. (A) TA MEPs following TSCS, (B) TA MEPs following TSCS–kHz, (C) SOL MEPs following TSCS, (D) SOL MEPs following TSCS–kHz. Mean ± SEM. MEP, motor-evoked potential; SOL, soleus; TA, tibialis anterior; TMS, transcranial magnetic stimulation; TSCS, transcutaneous spinal cord stimulation; TSCS–kHz, transcutaneous spinal cord stimulation with kHz frequency modulation. *represents results for which $p<0.05$.

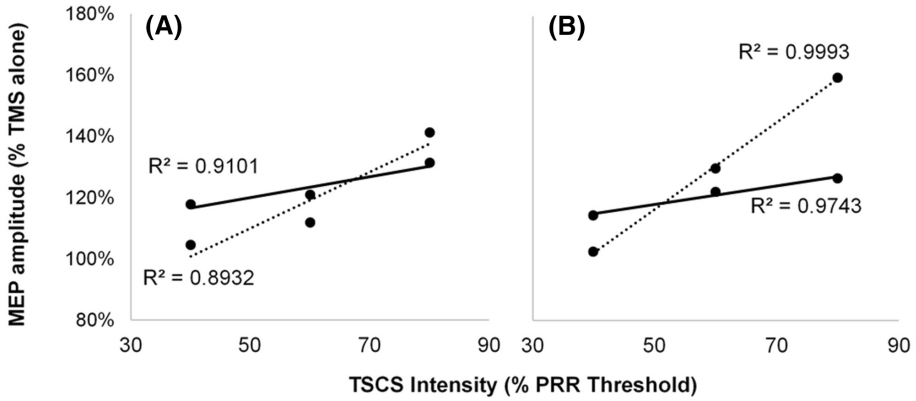

FIGURE 7 Relationship between stimulation intensity (% PRR threshold) and mean MEP amplitude (% MEP amplitude elicited by TMS alone) for TA (A) and SOL (B) muscles following a short (10-pulse) train of TSCS (solid line) and TSCS–kHz (dotted line). MEP, motor-evoked potential; PRR, posterior-root reflex; SOL, soleus; TA, tibialis anterior; TMS, transcranial magnetic stimulation; TSCS, transcutaneous spinal cord stimulation; TSCS–kHz, transcutaneous spinal cord stimulation with kHz frequency modulation.

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While the two interventions (TSCS and TSCS–kHz) were given at a similar intensity with respect to the PRR threshold, the greater charge per pulse with TSCS–kHz (see Table 1) may have altered the current field, causing its spread across a larger number of large-to-medium diameter afferent nerve roots, smaller diameter group II fibers, cutaneous afferents, and/or intraspinal connections and spinal interneurons,[32] resulting in stronger inhibition.

The reduction in spinal inhibition evidenced by reduced PRR amplitude was not reflected in the measured H-reflexes, which did not significantly reduce following the intervention. This disparity may be explained by the SOL PRR amplitude being influenced by a greater number of heteronymous projections activated by SCS, compared with the homonymous H-reflex.[33] Indeed, Andrews et al[33] reported greater post-synaptic inhibition in PRR amplitude, when it had been conditioned by a preceding PRR, compared with post-synaptic inhibition of the H-reflex, also conditioned by a preceding PRR, particularly when stimulation was given at a low or medium intensity.

Sub-threshold TSCS–kHz has been used in several studies exploring its therapeutic effects in people with SCI.[19,24,32,34] These small clinical trials report improved motor control in the presence of TSCS–kHz, delivered at similar absolute current amplitudes as we used here, which is thought to be due to the modulation of spinal networks. Our data indicate that TSCS–kHz at this intensity inhibits spinal excitability, which may, at least in part, enable voluntarily driven movements in people with SCI by reducing the neural hyperexcitability that causes spasticity in this population.[35] While this contradicts the widely held hypothesis that voluntary driven movements are enabled by SCS due to an increase in neural excitability, bringing membrane potential closer to the threshold,[18] the actual mechanisms are likely to be more complex, involving both spinal and supraspinal mechanisms.

