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A single session of motor imagery paired with spinal stimulation improves manual dexterity and increases cortical excitability after spinal cord injury

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ABSTRACT

Objective: Non-invasive stimulation of the spinal cord at the cervical level (TSCS) can induce neural plasticity and improve upper limb function in people living with cervical spinal cord injury (SCI) when paired with task practice. The aim of this study was to investigate the effects of a session of motor imagery (MI) paired with TSCS on manual dexterity, corticospinal and spinal excitability in people living with cervical SCI.
Methods: Eight participants (4 females, mean age 46yrs ± 17) completed three sessions of: 1) MI; 2) TSCS at C5–C6 level; 3) MI + TSCS, listening to the MI script while receiving TSCS. Manual dexterity was assessed with the Purdue Pegboard Test (PPT), corticospinal excitability was assessed with Transcranial Magnetic Stimulation (TMS) delivered at motor threshold and suprathreshold (120 % intensities, and spinal excitability delivered at motor threshold and suprathreshold (110 %, 120 %) intensities was assessed with single pulses of TSCS.
Results: Manual dexterity increased from baseline after all three conditions ($p = 0.016$). Corticospinal excitability increased from baseline after MI ($p = 0.002$] and MI + TSCS ($p = 0.031$], but not TSCS ($p = 0.343$). Spinal excitability was not affected by any of the conditions ($p = 0.425$).
Conclusions: These findings demonstrate that a single session of MI and TSCS, either alone or in combination, can increase manual dexterity in people living with cervical SCI. The increase in dexterity was paralleled by increases in corticospinal excitability for the MI and MI + TSCS conditions.
Significance: Our findings indicate that MI and TSCS improve manual dexterity and increase corticospinal excitability in people living with cervical SCI when employed in isolation or in combination.

1. Introduction

Spinal cord injuries (SCI) at the cervical level can lead to loss of function in the upper and lower limbs (tetraplegia) resulting in a detrimental effect on quality of life (Anderson, 2004). Given this, developing rehabilitation protocols specifically targeting upper limbs function after SCI is considered a clinical priority (Anderson, 2004). One of the therapies which yielded promising results across the last decades is epidural electrical stimulation (ES) of the spinal cord, in which electrical current is delivered at a specific spinal cord level through an electrode implanted in the epidural space (Lu et al., 2016). ES has been shown to provide beneficial effects across a wide range of domains including pain, spasticity, walking abilities and hand strength/control. Nevertheless, complications and risks can arise after ES due to the invasive procedure of implanting electrodes (Jervis Rademeyer et al., 2021). Therefore, in recent years non-invasive alternatives to ES not requiring electrode implantation, such as transcutaneous spinal cord stimulation (TSCS), have been implemented. TSCS employs low-intensity electrical stimulation delivered via surface electrodes placed directly on the spinal processes (Gad et al., 2018). When combined with task practice, TSCS applied at the cervical level of the spinal cord has been reported to improve grip strength and hand functions in cervical SCI participants (Inanici et al., 2021, Moritz et al., 2024). These findings support the use of TSCS as one of the most promising non-invasive techniques for upper limb rehabilitation after SCI (Inanici et al., 2021).

The use of functional task practice for upper limb rehabilitation after SCI is hindered by the limited residual function of some patients (Mateo et al., 2015). A promising approach for patients who cannot engage in task practice is the use of motor imagery (MI) (Gerasimenko et al., 2018). MI represents the "conscious access to the content of the intention of a movement" (Jeannerod, 1995). In a typical MI practice session, participants listen to an audio recording instructing them to internally

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visualise the execution of a movement/task in all his visual, motor and sensory components (Ingram et al., 2016). Thus, while research shows MI practice (Mateo et al., 2015) and TSCS when combined with structured rehabilitation (Moritz et al., 2024) or Robotic Exoskeleton Rehabilitation (García-Alén et al., 2023) promote upper-limb motor recovery after a SCI, to our knowledge no study has investigated the effects of combining motor imagery with non-invasive spinal stimulation on skilled behaviour and cortical/spinal excitability in the cervical SCI population.

Neuroimaging and non-invasive brain stimulation work have yielded data elucidating the neurophysiological mechanisms associated with the motor improvements observed after MI. For example, fMRI data showed that MI activates the same network of motor areas which are active during overt movement production (Lotze et al., 1999). The excitability of the corticospinal tract, as assessed via recording of motor-evoked potential (MEPs) upon transcranial magnetic stimulation (TMS), increases during (Facchini et al., 2002, Gerasimenko et al., 2018) and after MI practice (Leung et al., 2013), indicating that MI can affect cortical structures and influence the excitability of spinal motoneurons even in the absence of movement. This evidence is confirmed by neuroimaging data showing that MI induces activity in a large network of motor cortical areas, although to a lesser extent than the one produced by actual movements (Lacourse et al., 2004). While the underlying cortical mechanisms mediating the effects of MI on motor imagery have been extensively studied (Ladda et al., 2021), there is still debate regarding the role of spinal neuronal populations in mediating the behavioural effects observed after MI practice (Grosprêtre et al., 2016).

