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Effect of repetitive transcranial magnetic stimulation combined with robot-assisted training on wrist muscle activation post-stroke

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HIGHLIGHTS

- rTMS and robot-assisted training (rTMS+RW) improved voluntary wrist muscle activation post-stroke
- Reduced motor unit (MU) recruitment thresholds and increased MU firing rates were found compared to Sham rTMS+RW
- Further study of rTMS+RW as a neurorehabilitation strategy post-stroke is warranted

ABSTRACT

Objective: To compare the effects of active assisted wrist extension training, using a robotic exoskeleton (RW), with simultaneous 5 Hz (*rTMS+RW*) or Sham rTMS (*Sham rTMS+RW*) over the ipsilesional extensor carpi radialis motor cortical representation, on voluntary wrist muscle activation following stroke.

Methods: The two training conditions were completed at least one week apart in 13 participants >1 year post-stroke. Voluntary wrist extensor muscle activation (motor unit (MU) recruitment thresholds and firing rate modulation in a ramp-hold handgrip task), ipsilesional corticospinal excitability (motor evoked potential amplitude) and transcallosal inhibition were measured *Pre*- and *Post*-training.

Results: In MUs active both *Pre* and *Post* training, significantly greater reductions in MU recruitment thresholds *Post rTMS+RW* training ($p=0.0001$) were found compared to *Sham rTMS+RW* ($p=0.09$). MU firing rate modulation increased *Post rTMS+RW* (mean 2.5 Hz, $p=0.03$), but not *Post Sham rTMS+RW* (mean 0.8 Hz, $p=0.3$). No significant changes were seen in ipsilesional corticospinal excitability and transcallosal inhibition measures ($p>0.05$).

Conclusions: Changes were found in voluntary muscle activation of wrist extensor muscles but not measures of ipsilesional corticospinal or interhemispheric excitability following a single *rTMS+RW* session in people >1 year post-stroke.

Significance: The effects of *rTMS+RW* on muscle activation warrant further investigation as post-stroke rehabilitation strategy.

Key words: stroke; upper extremity; rehabilitation; robotic exoskeleton; repetitive Transcranial Magnetic Stimulation; motor unit

Abbreviations

CSE - Corticospinal excitability

CV – coefficient of variation

ECR- extensor carpi radialis muscle

EMG – electromyography

iSP - ipsilateral silent period

M1 - primary motor cortex

MEPs - motor evoked potentials

MVC – maximal voluntary isometric contraction

MU – motor unit

rTMS - repetitive transcranial magnetic stimulation

RMT - resting motor threshold

RW – robot-active assisted wrist extension training

Sham rTMS – sham repetitive transcranial magnetic stimulation

SD – standard deviation

TCI - transcallosal inhibition

UE-FMA - Fugl-Meyer Upper Extremity Motor Assessment

WMFTa – abbreviated Wolf Motor Function Test

1 INTRODUCTION

Stroke is the third leading contributor to Disability Adjusted Life Years in developed countries (Murray et al. , 2015). Over 60% of individuals do not fully recover arm and hand use despite rehabilitation efforts (Kwakkel et al. , 2003), resulting in ongoing activity limitations and reduced quality of life (Wyller et al. , 1997) . Prognosis for upper extremity recovery is particularly poor for those with severe paresis (Kwakkel et al. , 2003). This impairment in voluntary muscle activation has been attributed to reduced rate modulation, the ability to modulate motor unit firing (Li et al. , 2015), and alterations in motor unit recruitment post-stroke (Hu et al. , 2015, Li et al. , 2015). These individuals have little or no voluntary movement to facilitate independent repetitive task practice, or to incorporate their paretic arm and hand in everyday activities to promote motor recovery (Barker et al. , 2005, Gebruers et al. , 2014, Shim et al. , 2014) .

Robot-assisted upper extremity training and repetitive transcranial magnetic stimulation (rTMS) have been explored as potential means of rehabilitating arm and hand function following stroke. Robot-assisted upper extremity training, the use of electromechanical or robot-assisted devices for intensive practice of repetitive arm and hand movements (Kwakkel et al. , 2008), has shown potential for individuals requiring assistance to perform movements. A Cochrane systematic review found significantly greater improvements in activities of daily living, arm function, and muscle strength following robot-assisted upper extremity training compared to active control interventions post-stroke (Mehrholz et al. , 2015). However, the level of evidence supporting the intervention was judged as low to very low in quality; therefore, caution was recommended in adopting robot-assisted upper extremity training alone in clinic (Mehrholz et al.

, 2015). Robot-assisted upper extremity training combined with other therapeutic techniques has been suggested to enhance the robustness and durability of neuroplasticity and motor skill learning, beyond what might be achieved with repetitive, passive, or active assisted paretic upper extremity movements (Lotze et al. , 2003, Turner et al. , 2013).'

rTMS is a promising therapeutic technique to combine with robot-assisted upper extremity training. It is a non-invasive neuromodulatory approach, where electromagnetic pulses are applied over focal areas of the brain to induce changes in corticospinal excitability (Lefaucheur, 2012). In persons with chronic stroke, a single session of 5 Hz rTMS applied over the ipsilesional primary motor cortex (M1) led to a significant increase in motor evoked potential (MEP) amplitude compared to baseline sustained for up to 60 minutes afterward .(Goh et al. , 2015). Typically, rTMS has been applied over the primary motor cortex (M1), closely followed by conventional skilled repetitive upper extremity training, to capitalize on the after-effects of the rTMS, to augment motor skill learning and promote neuroplastic changes (Higgins et al. , 2013, Wessel et al. , 2015). Several small clinical trials have reported improvements in upper extremity impairment and changes in corticospinal excitability (motor evoked potentials) when high-frequency rTMS was followed by upper extremity training compared to sham rTMS and training post-stroke (Sasaki et al. , 2013, Li et al. , 2016). However, these protocols largely rely on participants having sufficient volitional muscle activation and control to participate in skilled motor practice.

