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Downregulating aberrant motor evoked potential synergies of the lower extremity post stroke during TMS of the contralesional hemisphere

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Abstract

Background—Growing evidence demonstrates unique synergistic signatures in the lower limb (LL) post-stroke, with specific across-plane and across-joint representations. While the inhibitory role of the ipsilateral hemisphere in the upper limb (UL) has been widely reported, examination of the contralesional hemisphere (CON-H) in modulating LL expressions of synergies following stroke is lacking.

Objective—We hypothesize that stimulation of lesioned and contralesional motor cortices will differentially regulate paretic LL motor outflow. We propose a novel TMS paradigm to identify synergistic motor evoked potential (MEP) patterns across multiple muscles.

Methods—Amplitude and background activation matched adductor MEPs were elicited using single pulse TMS of L-H and CON-H (control ipsilateral) during an adductor torque matching task from 11 stroke and 10 control participants. Associated MEPs of key synergistic muscles were simultaneously observed.

Results—By quantifying CON-H/L-H MEP ratios, we characterized a significant targeted inhibition of aberrant MEP coupling between ADD and VM ($p=0.0078$) and VL ($p=0.047$) exclusive to the stroke group ($p=0.028$) that was muscle dependent ($p=0.039$). We find TA inhibition in both groups following ipsilateral hemisphere stimulation ($p=0.0014$; $p=0.015$).

Conclusion—We argue that ipsilaterally mediated attenuation of abnormal synergistic activations post stroke may reflect an adaptive intracortical inhibition. The predominance of sub 3ms interhemispheric MEP latency differences implicates LL ipsilateral corticomotor projections. These findings provide insight into the association between CON-H reorganization and post-stroke LL recovery. While a prevailing view of driving L-H disinhibition for UL recovery seems

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expedient, presuming analogous LL neuromodulation may require further examination for rehabilitation. This study provides a step toward this goal.

Keywords

Transcranial Magnetic Stimulation; Muscle Synergies; Contralesional Hemisphere; Stroke; Lower Limb

Introduction

During walking, abnormal kinematic deviations are apparent following stroke¹ reflecting the loss of independent joint control^{2, 3}. Subsequent studies suggest these deficits are not compensatory strategies⁴, but involve abnormal neuromechanical interactions⁵. Moreover, observations of such neural constraints include coupled cross planar kinetic outflow^{6, 7} and altered muscle synergy structure^{8,9}. Recent evidence from our lab demonstrated that the lesioned hemisphere upregulates aberrant coupled LL corticospinal excitability¹⁰. There we reported that stroke subjects exhibit abnormal TMS induced corticospinal coupling of the quadriceps muscles while performing isometric hip adduction. These findings underscore the need to clarify neuromechanical constraints underlying hemiparetic gait for developing effective clinical interventions.

In this study we explore the potential role that acute modulation of motor cortices play in the regulation of lower limb (LL) neural coupling post stroke. Specifically, we examine the relative contribution of contralesional hemisphere (CON-H) activation to aberrant across joint coupling of ipsilateral lower-extremity muscles post stroke. While traditional TMS paradigms examine the cortical excitability of a single muscle, we propose a novel TMS paradigm that evaluates MEP patterns across multiple LL muscles. This differential MEP signature will be used to explore potential cortical regulation¹¹ of abnormal synergistic muscle activations while characterizing the modulatory effect of CON-H post-stroke. To our knowledge, no study has systematically evaluated synergistic LL MEP patterns to clarify cortically mediated motor adaptation.

Extensive evidence has implicated CON-H in mediating functional recovery post stroke^{12–17}, motivating investigations of the underlying neurophysiology. The association of increased CON-H activity with paretic hand movements have been documented using fMRI^{12, 13} and EEG^{14, 15}. However, post-stroke remapping of sensorimotor cortices results in simultaneous activation of various cortical regions and descending pathways^{16, 17}. TMS has been used to discern descending inputs to upper limb (UL) motor function in primates^{18,19} and humans²⁰. Previous inquiries of ipsilateral UL control^{21,22} using TMS of CON-H^{23,24} have presented mixed results. While some have indicated that CON-H modulates post-stroke motor plasticity transcallosally^{25, 26}, others observed intact cortico-motoneuronal circuitry^{27, 28}. It is unclear how CON-H governs across joint coupling of the LL post stroke.