While there is no consensus on the neurophysiological mechanisms underlying the effects of TSCS (Milosevic et al., 2019), a recent study investigated the effects of a session of 20-minutes of continuous cervical TSCS on cortical and spinal excitability in healthy and SCI participants (Benavides et al., 2020). Spinal responses recorded from upper-limb muscles upon cervicomedullary stimulation were observed to increase after TSCS compared to sham stimulation in healthy and SCI participants. This finding is in line with the emerging evidence that activating spinal cord networks through TSCS facilitate spinal excitability in able-bodied participants (Barss et al., 2022). However, Benavides et al. (2020), also reported that TMS-induced MEP amplitudes were not affected by TSCS or sham stimulation in healthy and SCI participants if a high-frequency carrier frequency was employed. Only when stimulation was delivered without the high-frequency component (5 kHz), facilitation of corticospinal output became evident (Benavides et al., 2020). Similarly, Kumru et al. (2021a) reported that corticospinal excitability (measured through MEP threshold) was not affected by cervical spinal stimulation when delivered alone, but increased when cervical spinal stimulation was delivered alongside hand training. In line with this result, we reported little effect of a single session of TSCS including a 5 kHz carrier frequency when delivered in isolation on cortical excitability, suggesting that TSCS exerted a limited effect on cortical excitability in neurologically intact participants (Capozio et al., 2023). However, when TSCS was paired with MI, changes in spinal and corticospinal excitability in healthy participants were observed (Capozio et al., 2023). Nevertheless, the short-term effects of MI and TSCS when delivered in combination on the excitability of cortical and spinal neuronal populations in people with SCI remain largely unknown.

The current study had three specific aims: (1) to compare the effects of a single session of MI, TSCS and MI + TSCS on manual dexterity, assessed with the Purdue Pegboard Test (PPT); (2) to compare the effects of a single session of MI, TSCS and MI + TSCS on cortical excitability, measured by cortical stimulation of forearm muscles; (3) to compare the effects of a single session of MI, TSCS and MI + TSCS on spinal excitability, measured by spinal stimulation of arm muscles in people with a cervical SCI.

2. Methods

2.1. Participants

Eight participants with a cervical SCI (4 females, mean age 46 ± 17) volunteered to the study. Participants characteristics are listed in Table 1. Participants were included in the study if right-handed, as assessed by the Edinburgh Handedness Questionnaire (M ± SD = 65 ± 19, scores higher than 40 indicate right-handedness) (Oldfield, 1971). Inclusion criteria included having sustained a SCI between the cervical levels of C2 and C7 at least six months before enrolling to the study, being aged between 18 and 80 and not having implanted metallic or magnetic devices. All participants had incomplete spinal cord injury (American Spinal Injury Association Impairment Scale B-D, Table 1). All participants completed four experimental sessions separated by at least 7 days to avoid the influence of carry-over effects of stimulating the brain (Nitsche et al., 2008). Sessions were scheduled at the same time of the day to control for potential influences of circadian rhythms (Capozio et al., 2023, Sale et al., 2007). The order of allocation to conditions was pseudo-randomised and counterbalanced across participants. Reporting of methods follows the guidelines for minimum reporting standard for spinal stimulation studies in SCI (Malik et al., 2024). All participants gave written informed consent to experimental procedures approved by the Faculty of Biological Sciences Ethical Review Committee (BIOSCI 20–020) at the University of Leeds and conformed to the Declaration of Helsinki.

2.2. Purdue pegboard test (PPT)

We assessed manual dexterity with the Purdue pegboard test (PPT) (Tiffin and Asher, 1948). The participants sat in front of a plastic board with two parallel rows, one on the left and one on the right, of 25 holes. On the top of the board are located small cylindrical metallic pegs placed in a shallow container. The participants were instructed to place as many pegs as possible in the holes on the right side of the board, picking them up one by one (Tiffin and Asher, 1948). Before starting the trial, the experimenter demonstrated the movement and then allowed participants to practice the trial three times (Desrosiers et al., 1995). Participants were instructed on when to start and end the trial via a stopwatch.

2.3. Electromyography (EMG) measures

We recorded surface EMG activity from the following right upper limb muscles: abductor pollicis brevis (APB), electrode placed at the midpoint between the first metacarpophalangeal joint and carpometacarpal joint (Perotto, 2011); flexor carpi radialis (FCR), electrode placed at one-third of the distance from the medial epicondyle to radial styloid (Capozio et al., 2021b); extensor carpi radialis longus (ECRL), electrode placed at one-sixth of the distance from the lateral epicondyle to radial styloid (Riek et al., 2000); biceps brachii (BB), electrode placed at one-third of the distance from cubital fossa to medial acromion (Madeleine and Arendt-Nielsen, 2005). For EMG acquisition, parallel-bar wireless sensors (3.7 × 2.6 cm) (Trigno, Delsys Inc., Natick, MA, USA) for APB, FCR and BB and a parallel- bar wireless mini sensor (Trigno, Delsys Inc., Natick, MA, USA) for APB were employed. Raw EMG recordings were pre-amplified (gain = 909), recorded with a 20–450 Hz bandwidth and digitized at 2 kHz using data 106 acquisition software (Spike2, Cambridge electronics Design, Cambridge, UK).

2.4. TMS

Single-pulse TMS was delivered at 5-seconds intervals to the left primary motor area (M1) via a Magstim Bistim^2 stimulator connected to a flat alpha coil (D70 Alpha Flat Coil, Magstim Company, Whitland, Dyfed, UK) being held by a support stand (Magstim AFC Support Stand, Magstim Company, Whitland, Dyfed, UK). In order to reduce the effects

Table 1
Demographic characteristics of the participants. AIS = American Spinal Injury Association Impairment Scale. VMIQ-2 = Vividness of Movement Imagery Questionnaire-2. GRASSP = Graded Redefined Assessment of Strength, Sensibility, and Prehension. MEP MT = corticospinal motor threshold at the baseline session. Spinal Th = spinal threshold at the baseline session. MSO = maximal stimulator output.