The challenge is how to provide rehabilitation training appropriate for individuals with a range of upper extremity impairment following stroke, including those moderate to severe motor impairment. A previous study in healthy participants applied TMS over the right first dorsal

interosseous muscle (FDI) M1 with a stimulus frequency of 0.1 Hz at a stimulus intensity of 150% of the active motor threshold in the right FDI applied synchronously with maximum voluntary contraction (MVC) efforts, and found a significant increase in right FDI MEP amplitudes and MVC compared to a sham TMS condition (Touge et al. , 2012). In this current proof of principle study, we compared the effects of *robot-active assisted wrist extension (RW)* training and simultaneous application of rTMS over the ipsilesional M1 (*rTMS+RW*) on voluntary muscle activation of the wrist extensor muscles, evidenced by changes in motor unit activity, to Sham rTMS with RW (*Sham rTMS+RW*) in participants with a range of impairment, including those with moderate to severe motor impairment following stroke. We also examined changes in corticospinal excitability (CSE) and transcallosal inhibition (TCI) for the extensor carpi radialis (ECR) muscle. The intention was to increase corticospinal excitability to augment the participant's efforts to activate the paretic wrist extensor muscle group with the assistance of the robotic device.

2 METHODS

2.1 Participants

Participants who had experienced a first-time middle cerebral artery stroke more than one year prior to the study, and had unilateral upper extremity motor impairment, were recruited from the community. The exclusion criteria for the study were: contraindications to TMS (Rossi et al. , 2009); significant cognitive impairment (<24 on the Montreal Cognitive Assessment) (Nasreddine et al. , 2005) or aphasia (<13 on the Frenchay Aphasia Screen) (Rossi et al. , 2009); a history of head trauma, major psychiatric diagnosis, neurodegenerative disorder, or substance abuse; or taking medications (e.g., GABAergic, NMDA-receptor antagonist) known to influence

neuroplasticity (Rossi et al. , 2009). The study was approved by institutional Research Ethics Boards and all participants gave informed, written consent for the study.

2.2 Procedures

The study used a cross-over design. Participants undertook two training sessions at least one week apart (order randomly assigned); 1) high-frequency (5 Hz) rTMS applied over the ipsilesional M1 cortical representation of the ECR during robot-assisted active wrist extension (RW) training (*rTMS+RW*); and 2) sham rTMS applied over the ipsilesional M1 during RW training (*Sham rTMS+RW*). Each session was 120-150 minutes in duration, including participant rest breaks as required.

At the beginning of the first session, the Fugl-Meyer Upper Extremity Motor Assessment (UE-FMA) (Fugl-Meyer et al. , 1975) was administered, as well as an abbreviated Wolf Motor Function Test (WMFTa) (Wolf et al. , 2005), consisting of three timed test items: a test of gross motor function (item 9: lift can); fine motor function (item 11: lift paper clip), and a functional task (item 16: fold towel). The movement time for each item (maximum of 120s) was averaged over three trials. This value was used to calculate the rate of performance for each item (60s/mean task performance time) (Hodics et al. , 2012).

2.2.1 Outcome measures

Measures of CSE, TCI, and voluntary muscle activation were recorded before (*Pre*) and after (*Post*) each training session in a consistent order: i) CSE, ii) TCI, and iii) muscle activation *Pre*; and i) muscle activation, ii) CSE, iii) TCI *Post*. These outcome measures and their timing were selected and adapted to capitalize on the after effects of the rTMS (approximately 20-60 minutes post-rTMS), as well as to reduce the testing burden for participants (Ziemann et al. , 2008).

2.2.1.1 Voluntary muscle activation

The paretic wrist and hand were placed in the custom-built wrist extension exoskeleton “RoboWrist” device^E (Figure 1A) on a height-adjustable table with forearm pronated and secured in the device with VelcroTM straps (Figure 1B). Given the importance of ECR for placing the hand for functional use in everyday activities, wrist extensor muscles were activated as synergists during a gripping task (Rose et al. , 2014). Participants gripped and squeezed a hand-held dynamometer to perform the following tasks:

1. *Maximal voluntary isometric contraction (MVC)*: participants performed two MVCs.
2. *Ramp-hold*: participants steadily increased their handgrip forces (0-30%MVC) over 5s, held at 30%MVC for 5s, and released to 0%MVC over 5s with real-time visual feedback on a screen in front of them. Three ramp-hold trials were recorded.

Rest periods (at least 30s) were given between contractions to reduce fatigue during testing.

Two high-density surface electromyography (EMG) grids^F (semi-disposable adhesive matrices) were positioned over the ECR, extensor carpi ulnaris, and extensor digitorum communis muscles of the paretic limb (Figure 1B). Each grid had 64 electrodes (5 columns and 13 rows with an electrode missing in one corner) with 8mm interelectrode distance. Two reference electrodes^C (2×3.5cm) were placed on the medial and lateral olecranon processes. Electrodes on the radial and ulnar styloid processes served as the amplifier grounds. Muscle activation from wrist and finger flexors was recorded with 2 bipolar surface EMG electrodes^C (2×3.5cm) placed over the common flexor origin, 3cm apart center-to-center. The EMG grids and bipolar electrodes were left in place during the training.

The high-density surface EMG grid signals were collected in monopolar modality and amplified (200×; EMG-USB^F). Differential wrist flexors EMG was band-pass filtered (10-1000 Hz) and amplified (10000×; Iso-DAM8^G) and the force signal was low-pass filtered at 0.1 KHz and amplified (1000×; Bridge-8^G). All signals were digitized at 2048 Hz using a 12-bit A/D converter (EMG-USB^F).

2.2.1.2 Corticospinal excitability and transcallosal inhibition

Participants sat in an adjustable chair. Measures of CSE and TCI were elicited by single pulse TMS from a Magstim Super Rapid2^A stimulator with a 70 mm figure-8 air-cooled coil in concert with theBrainsightTM neuronavigation software package^B. The ‘hotspot’ for eliciting motor evoked potentials (MEPs) in the contralateral ECR was found by positioning the coil over the scalp region to elicit the largest and most consistent MEPs. Standard procedures for determining resting motor threshold (RMT) (Rossini et al. , 1999) were performed. TMS pulses were delivered at a random rate between 0.15 and 0.2 Hz, with 20% variation, when assessing RMT, CSE, and TCI.