Few published studies elucidate contributions of CON-H to adaptive motor output in LL muscles following stroke. Accordingly, there is insufficient evidence supporting that CON-H activates analogous neural substrates for the UL and LL, given segmental modulation of

walking²⁹. We hypothesize that a biased stimulation of the lesioned and contralesional cortices will have differential effects on the MEP patterns in the paretic limb of stroke survivors, suggesting that each hemisphere plays different roles in regulating the expression of abnormal muscle couplings. We further characterize temporal features of the elicited MEPs to indirectly explore neural pathways proposed to mediate adaptive CON-H plasticity.

Methods

Participants

10 normal control participants (mean age 37, range 20–52) and 12 stroke participants (mean age 58, range 43–67) with single hemispheric stroke were recruited for this study (Table 1). All participants gave written informed consent according to the declaration of Helsinki. The study was approved by the Northwestern University Review Board. Stroke participants were included if 1) they had only one stroke at least one year prior to study participation 2) had the ability to perform hip adduction movements during body weight support standing on the non-paretic leg. All stroke participants presented with persistent hemiparesis due to chronic stroke. Further, all stroke participants were ambulatory and able to independently walk with or without the use of an orthotic or assistive device. Functional levels of the participants are tabulated when available. Exclusion criteria for both stroke and control groups include 1) contraindications for TMS 2) history of seizures and medications known to affect central nervous system excitability 3) history of orthopedic injury or surgery to their lower limbs.

Transcranial Magnetic Stimulation

Motor evoked potentials (MEP) were elicited in 8 LL muscles of the test limb using single pulse TMS. In stroke participants the test limb was the paretic leg whereas for control the test limb was the right leg. TMS was applied on separate trials to either the lesioned hemisphere (L-H) or contralesional hemisphere (CON-H). Surface electromyography (EMG) preamplifiers (Motion Analysis) were placed over the following muscles of the test limb: vastus medialis (VM), vastus lateralis (VL), rectus femoris (RF), adductor longus (ADD), tibialis anterior (TA), medial gastrocnemius (GAS), and biceps femoris (HAM). A common ground was placed over the dorsum of the hand. All electrodes were secured to the skin using adhesive tapes and self-adherent elastic Coban wrap (Dynarex Corp., Orangeburg, NY, USA). Single pulse TMS was delivered using a Magstim 200 stimulator (Magstim, Whitland, UK) via a 110 mm diameter double-cone coil. The coil was oriented to induce a posterior-to-anterior current flow over M1 region of each cortex. A tightly secured swimming cap placed over the participant's head was used to map and demarcate the optimal coil position and orientation for placement consistency. The intersections of the inter-aural and nasion-inion lines were first marked on the cap as the vertex. The coil position was incrementally adjusted from an initial starting position of 1cm posterior and 2 cm lateral to vertex¹⁰ to locate the hotspot of the targeted ADD muscle in the test limb (paretic limb for stroke, right limb for control) over the lesioned motor cortex (L-H). Optimal coil position and intensity was mapped as the lowest intensity of magnetic stimulation required to evoke ADD MEPs of 50 μ V in peak-to-peak amplitude in at least three of five consecutive trials. Coil positioning was performed in seated position to minimize subject fatigue. The lower motor hotspot for the test limb ADD was symmetrically

mapped for stroke contralesional hemisphere (CON-H) or control ipsilateral hemisphere to the test limb. The initial coil position placement was approximately 1 cm posterior and 2 cm lateral to vertex on the hemisphere ipsilateral to the test limb³⁰.

Experimental Procedure

Participants were secured in a motorized, instrumented exoskeleton (Lokomat; Hocoma, Zurich, Switzerland) and isometrically locked in the toeoff gait posture: 15° hip extension, 45° knee flexion, and 90° ankle dorsiflexion⁷. The dimensions of the orthosis were adjusted for each participant to align the orthosis joint centers with those of the participant. The participant's lower extremities were secured to the orthosis via cuffs instrumented with 3 total six-degree-of freedom load cells (JR3, Woodland, CA) to measure the interaction forces and moments of the paretic test limb (Figure 1). The test limb was completely unloaded by the Lokomat such that participants did not have to actively support the limb during torque production.