Participant	Sex	Age	AIS grade	Injury level	Years since injury	VMIQ-2	GRASSP (L/R)	MEP MT (%MSO)	Spinal Th (mA)
P79	M	46	B	C4-C5	26	2.33	12/15	62	135
P648	M	22	D	C4-C7	2	1.08	116/84	60	85
P177	F	51	C	C6-C7	9	2.61	55/84	62	100
P278	F	45	D	C5	3	3.00	59/47	47	90
P130	M	64	D	C5	3	2.31	93/101	48	80
P339	F	53	C	C4-C6	32	2.69	114/66	50	69
P725	F	22	C	C2	14	3.80	54/32	59	150
P500	M	72	C	C5-C6	6	2.08	107/93	60	85

of sound produced by stimulation, participants wore sound-attenuating headphones while receiving stimulation (Capozio et al., 2021a). The coil was oriented at ~45° to the midline to induce a posterior-to-anterior current flow perpendicular to the central sulcus (Janssen et al., 2015). The cortical hotspot, the site at which TMS evoked the largest MEPs in the APB muscle, was found by moving the coil over the scalp while delivering stimulation. The hotspot was marked with a non-permanent marker to ensure consistency of recordings over the session (Carson et al., 2021). The positions and orientations of the coil was monitored continuously, and if necessary, adjusted to align with the scalp markings (Carson et al., 2021). Resting motor threshold (MT) was defined as the smallest intensity of stimulation (in % of maximal stimulator output, MSO) that produced peak-to-peak MEP amplitudes of at least 50 μV in at least 5 out of 10 trials in the relaxed APB muscle (Rossini et al., 1994). The thresholding procedure was repeated on each session at baseline (prior to the Condition phase, Fig. 1). Ten MEPs traces were recorded and averaged at MT intensity and 120 % MT intensity for each participant/session. Given that stimulation at the optimal site to induce MEPs in the APB also elicits activity in the FCR muscle (Triggs et al., 1999), we simultaneously recorded MEPs from both muscles. The stimulation was controlled through Spike2 (Cambridge Electronic Design, Cambridge, UK) software.

2.5. TSCS

Single-pulse TSCS was carried-out with a 5-channels constant-current spinal stimulator (BioStim-5, Cosyma, Moscow, Russia). Stimulation was delivered through two self-adhesive electrodes: a 5x9 cm electrode (Axelgaard, ValuTrode Cloth) placed over the left iliac crest as anode and a 3.2 cm round electrode (Axelgaard, ValuTrode Cloth) placed at the midline between C5 and C6 spinous processes as cathode (Benavides et al., 2020). TSCS pulses were delivered as 1-ms biphasic square-wave pulses every 5 s (Capozio et al., 2023). Since the effects of TSCS delivered at the C5-C6 level can spread across multiple upper limb muscles (Benavides et al., 2020, Capozio et al., 2023), spinal responses were recorded simultaneously from the right APB, FCR, ECR and BB muscles. Spinal-evoked potential threshold was determined for each participant by increasing the current until spinal responses of amplitudes > 50 μV could be observed in each of the four muscles (Wecht et al., 2021). Five spinal-evoked potentials were recorded and averaged at threshold intensity, 110 % threshold intensity and 120 % MT intensity for each participant/session (Capozio et al., 2023). Participants were asked to rate their perceived level of discomfort using the visual analogues scale for pain (ranging from 0 to 10, where 0 indicates No pain and 10 Worst pain) at each intensity increase. Stimulation was halted immediately if participants rated their discomfort as level 8 out of 10.

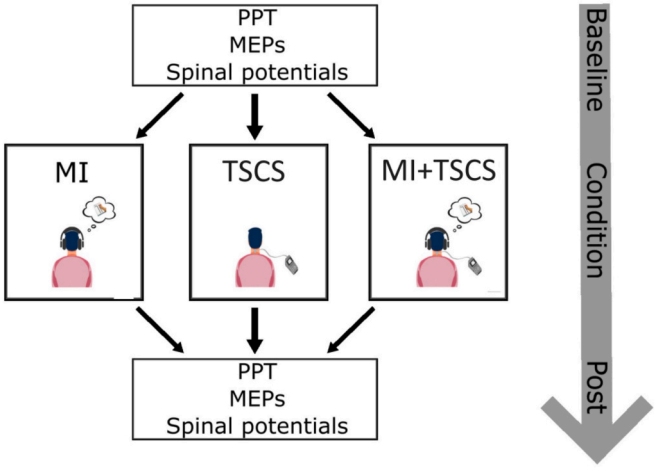

Fig. 1. Experimental design. Time course and graphical depiction of the three experimental conditions. PPT = Purdue pegboard test. MEPs = motor-evoked potentials.

2.6. Baseline sessions

A baseline session was completed at least a week before the start of the experimental sessions. The purpose of the baseline session was to familiarise participants with the TMS/TSCS experimental procedures and to ensure that MEPs and spinal-evoked potentials could be consistently recorded from each participant. In order to ensure that participants could internally visualise the movements and imagine the sensations associated with movements, participants filled the Vividness of Movement Imagery Questionnaire-2 (VMIQ-2) (Roberts et al., 2008). The VMIQ-2 assesses the vividness with which participants can: imagine completing movements from the first-person perspective (Internal visual imagery); imagine completing movements from the third-person perspective (External visual imagery); imagine the sensations associated with movements (Kinaesthetic imagery) (Roberts et al., 2008). Items are scored from 1 to 5, with 1 representing “perfectly clear and vivid as normal vision” and 5 “no image at all”. All participants but one (P725, injury at the C2 level) exhibited average VMIQ scores lower than 3 (“moderately clear and vivid”) (Table 1).