MEPs were recorded with surface bipolar recording electrodes^C (3 cm diameter) over the ECR muscle of the paretic and non-paretic arms. The surface EMG data were collected using LabChart 7.0^D software , sampled at 2000 Hz, pre-amplified (1000×) and band-pass filtered at 10-1000 Hz using a Powerlab^D data acquisition system and two biological amplifiers. Data were recorded in a 450 ms sweep from 100 ms before to 350 ms after TMS delivery. The EMG electrode location over the paretic ECR was traced using permanent marker. The electrodes were removed prior to the voluntary muscle activation assessments and returned to the original location for MEP collection after training.

MEPs were recorded from the paretic ECR using TMS elicited over the ipsilesional M1. For MEPs, single TMS pulses were applied over the ipsilesional M1 from 90-150% of RMT in increments of 10% (10 per intensity, 70 pulses total) while participants were at rest. When an ipsilesional MEP was present, the MEPs were collected up to 150% of RMT, or 100% of the maximum stimulator output (MSO), whichever value was lower.

For TCI assessment, participants were asked to produce an active isometric contraction of 50% maximum grip force output with the arm ipsilateral (non-paretic) to the identified ipsilesional ECR hotspot while 10 single TMS pulses were delivered at 150% RMT when possible (Fling et al. , 2012). If no ipsilesional MEP was present, or when 150% RMT exceeded 100% MSO, TMS was applied at 100% MSO during TCI collection (Hayward et al. , 2017).

2.2.2 Training sessions

The robotic system consisted of a powered exoskeleton and controller programmed to move the wrist alternately into 30° of flexion and extension, through a handle that participants were strapped to or gripped with their paretic hand (depending on their motor ability) (Figure 1A, B). The controller time-locked and synchronized the robotic and TMS systems. The participant attempted to activate their paretic wrist extensors to actively assist the robotic system as it moved their wrist into extension, while simultaneously 5 Hz rTMS (*rTMS+RW*) or Sham rTMS (*Sham rTMS+RW*) was applied over their ipsilesional ECR M1 representation. When an ipsilesional MEP could not be elicited, the contralesional M1 ECR representation was acquired and this location was mirrored to the ipsilesional hemisphere. Thirty trains of *rTMS+RW* (or *Sham rTMS+RW*) were applied over the 8min training session. Coil location was monitored in real-time using neuronavigation. Both sessions were identical, except sham rTMS stimulation

was performed with a coil that looked and sounded like active stimulation but did not induce any current. Active rTMS was applied at 80%RMT. The order of the sessions were randomized and counterbalanced.

2.3 Data Analysis

2.3.1 Voluntary muscle activation

EMG and force analyses for the voluntary muscle activation assessments were done using MatLab^H R2013b and Spike 2^I v.6.17 custom scripts. For MVC, the force maximum during each MVC was measured and the higher value *Pre* and *Post* was taken. For ramp-hold contractions, average rate of force rise during ramp and the mean force with standard deviation (SD) and coefficient of variation (CV=SD/mean), as an index of muscle contraction stability, were calculated over a 2s-moving window during hold. 2s-sections with CV< 10% were selected for analysis.

Single motor unit (MU) potentials were identified by decomposing the high-density surface EMG from wrist extensor muscles using DEMUSE software.(Holobar et al. , 2007) To identify MUs active both before and after training, one *Pre* ramp and one *Post* ramp from the ramp-hold contractions with comparable rates of force rise were spliced together and decomposed. Recruitment threshold of each MU was measured as the force (%MVC) when the recruited MU started to discharge steadily (at least 4-5 discharges with a firing frequency >4 Hz). Firing frequency for the first five MU discharges (initial frequency) and over 2s during the holding phase (mean frequency) were calculated for each MU *Pre* and *Post rTMS+RW* (or *Sham rTMS+RW*). The ability to modulate the firing rate of the MU was examined by subtracting the initial frequency from the mean frequency (mean frequency – initial frequency). Over the same

2s, during the holding phase, a root mean square of the bipolar surface EMG of the wrist flexor muscles was calculated to evaluate wrist flexor muscle activation.

2.3.2 Corticospinal excitability and transcallosal inhibition

We measured MEP peak-to-peak amplitude for each stimulus intensity (90-150%RMT) before and after *rTMS+RW* (or *Sham rTMS+RW*). The linear slope of the MEP recruitment curve was determined when possible. TCI was quantified by the ipsilateral silent period (iSP), defined as the transient reduction in volitional EMG activity elicited by TMS applied over M1 ipsilateral to the active muscle (Fling et al. , 2012). The magnitude of iSP was defined as the average EMG level during the iSP (iSP_{mean}) expressed as a ratio of the mean pre-stimulus EMG ($iSP_{mean}/pre-stim_{mean}$), where a lower value indicates more inhibition (Mang et al. , 2015, Neva et al. , 2016). Custom MatLab^H scripts were used to identify the MEP recruitment curve slope and iSP magnitude.

2.4 Statistical Analysis

Statistical analyses were performed using SPSS^J v.22. Force parameters (MVC, rate of force rise during ramps, mean force for 2s during hold, MEP recruitment curve slope, and iSP duration and magnitude) were compared before and after the robot-assisted practice using separate two-way repeated measures ANOVAs with TIME (*Pre*, *Post*) and TRAINING CONDITION (*rTMS+RW*, *Sham rTMS+RW*) as factors. Recruitment threshold (RT) and MU firing rate modulation for MUs active BOTH *Pre* and *Post* during ramp contractions were compared by two-way mixed model ANOVAs with the factors TIME (*Pre*, *Post*; repeated) and TRAINING CONDITION (*rTMS+RW*, *Sham rTMS+RW*). For MUs identified ONLY *Pre* or

Post, RT and MU firing modulation were compared with two-way ANOVAs with TIME (*Pre*, *Post*) and TRAINING CONDITION (*rTMS+RW*, *Sham rTMS+RW*) as factors. Post-hoc analyses were conducted using Newman-Keuls tests. Alpha level for significance was set at 0.05 for all comparisons. Partial eta squared (η_p^2) values were calculated to estimate effect sizes for sample size calculation for future studies (Cohen, 1988).