Participants were first asked to produce maximum voluntary isometric contractions (MVIC) in hip adduction, abduction, flexion, and extension directions with their test limb. MVIC were performed to obtain maximum torque and EMG signals. Participants were next instructed to match voluntary isometric hip adduction torques at 40% of MVIC levels (Figure 1) with their test limb. Differences in background activity across muscles may influence the evoked MEP amplitude³¹. Therefore a minimum target torque of 30% MVIC was needed to enable consistent matching of the pre-stimulus background EMG activity in the non-targeted (non-ADD) muscles across trials and coil placements¹⁰. Furthermore, previous investigations reveal no significant variations in both the isometric torque signal and the TMS elicited action potentials in the post stroke lower limb for levels up to 60% MVIC^{32,33,34}. Participants received instantaneous visual feedback of the hip joint torque magnitude and direction produced by moving a circular cursor on a LCD monitor to a circular target. A successful trial was considered if the participant was able to match the target adduction torque within $\pm 5\%$ of the torque magnitude hold for a minimum of 200ms. The torques generated at each joint were calculated from thigh and shank load cell signals using static equilibrium equations.

For each trial, the TMS coil was placed over the hotspot location for either hemisphere marked on the swim cap and secured to the participant's head. CON-H and L-H were stimulated on separate trials. Stability of the coil placement was improved using a custom designed overhead pulley system and cable supporting stand. A research assistant manually stabilized and positioned the coil over each target hotspot for each hemisphere for all trials along with Velcro straps. When the participants successfully matched the target adduction torque, the TMS device was triggered to deliver a single pulse using a custom automated software trigger (MATLAB v7.01). The TMS device was not triggered if the participant produced any off axis torque during the hip adduction or if the target torque magnitude exceeded $\pm 5\%$. MEPs for each trial were constantly monitored by oscilloscope to ensure that ADD amplitude cMEP and iMEP were matched for all trials (Figure 1, 2). Stimulator output was accordingly adjusted to meet this condition and was initially set at 110% of the estimated seated stimulator output. The adjusted stimulator outputs for each subject for both

L-H and CON-H coils placements while the participants were inside the Lokomat are listed in Table 1. 10 to 30 trials were performed for each hemisphere in blocks of 10, with two 5-minute rest periods between blocks.

Given the low spatial resolution of TMS, we expect that we will activate both hemispheres even after CON-H placement. This is further amplified by the complex representation of the lower limb muscles in the cortex. MEP responses recorded at the muscles during CON-H stimulation will therefore be the summated responses from the activation of both ipsilateral and contralateral descending drive. We optimize the CON-H coil location and orientation for the test limb ADD by normalizing the stimulation intensity to the increased distance of a CON-H coil placement (Figure 2). This was accomplished by incrementally increasing the stimulator output in order to match the contralateral ADD MEP (cMEP) amplitude evoked by stimulation of CON-H (Figure 2). Successful matching of ADD MEP across coil placements ensures that the combined stimulation of both L-H and CON-H results in the same summated downstream motor output of the targeted ADD MEP from both hemispheres. Thus any non-uniform change in the pattern of MEPs for other non-targeted lower limb muscles must be a result of additional CON-H activation, facilitating meaningful comparisons between the patterns of MEP modulation across multiple muscles.

MEP Analysis

A secondary analysis of onset latencies from TMS has been traditionally used to make inferences regarding putative neural pathways to the upper and lower limbs^{21,22,26}. While the primary goal of this investigation was to characterize the contribution of CON-H to aberrant synergistic muscle activation post stroke, the differential onset latency across hemispheres may provide indirect evidence regarding select neural pathways. To identify the onset latency of the TMS-induced motor evoked potential (MEP) in the EMG of each muscle, the EMG signal during the target matching task was filtered with an 8th order Butterworth, low-pass, zero-phase digital filter with a 220 Hz cutoff frequency. The filtered EMG signals were rectified and smoothed using the same digital filter with a 20 Hz cutoff frequency. MEP onset latencies were computed for each primary muscle in each participant from the ensemble of successful hip adduction trials (Figure 5). A pre-TMS background level of EMG activity was defined by calculating the mean and standard deviation of activity within a 100 ms window prior to a recorded TMS trigger pulse³⁵. An extended 80 ms window following the TMS trigger pulse was used to search for the onset of MEPs^{30,36} that were clear and distinguishable from background activation^{35,36}. Consistent with published onset latencies for LL muscles greater than 30ms^{10,29,35}, the initial 20 ms epoch following the TMS trigger was designated to account for any post stimulus artifact. Similar to a procedure outlined by Garvey et al³⁷, MEP onset was identified when five consecutive points in the EMG trace after this 20ms window were above three standard deviations of the mean pre-TMS EMG activity³⁷ on the ascending phase (Figure 5). MEP amplitudes were measured peak-to-peak and averaged off-line across successful trials³⁸. The MEP amplitude was calculated as the difference between the background activation during the target matching task and the average of a 20 ms time window centered at the peak MEP rectified pulse.