In addition, during this session participants completed the Graded Redefined Assessment of Strength,

Sensibility, and Prehension (GRASSP), a battery of tasks assessing: (1) Manual Muscle Testing in the upper limbs; (2) Dorsal and (3) Palmar sensation; (4) Qualitative prehension; (5) Quantitative prehension. Total Scores computed from summating the scores of each task and for each arm are provided in Table 1 (with total highest score possible being 116 for each arm and indicating no impairment).

2.7. Experimental sessions

Each of the three experimental sessions was divided in phases: Pre (approx. 40 min), Condition (20 min) and Post (approx. 40 min) (Fig. 1).

2.3.4. Pre

Single-pulse TMS, single-pulse TSCS and the PPT were administered during this phase according to the methods described above. The order of measures was pseudo-randomised across time, participants, and sessions.

2.3.4. Conditions

In each session, one of the following three experimental conditions was completed. The order of condition was pseudo-randomised and counterbalanced across participants and sessions.

2.7.1. TSCS

During this condition, TSCS was delivered in continuous mode using biphasic blocks of pulses at a frequency of 30 Hz, with a modulating frequency of 5 kHz (Benavides et al., 2020). Stimulation was delivered at the midline between C5 and C6 spinous processes for 2 min followed by 2 min of rest, repeated 5 times (20 min total length). Stimulus intensity was based on the threshold value estimated during the Pre phase. Intensity started at 20 mA and was ramped up in steps of 3 mA until reaching 90 % of the threshold value (Kumru et al., 2021b). Upon every increase of intensity, participants were asked to rate their perceived pain level from 0 to 10 using a visual analogue scale for pain, with 0 defined as "no discomfort at all" and 10 as "unbearable pain". Stimulation was halted immediately if discomfort reached level 8 out of 10. The first cycle (two minutes) of continuous TSCS started after reaching 90 % of the threshold value. No participants reported pain levels higher than 5 (moderate pain) in any of the sessions and it was never necessary to halt stimulation (individual pain levels are reported in Table S2).

2.7.2. MI

The MI script was played through wireless headphones (Philips 5000, Philips, The Netherlands) while participants sat comfortably at the table, with both hands resting pronated on the table. Participants were instructed by the script to close their eyes and imagine themselves completing the PPT test from the first-person perspective (internal MI) (Callow et al., 2013). The script incorporated proprioceptive ("take your left arm towards the edge of the pegboard"), visual ("visualise a pegboard in front of you on the table") and kinaesthetic ("feel the pressure of the peg") elements. Kinaesthetic MI related to the sensations associated with a specific task and has been proved to modulate the excitability of the motor cortex (Stinear et al., 2006). The MI script lasted for 2 min and was followed by 2 min of rest, both repeated 5 times for a total length of 20 min (Capozio et al., 2023).

2.7.3. MI + TSCS

Participants listened to the MI script delivered through wireless headphones (Philips 5000, Philips, The Netherlands) while receiving TSCS at the midline between C5 and C6 spinous processes. Concurrent MI + TSCS lasted for 2 min and was followed by 2 min of rest, both repeated 5 times for a total length of 20 min (Capozio et al., 2023) (Fig. 1).

2.3.4. Post

TMS, single-pulse TSCS and the PPT test were administered during this phase according to the methods described above. The order of measures was pseudo-randomised across participants and sessions.

2.8. Data analysis

For manual dexterity, the total number of pegs placed within 30 s on the pegboard on each session and pre-post phase was used as dependent variable. A linear mixed-effects model fit by maximum likelihood was run using SPSS software (Version 26.0) with an a priori significance level of 0.05. Participant was included as a random factor, with Condition (MI, TSCS, MI + TSCS) and Time (Pre, Post) included as fixed factors. The distribution of residuals was plotted to check for any violation of the assumption of normality.

For the TMS data, the average peak-to-peak amplitudes of the 10 MEPs collected at each intensity (MT and 120 % MT) were used as dependent variables Given that TMS amplitude data often reveal skewed distributions and deviations from normality (Nielsen, 1996), a logarithmic transformation was carried out. A linear mixed-effects model fit by maximum likelihood was run using SPSS software (Version 26.0) with an a priori significance level of 0.05. Participant was included as a random factor, with Intensity (MT, 120 % MT), Condition (MI, TSCS, MI + TSCS), Time (Pre, Post) and Muscle (APB, FCR) included as fixed factors. The distribution of residuals was plotted to check for any violation of the assumption of normality.

For the TSCS data, the average peak-to-peak amplitudes of the 5 spinal-evoked potentials collected at each intensity (100 %, 110 % and 120 % threshold) were used as dependent variables. Data were log-transformed to reduce right skewness (from 1.85 to −0.24) and kurtosis (from 3.40 to −0.23) (Capozio et al., 2023). A linear mixed-effects model fit by maximum likelihood was run using SPSS software (Version 26.0) with an a priori significance level of 0.05. Participant was included as a random factor, with Intensity (100 %, 110 % and 120 % of threshold), Condition (MI, TSCS, MI + TSCS), Time (Pre, Post) and Muscle (APB, FCR, ECR, BB) included as fixed factors. The distribution of residuals was plotted to check for any violation of the assumption of normality.

3. Results

3.1. Manual dexterity

The linear mixed-effects analysis revealed that the interaction between Condition and Time on the PPT scores was not significant [$F(2, 21) = 0.393$, $p = 0.676$]. The main effect of time was significant [$F(2, 21) = 6.686$, $p = 0.016$], while Condition did not significantly affect PPT scores [$F(2, 21) = 0.020$, $p = 0.981$] (Fig. 2). The normality test performed on the distribution of residuals suggested a violation of the normality assumption ($p < 0.001$), but no outliers were identified.