3 RESULTS

3.1 Participants

Thirteen participants (4 female/9 male; mean \pm SD of 65.9 \pm 8.7 years of age) who had experienced a first-time middle cerebral artery stroke 70.3 \pm 48.1 months prior took part in the study (Table 1). They had persisting unilateral upper extremity motor impairment (8 dominant/5 non-dominant hand) with an UE-FMA of 28.8 \pm 19/66 (Fugl-Meyer et al. , 1975). Nine participants had moderate to severe upper extremity motor impairment (UE-FMA<47/66) (Hoonhorst et al. , 2015). WMFTa rate of performance for participants was a median (IQR) of 2.7(17.2)/min. Six participants scored 0/min on the WFMTa (UE-FMA <16/66).

3.2 Voluntary muscle activation

Figure 2 depicts representative examples of ramp-hold contractions *Pre* and *Post rTMS+RW*.

A total of 413 MUs were identified, 293 MUs were identified ONLY *Pre* or *Post* and 120 MUs were active BOTH *Pre* and *Post* (Table 2). Of these, 35 MUs (RT< 0.1 %MVC) were excluded from the analysis because they were recruited prior to the ramp-hold task and were tonically active when the recording started.

Analysis of the subset of matched MUs that were active BOTH *Pre* and *Post* training showed a significant interaction between TIME and TRAINING CONDITION ($F_{(1,103)}= 4.698$, $p=0.03$, $\eta_p^2=0.04$) with post-hoc analyses revealing MU recruitment thresholds decreased significantly following the *rTMS+RW* training ($p=0.0001$), but not after the *Sham rTMS+RW* training condition ($p=0.16$) (Figure 3C). There was a significant difference in MU recruitment thresholds for TIME *Pre* and *Post* training ($F_{(1,103)}=18.860$, $p<0.001$, $\eta_p^2=0.16$), but not for TRAINING CONDITION ($F_{(1,103)}=1.676$, $p=0.198$, $\eta_p^2=0.02$). For firing rate modulation, the interaction between TIME and TRAINING CONDITION was non-significant ($F_{(1,103)}= 0.394$, $p=0.531$, $\eta_p^2<0.004$). However, a significant main effect of TIME ($F_{(1,103)}= 27.094$, $p<0.001$, $\eta_p^2=0.21$) for MU firing rate modulation was found in the subset of matched MUs active BOTH *Pre* and *Post* with frequency modulation significantly increasing (average 2 Hz) following both TRAINING CONDITIONS ($p=0.001$) (Figure 3D). No significant differences in MU firing rate modulation were seen by TRAINING CONDITION ($F_{(1,103)}= 0.037$, $p=0.85$, $\eta_p^2=0.003$).

When MUs identified only during either the *Pre* or *Post* training sessions were examined, a significant interaction was observed between TIME and TRAINING CONDITION (*rTMS+RW* or *Sham rTMS+RW*, $F_{(1, 269)}=6.482$, $p=0.01$, $\eta_p^2= 0.02$). Significant differences in MU recruitment thresholds were found again for TIME *Pre* and *Post* training ($F_{(1,269)}=18.719$, $p<0.001$, $\eta_p^2=0.07$), but not TRAINING CONDITION ($F_{(1, 269)}=0.051$, $p=0.822$, $\eta_p^2 <0.001$). Post-hoc analyses revealed significant reductions in MU recruitment thresholds following the *rTMS+RW* ($p=0.0001$) but not the *Sham rTMS+RW* training condition ($p=0.22$, Figure 3A). The TIME and TRAINING CONDITION interaction ($F_{(1, 269)}=0.235$, $p =0.63$, $\eta_p^2=0.001$) was not statistically significant for MU firing rate modulation in this MU group. However, Figure 3B

illustrates that MU firing rate modulation increased by an average of 1.5 Hz following *rTMS+RW* but only by a mean of 0.5 Hz following *Sham rTMS+RW*.

Reductions in recruitment thresholds were observed for MUs identified ONLY *Pre* or *Post* following *rTMS+RW* training ($-5.86 \pm 13.69\% \text{MVC}$) even in the six participants who had the greatest motor deficits ($\text{WMFTa} = 0/\text{min}$). The mean change in recruitment threshold following the *Sham rTMS+RW* training appeared relatively smaller ($-1.93 \pm 13.14\% \text{MVC}$).

No significant changes between *Pre* and *Post* training were observed in MVC, the rate of force change and average force during the ramps for the *rTMS+RW* or *Sham rTMS+RW* training conditions (Table 2). During the ramp-hold contractions, there was no change in the root mean square of the bipolar surface EMG of the wrist flexors in either training condition.

3.3 Corticospinal excitability and transcallosal inhibition

An ipsilesional MEP was elicited in the more affected hemisphere *Pre* and *Post* in both the *rTMS+RW* and *Sham rTMS+RW* training sessions in only 5/13 participants (Table 2). Following training, MEPs were elicited in two additional participants (7/13 total). There was no change in the MEP amplitude for these individuals, suggesting no change in this measure of CSE. For those with *Pre* and *Post* recruitment curves, there was no significant change in the slope of the recruitment curve either across conditions or after training.

TCI was evaluated based on the iSP_{mean} elicited from the lesioned M1 obtained in all participants in all training sessions. No significant differences were found for TIME *Pre* and *Post* ($F_{(1,12)}=1.422$, $p=.256$, $\eta_p^2=0.106$), TRAINING CONDITION ($F_{(1,12)}=0.003$, $p=.954$,

$\eta_p^2=0.0003$), or TIME \times TRAINING CONDITION tests ($F_{(1,12)}=0.382$, $p=.548$, $\eta_p^2=0.031$) (Figure 4).

3.4 Individual response to training

Figure 5 illustrates the ranges of responses of the individual participants to the two training conditions (*rTMS+RW* and *Sham rTMS+RW*) by UE-FMA scores. Post-training reductions in MU recruitment thresholds and increases MU firing rate modulations were observed across the spectrum of motor impairments. Similarly, changes in MU firing rate modulation and MU recruitment were observed post-training in participants with and without ipsilesional MEPs.