Pre-stimulus background EMG activation of ADD was matched across trials for across coil placements through the torque matching paradigm. A post-hoc analysis was performed to ensure that all successful trials included in the ensemble averages achieved statistically equivalent ADD background activations. Similarly, a post hoc analysis of the background activation of any coupled non-targeted muscles was performed. The ratio of the mean iMEP amplitude over the mean cMEP amplitude will be quantified for each muscle for each participant. A statistical value less than one indicates reduction in iMEP amplitude relative to the cMEP amplitude following TMS of the ipsilateral hemisphere.

Statistical Analysis

Paired t-tests were used to evaluate if the ensemble averaged cMEP and iMEP ADD amplitudes were statistically different. To assess the potential confounding effect of differences in background activations of the non-targeted muscles for the different stimulation conditions, a paired t-test was used to test if the mean ADD, VM, and VL background activation in the 100ms prestimulus window during contralateral and ipsilateral hemisphere trials were statistically different. A two-way ANOVA was performed to examine the effect of muscle on iMEP/cMEP ratios, with group (stroke vs control) as the between subjects factor. Post hoc analysis using one-way analysis of variance (ANOVA) was used to detect significant differences in MEP ratios within the stroke group. Further post hoc analysis using pairwise comparisons were used to determine significant differences in MEP ratios across muscles using Fishers Least Squares Difference. Finally, one sample t tests were used to evaluate if the iMEP/cMEP ratio for each muscle's ratio is statistically different than 1 for each group. All statistical analysis was performed using SPSS for windows v 19.0 (NCSS 9 LLC, Kaysville, Utah USA). A significance level of $\alpha = 0.05$ was set for all statistical analyses.

Results

Consistency in Background EMG Activation

MEP traces across multiple muscles following coil shift from a typical stroke participant can be seen in Figure 3. For stroke participants, the average stimulator intensity on the CON-H was 71.25%, with a range of 50% to 85% of max stimulator output. For control subjects, the average stimulator intensity was 57.1 %, with a range of 40% to 66% of max stimulator output. For the 100 ms prestimulus window, no statistical difference was detected in the mean background activations for the targeted ADD muscle between L-H and CON-H trials for all participants ($p > 0.05$, $p = 0.241$). This indicates that after shifting the coil to the opposite hemisphere, all targeted ADD trials were successfully matched in background activation eliminating the confounding effect of background activation on cortico-motor excitability of the ADD. Similarly no statistical difference in background activation was detected for VM ($p = 0.213$) and VL ($p = 0.269$). Furthermore, the mean ADD MEP amplitude was not statistically different between hemispheres for all participants ($p > 0.05$, $p = 0.618$) such that cMEP = iMEP for all trials (Figure 3, 4). Exemplar data from one stroke participant depicts the quantification of latency differences between iMEP and cMEP (Figure 3). The distribution of the MEP latencies for all trials is shown in Figure 6. A paired

t-test of CON-H and L-H latencies for all muscles shows no significant difference in onset latencies ($p < 0.000$).

Effect of Stimulated Hemisphere on MEP Ratio

Average iMEP/cMEP ratios across participants can be seen in Figure 4 for stroke and control participants. MEP ratios for stroke VM and VL were significantly lower than all other muscles in the stroke group only. The MEP ratio for TA was also significantly lower for both groups (Figure 3,4). Several statistical outcomes confirm these findings. Results from a second two-way factorial ANOVA between the main effects of group and muscle location revealed a significant effect of group on MEP ratio ($p = 0.028$) and a significant effect of muscle location ($p = 0.039$). No significant interaction was detected between group and muscle, indicating that for both groups, responses in the same muscles were affected by changing the stimulated hemisphere. Specifically, a Tukey post hoc test revealed a significant reduction in TA MEP ratio in both groups ($p < 0.05$). Furthermore, a one-way ANOVA of the stroke group revealed significant differences between muscle MEP ratios ($p = 0.0331$). MEP ratio in the TA of stroke survivors was significantly lower from the same ratio in all other muscles in the same group except VM and VL ($p < 0.05$). Reductions in TA MEP amplitude for a typical stroke participant are plotted in Figure 3. Finally, one sample t tests of the MEP ratios for each muscle were not statistically different than 1 ($p > 0.05$) with the exception of stroke VM ($p = 0.0078$), stroke VL ($p = 0.047$), stroke TA ($p = 0.0014$), and control TA ($p = 0.015$).