3.2. Cortical excitability

Cortical excitability was assessed via MEP amplitudes recorded at 100 % MT and 120 % MT intensities from the APB muscle (average data are reported in Table S1). Mean and SD of the MT values across all

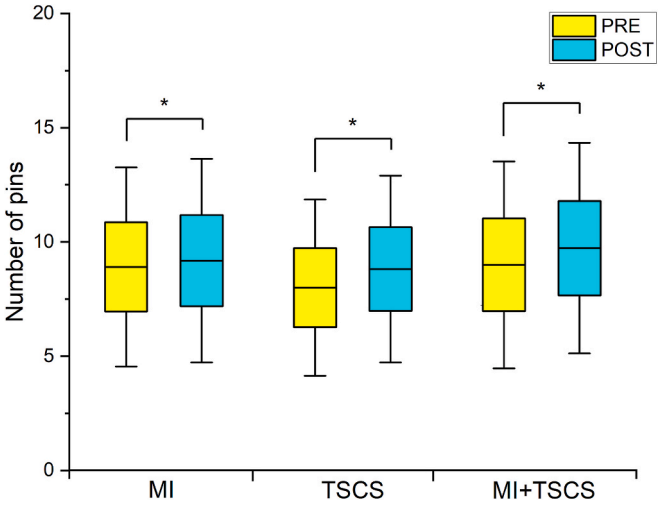

Fig. 2. Behavioural results. Mean number of pins correctly placed during the PPT test across time and conditions. Boxes represent the associated standard error (SE) and whiskers represent the associated 95 % confidence interval. Asterisks denote a statistically significant ($p < 0.05$) effect.

participants were 56 ± 6 %MSO. Responses could be evoked in all participants from the right APB muscle. The results from likelihood-ratio tests of goodness-of-fit revealed that the model including only the two-way interactions provided a better fit of the dataset (Log L = 143.32, AIC = 181.33, N of parameters = 15) than the model including three-way interactions (Log L = 184.59, AIC = 190.59, N of parameters = 22) and the four-way interaction (Log L = 182.23, AIC = 188.23, N of parameters = 24). The linear mixed-effects analysis run on the MEPs revealed that the main effect of Intensity was significant [F (1, 86) = 10.582, p = 0.002], as MEP intensities were higher when recorded at 120 % MT than at 100 % MT. While there was a main effect of Time [F (1, 91) = 6.315, p = 0.014], this also interacted significantly with Condition [F (2, 91) = 4.548, p = 0.013]. Pairwise comparisons showed a significant effect of Time on MEPs for MI [F (1, 91) = 9.714, p = 0.002] and MI + TSCS [F (1, 91) = 4.790, p = 0.031], but not TSCS [F (1, 91) = 0.908, p = 0.343] condition (Fig. 3). The effect of Muscle was non-significant [F (1, 86) = 2.829, p = 0.096]. All the other interactions and main effects were non-significant (Table 2). No violation of normality of the distribution of residuals could be inferred from the results (p = 0.665).

3.3. Spinal excitability

Spinal excitability was assessed via spinal-evoked potential amplitudes recorded at 100 %, 110 % and 120 % of spinal threshold intensities, recorded upon stimulation at the C5-C6 level from the APB, FCR, ECR and BB muscles (average data are reported in Table S1).. Mean and SD of the threshold intensity for TSCS across all participants were 99 ± 28 mA. Responses could be evoked in all participants from the right APB muscle. The results from likelihood-ratio tests of goodness-of-fit revealed that the model including only the two-way interactions provided a better fit of the dataset (Log L = 415.20, AIC = 421.20, N of parameters = 35) than the model including three-way interactions (Log L = 459.26, AIC = 465.26, N of parameters = 63) and the four-way interaction (Log L = 469.04, AIC = 475.04, N of parameters = 75). The main effect of Intensity was significant [F (1, 264) = 3.626, p = 0.028], which indicates that spinal responses were higher when recorded at 120 % threshold compared with the ones recorded at 100 % threshold intensities (p = 0.023), but not at 110 % threshold intensities (p = 0.637). The main effect of Muscle was significant [F (1, 264) = 3.932, p = 0.009], showing that stimulation induced higher activity in APB than in ECR (p = 0.035) and BB (p = 0.038) (Fig. 4). All the other interactions and main effects were non-significant (Table 3). No violation of normality of the distribution of residuals could be inferred from the results (p = 0.343).

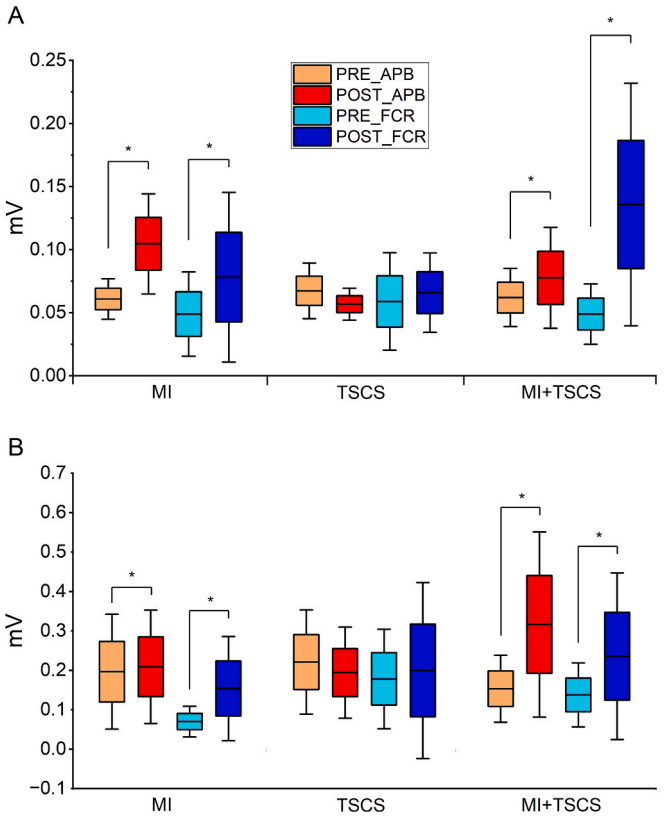

Fig. 3. Brain stimulation results. Mean amplitude values of the MEPs recorded from APB and FCR muscles *at (A)* 100 % MT and *(B)* 120 % MT intensities across time and conditions. Boxes represent the associated standard error (SE) and whiskers represent the associated 95 % confidence interval. Asterisks denote a statistically significant ($p < 0.05$) effect.