4 DISCUSSION

This proof of principle study compared changes in voluntary muscle activation of wrist extensor muscles following a single session of robot-active assisted wrist extensor training paired with sham (*Sham rTMS+RW*) versus 5 Hz rTMS (*rTMS+RW*) in participants with persisting post-stroke upper extremity motor impairment. Greater reductions in recruitment thresholds of wrist extensor MUs were found following *rTMS+RW* compared to *Sham rTMS+RW* training condition. Modulation of MU firing rates was observed following *rTMS+RW*, but not *Sham rTMS+RW* for wrist extensor MUs identified ONLY *Pre* or *Post* training. Firing rate modulations following both training conditions were observed in the subset of MUs that were active BOTH *Pre* and *Post* ramp contractions. Muscle activation changes were observed even in those individuals with moderate to severe motor impairment. No significant changes were found in measures of corticospinal excitability or transcallosal inhibition. This is the first study, to the authors' knowledge, to examine the synergistic effects of combining robot-assisted upper

extremity training with high-frequency rTMS on upper extremity muscle activation following stroke.

4.1 *Voluntary muscle activation*

Disturbances in MU firing rate modulation and MU recruitment are observed in the paretic upper extremity following stroke, and they have been associated with impairments in force generation and voluntary movement (Mottram et al. , 2014, Hu et al. , 2015, Hu et al. , 2016). We combined high frequency rTMS over ipsilesional extensor carpi radialis M1 with volitional efforts to move into wrist extension with the assistance of the robotic device intending to increase corticospinal excitability of the damaged hemisphere (Calvin & Stevens, 1968; Dartnall et al., 2009; Matthews, 1996). Ultimately this activity converges upon motoneuron pools innervating muscles (Burke et al, 1981) with the intention of augmenting the efforts of the participants to activate the paretic wrist extensor muscle group (Thompson et al. , 1991). Increases in motor unit firing rate modulation and recruitment have been linked to increases in excitatory corticospinal inputs (depending upon the percentage MVC and the muscle group examined) (Martin et al. , 2006). In this study, the observed lowering of MU recruitment thresholds suggests an improvement in the ability of the participants to activate their MUs in their wrist extensor muscles. The reduction of the MU recruitment threshold, along with the enhanced MU firing rate modulation, suggest that combined use of high-frequency rTMS and robot-assisted intensive movement training may have potential to improve MU behaviour and facilitate the activation of paretic wrist extensor muscles following stroke compared to robot-assisted movement training alone. The implications of these changes on wrist extensor muscle force and the quality of voluntary wrist and hand movements remain to be explored. That said,

improved MU activation was shown when the wrist extensors were activated as synergists during a functional gripping task.

Changes in MU recruitment thresholds and firing rate modulation were observed even in participants with UE-FMA < 47. This observation suggests there may be capacity for remodeling MU behavior even in those with the most severe upper limb impairment, to support rehabilitation efforts following stroke. It is acknowledged that even within individuals with severe motor impairment there is a large amount of variability in recovery that is not fully explained by clinical measures (Barker et al. , 2008) or by corticospinal tract indicators alone (Rondina et al. , 2017). We observed considerable inter-individual differences in training response highlighting the need for further study in this area to ‘tailor’ interventions and understanding differences between responders and non-responders.

The reduction in MU recruitment threshold and increased firing rate modulation observed following the *rTMS*+*RW* training in this study could be the result of a number of potential mechanisms. Edwards and colleagues (2014) reported that pairing of cyclic passive wrist movements with low frequency *rTMS* (1 Hz) applied over the flexor carpi radialis representation in M1 was accompanied by greater reductions in MEP amplitude than *TMS* alone in healthy subjects. These authors suggested that the repeated pairing of repetitive movement with *TMS* could lead alterations in spinal and supraspinal excitability, possibly accompanied by neuroplasticity-like changes (Edwards et al. , 2014). In our study, it is possible that the repetitive association between the somatosensory afferent input from the wrist movements imposed by the *RW*, with high-frequency *rTMS*, was associated with facilitation at the spinal level manifested as

a reduction in MU recruitment thresholds and increased MU firing rate modulation. However, this is speculative as we did not directly measure spinal excitability in this study.

Alternatively, the repeated stretch of wrist and finger flexors accompanying the RW imposed wrist movements may have reduced stiffness in these muscles (Crago et al. , 1980). The repeated active assisted wrist movements may have also reciprocally inhibited antagonist flexor muscles through spinal mechanisms such as 1A afferents (Berardelli et al. , 1987). These proposed mechanisms have potential to place the wrist extensor muscles in a more effective working position for activation (Fan et al. , 2006), contributing to the changes in firing rate modulation observed in the subset of synergist wrist extensor MUs that were active BOTH *Pre* and *Post* in the *rTMS+RW* and *Sham rTMS+RW* training conditions. Differential effects on MU firing rate modulation between these two training conditions were found when MUs identified ONLY *Pre* or *Post* were examined, with significantly increased firing rate modulation found only after *rTMS+RW*. This difference in findings may reflect changes in the population of MUs recruited *Pre* and *Post* training. No statistically significant changes were found in the wrist flexors bipolar surface EMG, and the effect of the training conditions on MU behavior in wrist flexors could not be evaluated. As the ramp-hold task used the flexors as a prime mover and the extensors as a synergist, it is impossible to determine if the *rTMS+RW* modulated any impairment of co-contraction of agonist and antagonist muscles.

4.2 Corticospinal excitability and transcallosal inhibition

In our study, no statistically significant changes in measures of corticospinal excitability and transcallosal inhibition were detected. Nonetheless, alterations in corticospinal and intracortical excitability, as well as spinal excitability changes, cannot be discounted as potential

mechanisms for the increased voluntary wrist extensor muscles activation observed following the *rTMS+RW* training. Indeed, our findings of decreased motor unit recruitment threshold and increased firing rate modulation after *rTMS+RW* reflect an increase in efferent neural drive during the tasks employed (MVC and ramp-hold) (Calvin & Stevens, 1968; Dartnall et al., 2009; Matthews, 1996). Detection of these potential changes was potentially compromised for a number of reasons: Evaluation of corticospinal excitability was underpowered as many participants had moderate to severe upper extremity motor impairment and an MEP was elicited *Pre* and *Post* training in the more affected hemisphere in only 5/13 participants. Furthermore, substantial inter-participant differences in intracortical excitability were likely present, as previously reported across levels of stroke severity and motor impairment by Hayward and colleagues (2017). Thus, any effects of *rTMS+RW* on corticospinal excitability or transcallosal inhibition may have been underrepresented due to low sample size and high inter-individual variability. To keep our assessment protocol to a tolerable length for our stroke participants and within the anticipated window for the after-effects of the rTMS, measures used in our study focussed on corticospinal excitability and interhemispheric inhibition (via transcallosal inhibition) elicited over the ipsilesional hemisphere only. While it is possible that changes in intracortical inhibitory interneurons could also underlie changes in motor unit activation, we did not directly evaluate modulation of intracortical excitability (Buetefisch et al. , 2011).