Discussion

The present study characterizes differences in the contribution of the lesioned and contralesional motor cortex to abnormal LL muscle activations following stroke. By quantifying the ratio of the iMEP to cMEP amplitudes, we observe a significant attenuation of aberrant MEP coupling between ADD, VM and VL following stimulation of the contralesional primary motor cortex that is exclusive to the stroke group. This confirms our hypothesis of a differential effect of lesioned and contralesional hemisphere stimulation on LL muscles and supports the idea that motor signals from CON-H reduce the expression of abnormal synergistic corticomotor excitability that impair stroke gait. The predominance of sub 3ms iMEP latencies together with a significant reduction of TA ratios across groups suggests evidence of direct LL ipsilateral projections from CON-H. As such our observations seem incongruent with the notion of maladaptive motor output in CON-H post stroke suggested in the UL³⁹ and LL³⁰. Our observations indicate that fundamental differences exist between CON-H contributions to functional recovery in the upper and lower limbs. The primary finding reported here is the reduction of abnormally coupled corticomotor output across lower limb muscles post stroke resulting from a biased stimulation of each cortex. Within the context of TMS, it is not possible to further discern between putative descending pathways given the limited spatial resolution. By combining both the targeted attenuation and interhemispheric onset latency data, we make informed interpretations regarding specific neural pathways that may or may not have contributed to the observed modulation.

Targeted Inhibition of Abnormal Synergistic Coupling

Several TMS paradigms have been used to investigate UL cortico-motoneuronal connections from the CON-H post stroke⁴⁰. However a systematic evaluation of coupled iMEP patterns in the post-stroke LL is lacking. While other groups have characterized changes in ipsilateral vs contralateral CST conductivity in the post stroke LL⁴¹, the iMEP/cMEP ratio employed here specifically highlights the reduction of coupled motor output across LL muscles resulting from biased activation of each motor cortex. We demonstrate a novel multi-muscle TMS paradigm that uniquely evaluates cortical modulation of abnormal synergistic neuromotor coupling. Our data elucidates a significant differential effect of hemisphere on the evoked MEP pattern across LL muscles. Two important findings argue for this interpretation: 1) consistent with a previous investigation¹⁰, stimulation of the lesioned hemisphere amplified abnormal adductor and knee extensor EMG activation coupling relative to controls 2) stimulation of CON-H significantly reduced this activation coupling only in stroke. Crucially, the observed inhibition was specific to muscles previously reported to be expressed in abnormal LL synergies^{2, 8}. Our observations corroborate evidence of neurally constrained muscle synergies⁹ previously proposed to constrain paretic gait subtask performance⁴². These findings support the idea that neuromodulation from CON-H may yield a functionally relevant output of CON-H hyper excitability for hemiparetic LL gait despite mixed evidence for UL recovery^{43,44}.

One could argue that ipsi MEPs from the lesioned hemisphere to the non-paretic muscle could potentially be a more appropriate control for changes in muscle activation coupling. It has been suggested that the unaffected side of stroke participants may not be completely unaffected given evidence of neural organization^{16,17}. Applying the current methodology to healthy subjects arguably yields a more effective control case to investigate ipsilateral hemisphere contributions to non-paretic muscle. In healthy subjects we do not see reductions in muscle activation coupling following CON-H stimulation. However it must be acknowledged that the iMEP/cMEP ratio cannot directly apportion the relative effect the stimulation to ipsilateral vs contralateral conductivity. In the absence of bilateral assessments of TMS connectivity and imaging analysis of CST integrity, inferences regarding asymmetries in contra vs. ipsilateral connectivity post-stroke will prove difficult in the context of this study.