4. Discussion

MI and TSCS are becoming increasingly popular rehabilitation strategies to promote neural plasticity and functional improvements in people living with SCI (Megía García et al., 2020, Opsommer et al., 2020). The effects of combining these two rehabilitation strategies on neural excitability and manual dexterity has not yet been investigated. Thus, the aim of the present study was to investigate the within-session effects of TSCS when paired with MI on: (1) manual dexterity, (2) cortical excitability and (3) spinal excitability as measured before and after 20-minutes of TSCS, MI or MI + TSCS. Manual dexterity significantly increased from pre to post independently on the condition. The effects of threshold and suprathreshold stimulation of the motor cortex depended on the condition participants completed, with increases in corticospinal excitability observed after MI and MI + TSCS but not after TSCS. Finally, none of the three conditions significantly affected spinal excitability as assessed via spinal-evoked potentials evoked upon TSCS at threshold and suprathreshold intensity.

4.1. Manual dexterity

Results from the analysis of PPT scores used to quantify changes in manual performance before and after the three conditions demonstrate that all three conditions (e.g., MI, TSCS and MI + TSCS) improved manual dexterity in people with a cervical SCI. In this study, participants practiced for three trials before completion of the test trial in order to reduce task familiarisation effects (Tiffin and Asher, 1948) and ensure that improvements in PPT scores are not the result of becoming familiar with the task. This combined with other work that has shown no changes in PPT scores after a single session of repetitive TMS suggests that the improvements we observed are not merely the results of task practice (Alexeeva and Calancie, 2016). In addition, our previous work demonstrated that a single session of MI, TSCS or MI + TDCS did not affect manual dexterity in healthy participants (Capozio et al., 2023). Other authors suggested that when using PPT as a motor learning task, ceiling effects can occur in healthy participants (Jelić et al., 2013). Considering that baseline performance was substantially lower for people with cervical SCI (average of 6 pegs across all sessions) compared to their healthy counterpart in our previously published work (e.g., Capozio et al., 2023; mean = 16 pegs across all sessions), our results indicate that there was still wide room for improvement for people with cervical SCI.

4.2. Corticospinal excitability

We investigated the effects of a 20-minutes session of MI, TSCS and MI + TSCS on corticospinal excitability, assessed by stimulating the left motor cortex with threshold and suprathreshold TMS and recording evoked responses in the contralateral APB and FCR. Despite the growing evidence that MI can promote functional improvements after SCI (Opsommer et al., 2020), to our knowledge this is the first study

Table 2
Fixed-effects table for the linear mixed model run on the MEPs collected across Time, Intensity, Muscle and Condition.

Parameter	Numerator df	Denominator df	*F*	Sig.	*η*2
Time	1	91	6.315	0.014	0.06
Condition	2	86	0.044	0.957	0.001
Muscle	1	86	2.829	0.096	0.03
Intensity	1	86	10.582	0.002	0.11
Intensity*Condition	2	86	0.210	0.811	0.001
Muscle*Condition	2	86	0.348	0.707	0.01
Time*Condition	2	91	4.548	0.013	0.09
Intensity*Muscle	1	86	0.086	0.770	0.001
Intensity*Time	1	91	0.055	0.815	0.001
Muscle*Time	1	91	0.236	0.629	0.001