4.3 Limitations

There are additional limitations to this study. As this was a proof of principle study, only the effects immediately following training were assessed. We do not know if changes were sustained or if greater intensity or dosage of training would have altered our findings. The

outcome measures selected were targeted and shortened; however, each testing session was up to 150 minutes in length. While handgrip MVC and ramp contractions where wrist extensors served a synergistic function were evaluated, potential changes in wrist extensor muscle force generation and hand function associated with the observed improvements in wrist extensor muscle activation remain to be established. Moreover, for clinical application of the current intervention the long-term effects *rTMS+RW* delivered over multiple sessions would be valuable to ascertain.

5 CONCLUSIONS

People with persisting upper extremity motor impairment following stroke who participated in a single session of robot-assisted active wrist extension training combined with simultaneous application of high-frequency rTMS over the ipsilesional motor cortex demonstrated greater changes in voluntary wrist extensor muscle activation compared to a session of robot-assisted active wrist extension training combined with Sham rTMS. Reduced MU recruitment threshold and increased MU firing rate modulation were found, but statistically significant changes were not detected in corticospinal excitability or transcallosal inhibition measures. These results are encouraging for the combined use of these innovative therapeutic interventions for upper extremity rehabilitation following stroke. However, the durability of these changes, the underlying mechanisms and their potential impact on upper extremity activities remain to be investigated.

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Table 1. Individual participant information (n = 13)

ID	Sex	Age (y)	Time Post- stroke (mo)	Lesion location (C/SC)	UE- FMA (/66)	WMFTa total score (#/min)	RMT	Paretic arm (R/L- D/ND)	MUs (n)					
									rTMS+RW			Sham rTMS+RW		
									Pre	Post	Matched	Pre	Post	Matched
RW01	M	62	108	C/SC	8	0	-	R-D	12	11	7	17	17	17
RW02	M	64	48	C/SC	16	0	82	L-ND	3	15	3	6	3	2
RW03	F	57	180	SC	30	0	100	R-D	9	10	8	3	4	1
RW04	F	51	53	SC	29	2.70	-	L-ND	6	8	6	4	5	4
RW05	M	61	95	NA	9	0	-	L-ND	13	14	0	21	17	0
RW06	M	67	92	C/SC	51	13.16	48	L-ND	14	22	2	4	6	4
RW07	F	77	14	SC	18	5.21	-	R-D	5	3	1	9	13	3
RW08	M	64	21	SC	16	0	100	R-ND	7	13	3	9	6	5
RW09	M	58	103	C/SC	59	69.45	65	R-D	28	27	25	11	11	9
RW10	M	73	76	SC	61	59.37	65	R-D	14	9	1	15	10	6
RW11	F	79	15	SC	6	0	-	L-ND	6	6	6	4	7	1
RW12	M	66	81	SC	23	21.40	-	L-D	4	5	1	12	8	2
RW13	M	79	79	SC	58	36.84	76	R-D	18	4	3	11	14	0

Lesion location: identified by functional magnetic resonance imaging (fMRI); C – cortical; SC – subcortical; C/SC –cortical & subcortical involvement; NA: lesion location unknown (fMRI contraindicated); UE-FMA - Fugl-Meyer Upper Extremity Motor scores; WMFTa –Wolf Motor Function Test abbreviated; RMT – Resting Motor Threshold; R – right/L – left; D –dominant/ ND –

non-dominant; MUs – motor units; Pre – prior to training session; Post – following training session; Matched – MUs active both Pre and Post; rTMS: repetitive transcranial magnetic stimulation; RW– Robowrist wrist extension exoskeleton

Table 2. Force, number of motor units and ipsilesional motor evoked potentials for participants (*n* = 13)

	rTMS+RW		Sham rTMS+RW	
	Pre	Post	Pre	Post
Voluntary force parameters				
MVC (V)	1.1 ± 0.7	1.2 ± 0.8	1.2 ± 0.9	1.2 ± 0.9
Rate of force rise in ramp (% MVC/s)	4.6 ± 1.7	4.7 ± 1.3	5.1 ± 1.3	5.0 ± 1.3
Force for 2s in hold (% MVC)	29.8 ± 4.7	31.8 ± 4.3	32.6 ± 10.9	29.9 ± 1.6
Motor units identified (<i>n</i>)				
All MUs	139	147	126	121
MUs with RT > 0.1 % MVC				
MUs active ONLY <i>Pre</i> or <i>Post</i>	65	80	65	63
MUs active BOTH <i>Pre</i> and <i>Post</i>	58	58	47	47
Ipsilesional MEP elicited (number of participants)	5	7	6	8

Pre – prior to training session; Post – following training session; rTMS – repetitive transcranial magnetic stimulation; RW – Robowrist wrist extension exoskeleton; MVC – Maximal Voluntary Contraction; MU-Motor Unit; RT– recruitment threshold; WMFTa – Wolf Motor Function Test abbreviated; MEP– Motor Evoked Potential.