Intracortical Inhibition

The specificity of the observed attenuation further suggests that TMS potentially activated inhibitory pathways from ipsilateral cortex to LL muscles. In the absence of more invasive recordings²⁰, we can only speculate regarding the mechanism of inhibition along the neuraxis. One possible neural substrate underlying our findings is the widely observed intracortical inhibition (ICI) mediated by GABA-ergic activity of cortical interneurons⁴⁵ and directly demonstrated in humans²⁰. Stimulation of CON-H may have activated intracortical inhibitory circuits that reduced the expression of abnormal muscle synergies. Investigations of UL cortico-cortical inhibitory circuits reported inhibitory modulation onsets of 1–3ms following a conditioning stimulus^{46, 23}. The similar timescale of our iMEP latencies suggest that circuits pertaining to ICI may potentially account for the targeted attenuation via inhibitory circuits that decrease the excitability of cortical cells²⁹. Although our paradigm

does not directly test this hypothesis, short latency ICI has been demonstrated in LL muscles⁴⁷ in which ICI was not strongly related to motor threshold or MEP recruitment⁴⁷. There are limited studies investigating intracortical mechanisms in the LL post stroke and the relation to clinical recovery measures in the UL is equivocal^{28, 40, 45}. Nonetheless the observed multi-muscle iMEP signature may be reflective of an inhibitory connectivity between the cortical representations of abnormally synergistic muscles post stroke.

Latency of Ipsilateral Responses to TMS

Given the multiplicity of pathways through which CON-H can access the LL, the skewed distribution of the observed iMEP latencies (Figure 6) favor certain neurophysiological interpretations. Short interhemispheric iMEP onset latencies suggest that TMS of the ipsilateral cortex may have excited a fast conducting corticospinal pathway to LL muscles. We observe short latencies from all LL muscles, whereas previously reported UL iMEPs were rarely found in distal muscles but reported in proximal muscles in normal participants^{48, 49}. Direct cortical control of ipsilateral limb muscles has been previously proposed from TMS studies of UL CON-H projections^{17, 28}. Moreover anatomical evidence from primates has established direct monosynaptic corticospinal projections to hindlimb motoneurons⁵⁰ along with an uncrossed corticospinal tract that descends ipsilaterally⁵¹. Therefore, it seems plausible that unlike the UL⁵², ipsilateral pathways from CON-H motor cortex play an important role in LL functional recovery post-stroke. Nevertheless, the dominance of short onset latency differences provides indirect evidence that ipsilateral corticospinal inputs to the human LL drive the observed inhibition, a notion debated in the healthy UL⁵³. Improving the sampling resolution may yield additional insight regarding pathways that may or may not contribute to the observed responses.

Differential Contributions of Each Hemisphere

Given that both cortices are activated even after a biased placement of the coil, the extent to which each hemisphere contributes to the lower limb expression of MEP coupling cannot be discriminated by the data. Nevertheless, biasing the stimulation of one hemisphere via coil placement has previously been employed in TMS studies investigating the underlying electrophysiology of both healthy and pathological cohorts^{17, 21, 22, 26}. To account for the increased distance of the coil to the targeted ADD representation, amplifying the stimulation output to match ADD cMEP and iMEP ensures that the aggregate effect of stimulating both L-H and CON-H results in the same motor output for the target ADD across coil placements. However, if distance from the lesioned hemisphere was the *only* parameter that changed following coil shift, then an increase in stimulator output should modulate iMEPs for all non-targeted muscles. To this end, we do not observe a modulatory effect on the iMEP amplitudes across non-targeted muscles. If CON-H has no role to play in the targeted attenuation, we would alternatively expect an amplification of abnormal knee extensor/hip adductor coupling consistent with previously reported results¹⁰. Our findings in Figure 4 presents the opposite case in which the inhibitory effect of CON-H stimulation was specific to the knee extensor muscles. One could also argue that the observed targeted attenuation could potentially be attributed to the relative spatial location of each muscle's cortical neurons. The representations of VM and VL in motor cortex may be located further away from the other lower limb muscles and experience a diminished stimulation. This seems

unlikely given anatomical studies in primates where the LL representations are clustered together⁵⁴. Together, these controls undermine the possibility that current spread merely activated contralateral cortex.