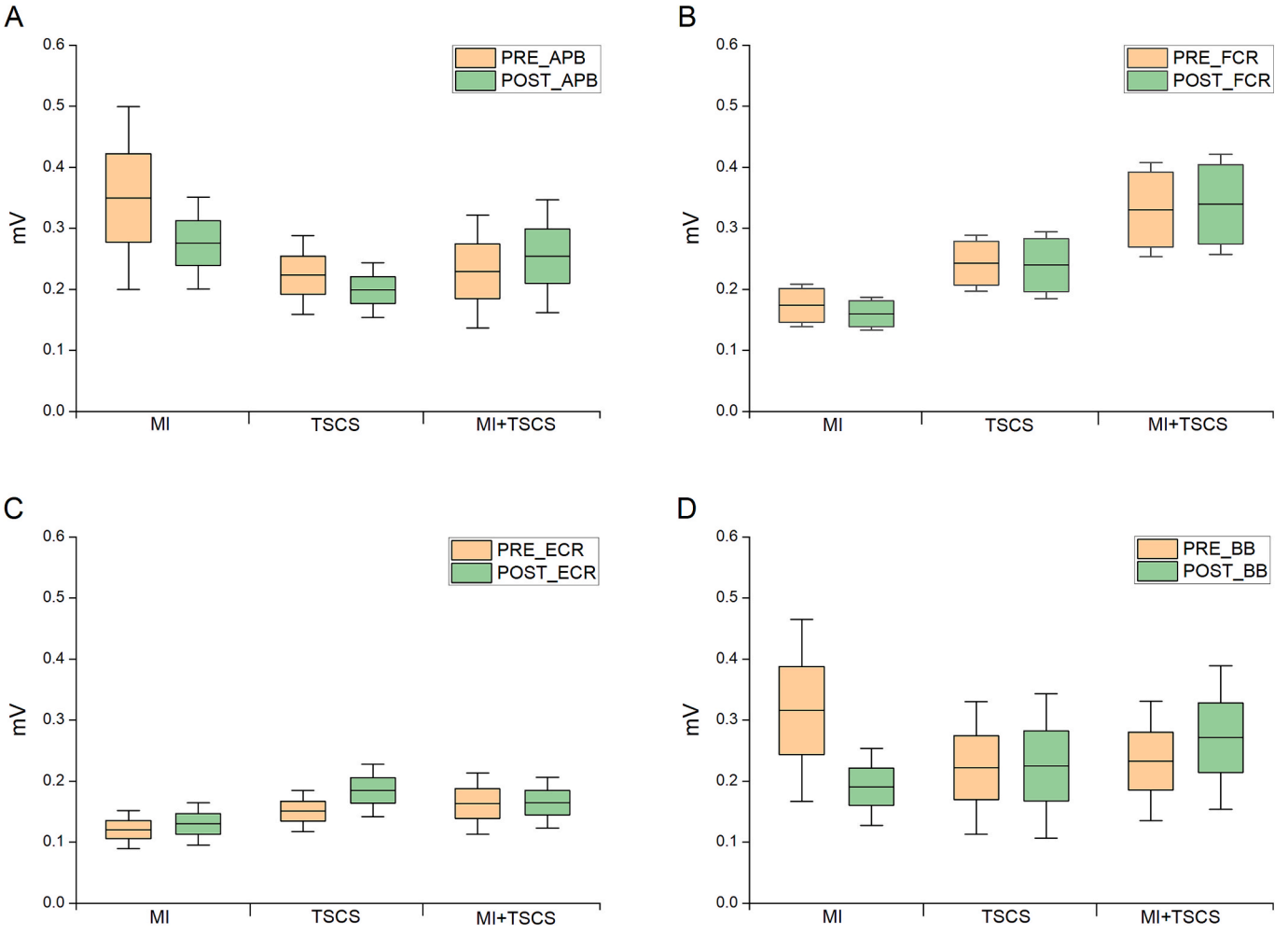

Fig. 4. Spinal stimulation results. Mean amplitude values of the spinal responses recorded from APB (*A*), FCR (*B*), ECR (*C*) and BB (*D*) muscles across time and conditions. Boxes represent the associated standard error (SE) and whiskers represent the associated 95 % confidence interval. Asterisks denote a statistically significant ($p < 0.05$) effect (if present).

assessing cortical excitability after a session of MI in cervical SCI. In the lower limbs, previous work by Cramer et al. (2007) reported that MEPs could not be elicited in the right tibialis anterior muscle in participants living with complete SCI. However, Cramer and colleagues attempted to elicit lower-limbs MEPs in a population of complete SCI participants and it is conceivable that MEPs could not be evoked because of the completeness of the injury. Contrarily, we demonstrated that corticospinal excitability increases immediately after a session of MI when MEPs are elicited at threshold intensity in participants living with incomplete SCI. These results confirm our previous finding that MEPs recorded at threshold intensity increase after MI (Capozio et al., 2023) and are in line with the evidence that corticospinal descending activity is increased during MI (Gerasimenko et al., 2018). Similarly, we confirmed that 20 min of TSCS are unlikely to affect the pyramidal drive to motoneurons nor the excitability of intracortical circuits which constitute the MEPs recorded at 120 %MT intensity (Capozio et al., 2023, Kumru et al. 2021a, Kumru et al., 2021b, Sasaki et al., 2021). Characterising the neural substrates responsible for the improvements in function observed after TSCS would require the use of more elaborate measures such as TMS-conditioning of the monosynaptic reflex (Hannah et al., 2018), but these mechanisms remain unknown. Finally, our research shows that combining TSCS with MI offer no advantage over using MI in isolation

Table 3
Fixed-effects table for the linear mixed model run on the spinal-evoked potentials collected across Time, Intensity, Muscle and Condition.

Parameter	Numerator df	Denominator df	*F*	Sig.	*η*2
Time	1	280	0.072	0.788	0.001
Condition	2	264	1.037	0.356	0.01
Muscle	3	264	3.932	0.009	0.04
Intensity	2	264	3.620	0.028	0.03
Intensity*Condition	4	264	0.020	0.999	0.001
Muscle*Condition	6	264	1.520	0.172	0.03
Time*Condition*	2	280	0.858	0.425	0.01
Intensity*Muscle	6	264	0.410	0.872	0.01
Intensity*Time	2	280	0.151	0.860	0.001
Muscle*Time	3	280	0.725	0.538	0.01

for modulating corticospinal excitability (see Fig. 3). When TMS was used to assess corticospinal excitability after a single session of hand training paired with spinal stimulation (Kumru et al., 2021b), similar increases in MEP amplitudes were reported to those in the present work. Taken together, these findings further strengthen the hypothesis that MI can activate the same structures which are active during overt movement (Grezes and Decety, 2001) even in people living with cervical SCI. As for the finding in healthy participants (Capozio et al., 2023), the lack of a significant difference between results from MI and MI + TSCS suggests the increase in cortical excitability is mainly brought upon by the MI element, with no further effect of the combinatorial element (Saito et al., 2013).