FIGURE LEGENDS:

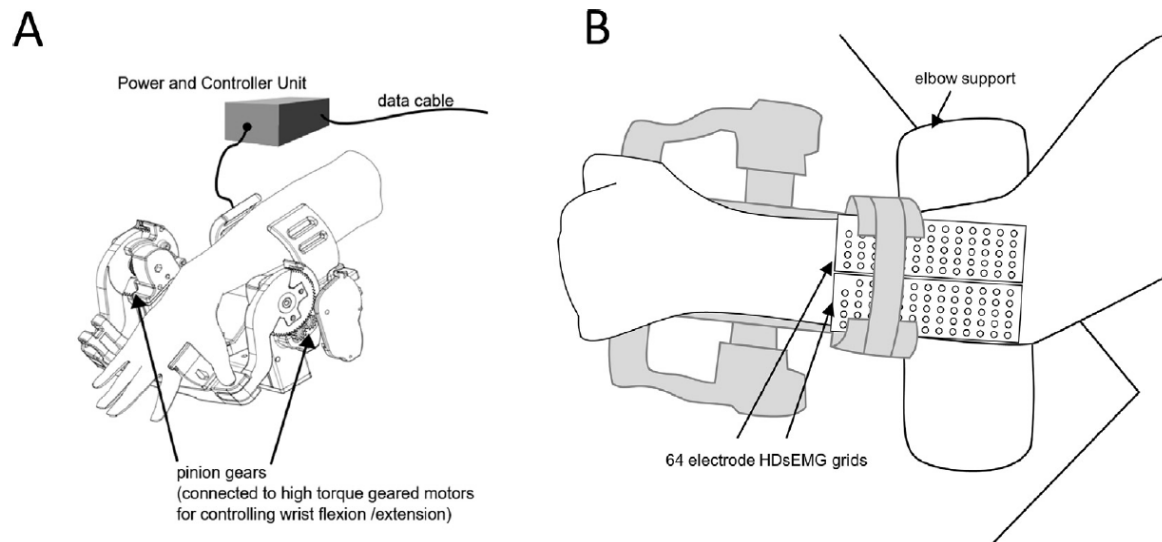


Figure 1. *Set up for robot-assisted active wrist extension practice.* Schematic of the custom built wrist extension exoskeleton “RoboWrist” (RW) device (A). Schematic of arm and wrist positioning in the RW device (B) with two high density surface EMG (HDsEMG) grids used for voluntary muscle activation assessment.

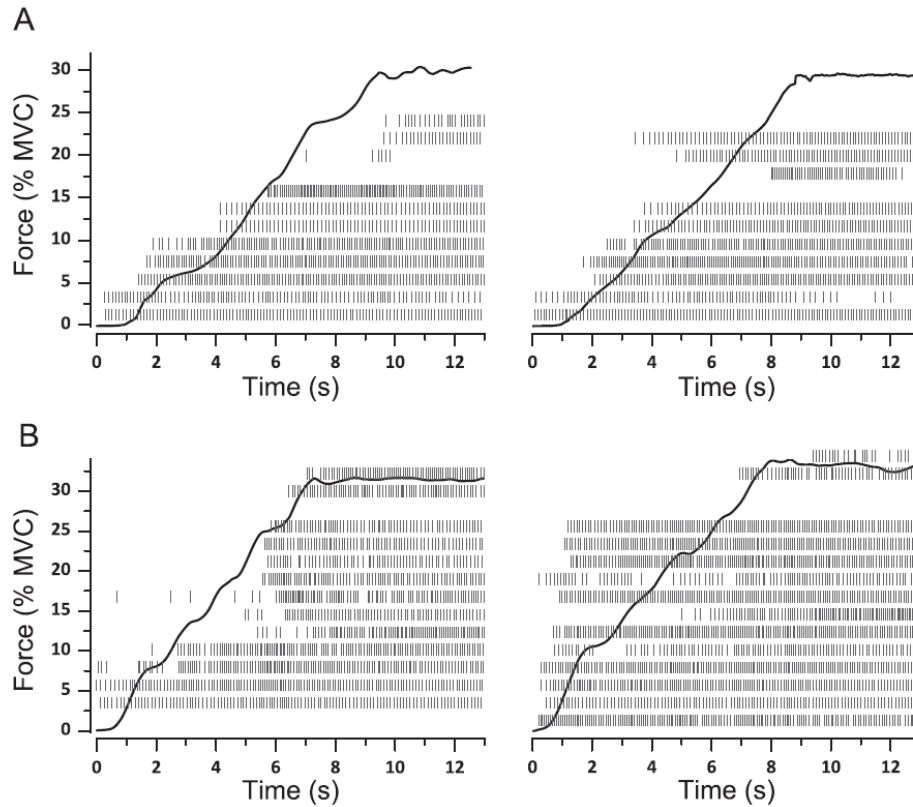


Figure 2. Motor unit activation in ramp-hold contractions *Pre* and *Post* TMS+RW

Examples *Pre* (left) and *Post* (right) *rTMS*+*RW* for two participants. Thicker lines depict the force during the ramp-hold contractions. The thin vertical lines depict motor unit (MU) firing times for each MU firing. MUs active both *Pre* and *Post* are aligned horizontally on the same row. A. Fugl-Meyer Upper Extremity Motor Score = 8/66; B. Fugl-Meyer Upper Extremity Motor Score = 59/66; *not all MUs are shown*). The rate of force change for the ramps was comparable *Pre* and *Post* within participants (A: *Pre* 3.1 % MVC/s; *Post* 3.2% MVC/s and B: *Pre* 5.0 % MVC/s; *Post* 4.9% MVC/s). Arrows show MUs that were active both *Pre* and *Post* and were recruited earlier after the intervention. Note the larger number of MUs that were recruited earlier in participant B.