CON-H Influence of Lower Limb

A significant feature of our findings was that we robustly evoked amplitude matched adductor iMEPs in all study participants by stimulating ipsilateral motor cortex. This finding seems incompatible with previous UL studies where some authors elicit iMEPs in the healthy UL^{53, 22} while others have failed to evoke ipsilateral responses^{55, 56, 27} in stroke participants. These equivocal results may be explained by predominant plastic reorganization in LH⁵⁷ or differential inputs to distal muscles²¹. The lack of correlation between CON-H activity and UL motor recovery^{58, 26} undermine a role of CON-H in shaping functional recovery. However, the neurophysiological substrates underpinning LL control are different given interactions with segmental modulation of neural walking circuits⁵⁹. The ability to preferentially modulate one lower limb cortex has been demonstrated in a limited number of studies using tDCS⁶⁰, rTMS⁶¹, and paired associative stimulation⁶². Reliable ADD iMEPs were elicited in all participants at 40% maximum torque, suggesting that the proximal LL joints may be more readily excitable post stroke than the distal UL.

In our study, we remarkably observe a targeted inhibition of TA across groups following coil shift. This corroborates previous research supporting privileged cortical control for TA^{63,64,65} where direct monosynaptic connections have been implicated in the healthy TA^{63, 29}. This reduction in TA across groups may thus represent a preserved feature of healthy motor control where post-stroke plasticity has limited effect. These findings imply that direct inhibitory pathways exist in healthy participants and implicate the ipsilateral motor cortex in the regulation of the lower limb.

Transcallosal Pathways

While transcallosal pathways and their inhibitory effect^{58, 66} have been widely documented during paretic hand movements^{20, 67}, their influence in LL pathways remains unclear. A majority of the observed iMEP latency differences were below previously estimated minimum UL interhemispheric conduction times ranging from 5–8ms⁶⁸. The minority of long latency differences in our data undermines the idea that the inhibition was mediated transcallosally. However, this does not conclusively preclude contributions of IHI as our data represents an aggregate effect of both excitatory and inhibitory activity. While reducing IHI from CON-H has been leveraged to enhance paretic hand function^{69, 39}, the inhibition from CON-H seems advantageous. Contrary to the UL, our data suggests that downregulating abnormally high IHI drive from CON-H^{70, 71} may be beneficial for LL gait.

Subthreshold Responses

One could argue that increasing the stimulator output could have differentially biased the electrophysiology of the underlying intracortical circuitry. Interestingly for control participants, while the stimulation needed to evoke an MEP in the contralateral hemisphere

is lower, a similar increase in stimulator output was needed to match ADD MEPS for ipsilateral coil placement (~6% stimulator output increase, TABLE 1). It has been reported in healthy subjects that short interval intracortical inhibition can be achieved in the upper limb using conditioning pulse intensities ranging from 0.7 to 1.0 of active motor threshold (AMT)^{45,46} within a paired pulse paradigm. However, there is currently no consensus regarding a shifted balance in intracortical inhibition/facilitation post stroke, especially in the lower limb at these stimulation ranges. We are aware of only one lower limb study that reports a shift toward lower intracortical facilitation in TA post-stroke using a 1.2 AMT conditioning pulse⁷². The maximum stimulation intensity used in our study was 1.12 AMT. Nevertheless, Chen and colleagues reported that the absolute conditioning stimulus intensities required to elicit intracortical inhibition may not be related to active motor threshold or MEP recruitment in both lower and upper limb muscles⁴⁷. Given that our CONH stimulation intensities never exceeded 1.2 AMT, there is no available evidence to suggest that stimulus levels used shifted the balance in intracortical circuitry toward facilitation or inhibition.

Future Directions

Other limitations may potentially confound the generalizability of the observed MEP patterns. Given that the current results focus on a hip adduction/knee extension synergy, it remains to be seen if CON-H also modulates other abnormal across joint kinetic coupling. Additionally, the stroke participants tested here are relatively high functioning and well recovered given their ability to independently ambulate in the community. Furthermore, individuals with only brain stem and cerebellar stroke were not tested in this study. Experiments with stroke subsets may clarify inferences on brain stem pathways as generalizations to the broader stroke population must be further qualified. Correlations with clinical recovery level and other gait related performance metrics are an important topic of future research.

Conclusion

While a prevailing view of driving LH disinhibition for UL recovery seems expedient, we argue that presuming analogous neuromodulation for the LL may adversely affect rehabilitation outcomes. Our results demonstrate that neural substrates underlying LL post-stroke muscle recovery may activate alternative pathways than the UL following CON-H stimulation. Therefore, developing appropriate gait therapies requires further clarity of post-stroke neural plasticity specific to LL motor pathways. This study takes an important step in exploring the relationship between CON-H cortical reorganization and the modulation of abnormal LL synergies post-stroke.