However, we also observed increase in excitability when suprathreshold (120 % MT) intensities were employed, a finding which is at odds with the results in our previously published work with healthy participants (Capozio et al., 2023). While effects observed when TMS is delivered at threshold levels are usually attributed to changes at the monosynaptic level of the descending signal, with increasing stimulus intensities polysynaptic circuits can also be activated (Di Lazzaro et al., 2012). The lack of effects observed in healthy participants with 120 % MT stimulation were interpretated as evidence that MI does not alter the excitability of intracortical and intraspinal circuits (Capozio et al., 2023). Conversely, our results in SCI participants suggest that excitability in neural populations activated by suprathreshold stimulation, whether intracortical or intraspinal in their origins, can be upregulated by MI. A potential explanation for the discrepancy between the effects observed in the healthy and SCI population might lie in the recruitment patterns of these two populations: in thenar muscles, participants living with SCI were shown to exhibit substantially reduced MEP amplitudes compared to healthy controls at 120 %MT stimulation intensity (Davey et al., 1999). These findings are in line with our previous and current results (average values across all conditions at baseline: 0.38 mV in healthy participants and 0.19 mV in participants living with cervical SCI) (Capozio et al., 2023). This suggests that suprathreshold stimulation activated fewer motor units at baseline in people living with SCI, but the excitability of these motor units was upregulated, and higher responses were evoked after MI and MI + TSCS (Davey et al., 1999).

4.3. Spinal excitability

In the present study, no differences in the amplitude of spinal responses evoked from cervical TSCS were observed after any of the conditions. The only study that, to our knowledge, assessed changes in spinal excitability in upper limb muscles after a single session of cervical TSCS in SCI was conducted by Benavides et al. (Benavides et al., 2020). The authors assessed changes in spinal excitability after 20 min of TSCS in a group of participants living with SCI and a control group. Spinal excitability was found to be increased for up to 75 min after the end of the stimulation in both groups, whereas sham stimulation did not induce any change in the evoked spinal responses. While the results are inconsistent with our finding of a lack of spinal modulation after TSCS, a number of methodological differences need to be taken into account: spinal responses were recorded upon cervicomedullary stimulation with 100 μs stimuli, as opposed to the stimulation at C5-C6 with the 1 ms pulses employed in our study; stimulation was delivered continuously for 20 min, rather than in a distributed fashion alternating 2 min of stimulation with 2 min of rest (10 min total stimulation time). The difference in stimulation time seems the most likely explanation for the discrepancy in findings, supported by other work that suggests that 10 min of TSCS is not sufficient to induce changes in spinal excitability in healthy participants (Sasaki et al., 2021). Similarly, TSCS delivered concurrently with maximal (Kumru et al. 2021a) or submaximal (Kumru et al., 2021b) grasp (6 min total stimulation time) had no effect on spinal evoked potentials collected after training in healthy participants. In able-bodied participants, Gerasimenko and colleagues (2018) observed a non-significant tendency for inhibition in spinal excitability of the lower limbs during imagining of visual imagery stepping compared to the excitability at rest. A recent systematic review on the neurophysiological effects of TSCS found high heterogeneity in the intervention parameters employed in the literature to date (Tajali et al., 2024), an issue which can explain the inconsistent evidence accumulated. García-Alén and colleagues (2024) conducted a systematic review and *meta*-analysis on the effects of noninvasive neuromodulation in cervical SCI and underlined the need for standardized outcome measures to further elucidate the mechanisms of TSCS. Given this, we reiterate the call for further, high-quality papers specifically assessing which stimulation modalities can promote neural plasticity in people living with SCI.

4.4. Limitations

There are several limitations to be considered in the present study. First, the study design did not include a control condition in which participants did not receive MI nor TSCS. Similar study designs have been previously employed for studies specifically comparing the effects of spinal stimulation on neural excitability (Kumru et al., 2021b, Sasaki et al., 2021), and we decided for pragmatic reasons to not include a control group as doing so reduced the number of times participants (and carers) had to travel to our lab, which could potentially drive up drop out and participant and carer fatigue. Second, the sample size from which our conclusions were drawn is relatively small, which might limit the generalisation of our findings to the broader population of people living with a cervical SCI. Nevertheless, the number of participants is in line with multiple studies involving people living with SCI (Islam et al., 2021, Meyer et al., 2020, Powell et al., 2018). These relatively small sample sizes underline the difficulty of conducting experimental studies in this clinical population. Third, while we observed statistically significant improvements in hand dexterity as measured via the PPT, the mimimal clinically important difference for this test in neurological populations has yet to be determined (Sigirtmac et al, 2021) and the functional impact of our interventions on hand dexterity remains unknown. Finally, we cannot exclude that the order in which parameters were collected at baseline and after the interventions did not affect the results, since previous neural activation can influence the following measurements (Kumru et al., 2021b). To partially overcome this issue, we randomised the order in which behavioural and neural outcome measures were collected across sessions and participants.

5. Conclusions

This study was the first to investigate the effects of MI paired with TSCS on manual dexterity and neural excitability in people living with cervical SCI. We showed that 20 min of MI and TSCS, either alone or in combination, are sufficient to increase manual dexterity. We also demonstrated that the behavioural effects are paralleled by an increase in cortical excitability after MI and MI + TSCS. In addition, and in contrast with the results obtained when testing healthy participants,

none of the conditions influenced spinal excitability which were assessed through recording of spinal evoked potentials. As the neural substrates responsible for the improvements in function observed after TSCS at the acute level (after a single session) in people living with SCI remain unknown, further research is needed to optimise rehabilitation paradigms for SCI by maximising neural plasticity through stimulation. Taken together, our evidence further supports the promising use of MI as a rehabilitation treatment for people living with chronic SCI.

CRediT authorship contribution statement

Antonio Capozio: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Madison Graham:** Investigation, Data curation, Writing – review & editing. **Ronaldo Ichiyama:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition. **Sarah L. Astill:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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