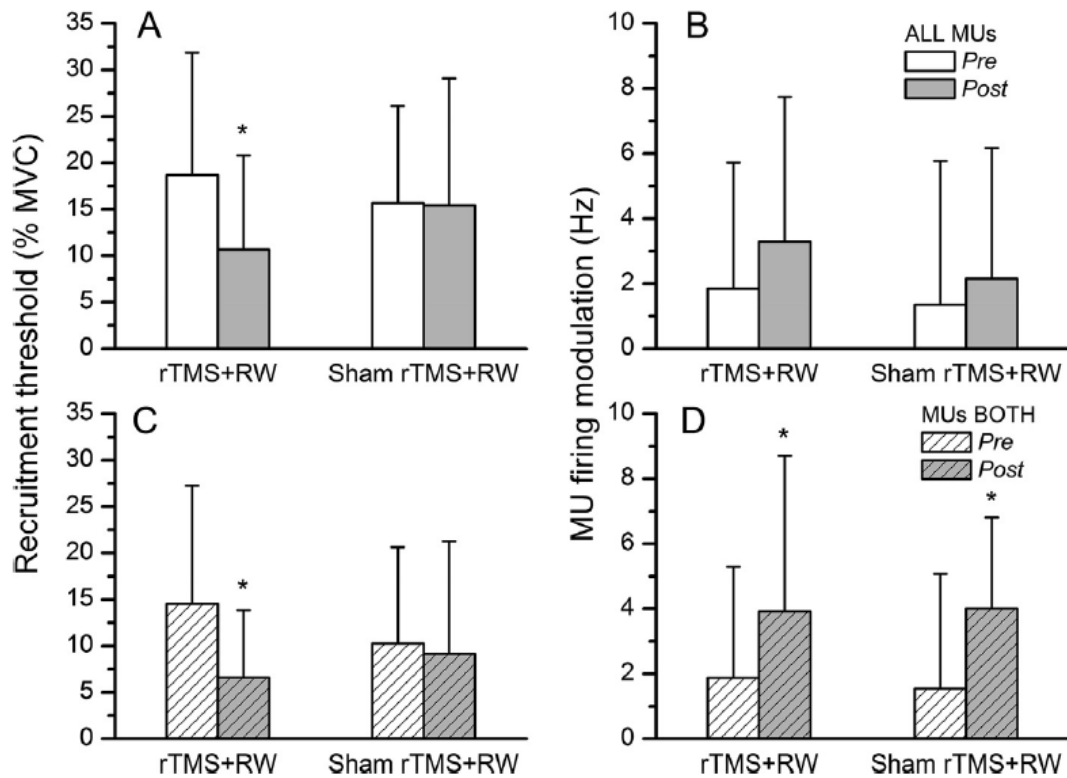


Figure 3. Motor unit recruitment thresholds and firing modulation Pre and Post training

Motor unit (MU) recruitment thresholds (A and C) and MU firing rates modulation (B and D)

Pre (white, solid and diagonally striped) and *Post* (grey, solid and diagonally striped)

rTMS+RW and *Sham rTMS+RW* training. All motor units with recruitment thresholds

>0.1%MVC are shown in panels A and B (solid bars), and the subset of matched motor units

active both *Pre* and *Post* training are shown in panels C and D (diagonally striped bars). Data

presented are mean \pm SD. * $p < 0.05$

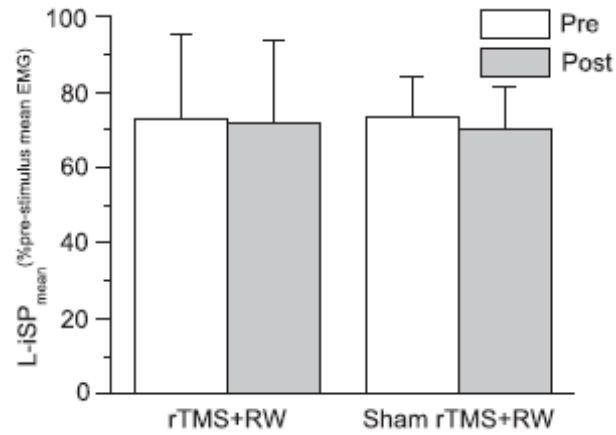


Figure 4. Transcallosal Inhibition (TCI). Ipsilateral silent period (iSP) elicited from the lesioned (L) hemisphere *Pre* and *Post* rTMS+RW (black) and Sham rTMS+RW (white) training sessions. L-iSP_{mean} EMG is presented as a percentage of pre-stimulus mean EMG. Data are mean \pm SD.

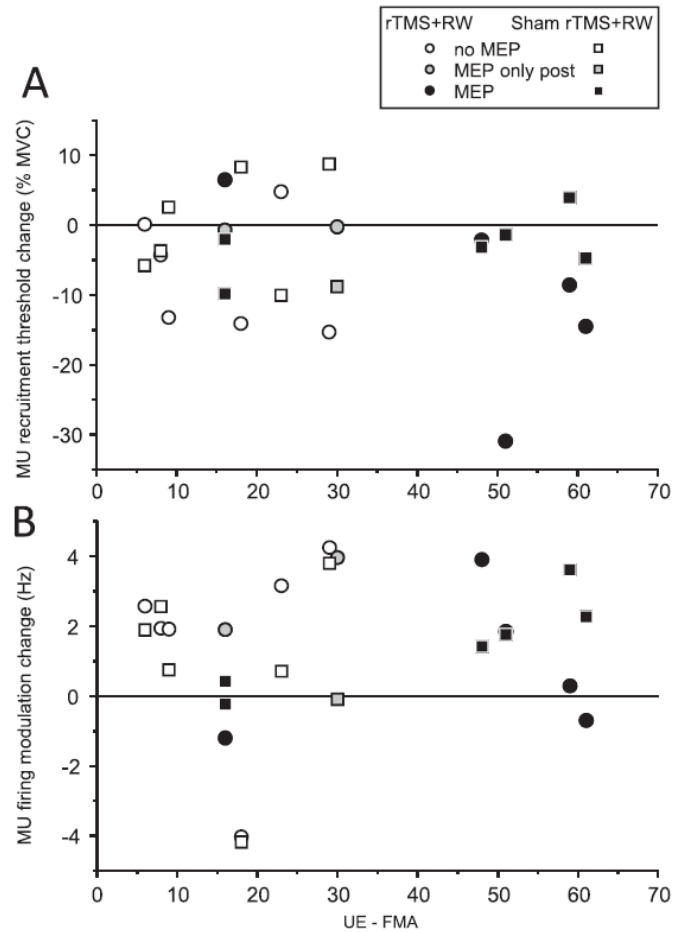


Figure 5: Individual response to training. Individual participant changes from pre- to post-training in (A) mean motor unit (MU) recruitment threshold (as a % of maximal voluntary contraction [MVC]) and (B) mean MU firing rate modulation for ALL identified MUs are presented for the two training conditions (rTMS+RW and Sham rTMS+RW) by their Fugl-Meyer Upper Extremity Motor score (UE-FMA). Data points are labelled based on the presence of an ipsilesional motor evoked potential both Pre and Post (MEP), Post only (MEP only post) or absence of an MEP.