4.2 | Experiment II

This experiment explored the dose–response relationship of short (10-pulse) trains of TSCS and TSCS–kHz on corticospinal excitability. For both TSCS and TSCS–kHz, there was a strong relationship between SCS intensity and the change in MEP amplitude ($R^2 > 0.89$). While short trains of TSCS tended to increase corticospinal excitability (Figure 6A,C), there was no statistically significant differences in the change in corticospinal excitability between the three intensities. Short trains of TSCS–kHz, however, delivered at 60% or 80% PRR threshold, did significantly increase corticospinal excitability compared with 40%. MEP amplitude was also increased to a greater extent following TSCS–kHz compared to TSCS when delivered at 80% PRR threshold ($p < 0.01$). Given the reduction in spinal excitability following 20 min of TSCS–kHz (delivered at 40% PRR threshold), observed in Experiment I, it is possible that the supraspinal effects of TSCS–kHz are actually greater than those reported here.

We have previously reported[22] that MEP amplitude significantly increased following a short train of TSCS when delivered at supra-threshold intensity. In the present study, we observed an increase in MEP amplitude immediately following TSCS–kHz, when delivered at sub-threshold intensities, between 60% and 80% PRR threshold, compared with 40% threshold, and the increase was greater than following TSCS. Presumably, the increased charge delivered using TSCS–kHz (Table 2) affected a larger area of neural tissue, influencing a greater number and/or type of nerve fibers,[32] as described earlier. This requires further investigation to better understand the clinical implications.

Taken together, the results of the two experiments highlighted differences in the modulation of the corticospinal system when TSCS and TSCS–kHz are delivered at similar sub-threshold intensities with respect to their own PRR threshold. It is possible that TSCS and TSCS–kHz need to be delivered at different intensities with respect to the PRR threshold to be effective rehabilitation interventions. However, future experiments should compare the effects of TSCS and TSCS–kHz when delivered with similar charges per pulse, to better understand these differences. It is possible that each form of SCS may have different applications for clinical rehabilitation.

4.3 | Limitations

There are some limitations to the studies we have carried out, which need to be acknowledged. First, the experiments were conducted on healthy, able-bodied human participants, who may have different neurophysiological responses to the electrical stimulation, compared to individuals living with SCI. In this article, we aimed to directly compare the neurophysiological effects of TSCS and TSCS–kHz, which can be done in a controlled way in healthy participants. However, we acknowledge that similar results may not be found in the SCI population, and it is important to repeat this research in people with SCI. Second, we provided an intervention of TSCS or TSCS–kHz, which was not combined with any rehabilitative intervention, Again, we did this to control as many variables as possible when comparing the two TSCS modalities. However, the importance of combining therapeutic SCS with task-directed training has been highlighted,[36] and this comparison should be further explored when combined with rehabilitation. Finally,

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the effects of a short train of TSCS and a 20-min intervention may be substantially different, therefore, the comparison of findings between Experiments I and II should be taken with caution, and further investigations are required to fully understand the neurophysiological changes in the short and longer terms.

5 | CONCLUSION

The present studies have shown, in healthy human volunteers, that 20 min of TSCS–kHz delivered at 40% PRR threshold caused a greater reduction in PRR amplitudes than TSCS delivered at a similar intensity with respect to PRR threshold. In addition, short trains of TSCS–kHz, delivered at 60%–80% PRR threshold, increased corticospinal excitability to a greater extent than TSCS–kHz delivered at 40% PRR threshold, and TSCS–kHz increased corticospinal excitability to a greater extent than TSCS, when delivered at 80% threshold. It is possible that these two forms of non-invasive SCS need to be applied at different intensities with respect to PRR threshold when used in a clinical setting.

AUTHOR CONTRIBUTIONS

None.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

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