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Highlights

- Novel multi-muscle magnetic stimulation paradigm used to evaluate abnormal muscle synergies
- Targeted inhibition of stroke specific synergy during contralesional hemisphere stimulation
- Short latency motor evoked potentials across muscles imply direct lower limb ipsilateral pathways
- Clarify differences between contralesional hemisphere contributions to upper and lower limbs
- Insight into contralesional hemisphere reorganization and lower limb recovery post stroke

Figure 1. Experimental Paradigm

A volitional torque generation task under isometric conditions with visual feedback. Participants match a target hip adduction torque magnitude that is normalized to 40% of maximum voluntary contraction. The test limb is the affected limb for stroke participants and the right leg for controls participants. Visual feedback of hip joint output torque in the form of a position cursor is provided. Subjects are locked in toeoff gait position with instrumented exoskeleton worn by participants (not shown). Torques were calculated using static equilibrium equations from a set of 6 degrees of freedom load cells attached to the orthosis (see Krishnan & Dhaher 2010 for details). While subjects successfully matched target torque, the TMS device was triggered over the hemisphere contralateral (lesioned) or ipsilateral (nonlesioned) to test limb. The TMS device was not triggered if the participant produced any off axis torque during the hip adduction or if the target torque magnitude exceeded $\pm 5\%$. MEPs were recorded across 8 lower limb muscles during target stimulation of adductor longus.

Figure 2. Schematic representation of TMS stimulation paradigm

Adductor longus motor evoked potentials were elicited during an adductor torque target matching exercise at 40% maximum voluntary contraction. The distance of individual lower limb muscle representations, such as adductor longus and vastus medialis, to the magnetic field (B) produced by TMS is represented as a vector r . This distance is assumed to increase following contralesional coil placement (ipsilateral in control) r_{nl} relative to coil placement over the lesioned hemisphere r_l . In order to normalize the strength of the magnetic field to the increased distance, stimulator output of B_{nl} was increased relative to B_l such that the resulting evoked adductor amplitude was matched across coil placements. MEP amplitudes for other lower limb muscles were simultaneously recorded during the same stimulation. Abbreviations: B = magnetic field, nl = nonlesioned, l = lesioned, ADD = adductor longus, MEP = motor evoked potential.

Figure 3. Exemplar stroke participant

Rectified ensemble averaged motor evoked responses from a stroke participant during a hip adduction torque matching task of 40% maximum voluntary contraction across several lower limb muscles. Each EMG trace plots a 200ms pre and 200ms post TMS stimulus window with TMS stimulus artifacts removed for clarity. The green traces indicate responses from lesioned hemisphere coil placement. Purple traces indicate responses from contralesional hemisphere coil placement. Note that the adductor amplitude was not statistically different ($p = 0.241$) following coil shift by increasing stimulator output. Significant reductions in knee extensor muscles MEP amplitude, VM ($p = 0.0078$), VL ($p = 0.047$) was detected only in the stroke group. A significant reduction in TA MEP amplitude was detected for both stroke ($p = 0.0014$) and control groups ($p = 0.015$) following ipsilateral coil placement (contralesional in stroke). Other lower limb muscles were not significantly attenuated across the stroke group.

Figure 4. Targeted inhibition following CON-H stimulation

Bar graphs represent the mean ratio of motor evoked potentials evoked from contralesional hemisphere (ipsilateral in control) coil placement over lesioned hemisphere (contralateral in control) coil placement across multiple lower limb muscles each group. A ratio less than one (horizontal broken line) indicates a reduction in the average MEP amplitude evoked following contralesional coil placement. Each bar represents the group average ratio for a particular muscle of successful trials where both ADD iMEP and ADD cMEP were matched in amplitude and background EMG activity during a 100ms prestimulus window. Asterisks indicate a statistically significant reduction in MEP amplitude for VM ($p = 0.0078$), VL ($p=0.047$), and TA ($p=0.0014$, $p=0.015$ control). Error bars represent standard error of the mean. Note that ADD amplitudes were statistically equivalent across coil placements as indicated by a MEP ratio of one.

Figure 5. Interhemispheric Onset Latency Identification

Quantification of interhemispheric onset latency differences between motor evoked potentials elicited via lesioned and contralesional hemisphere placement from a stroke participant. Rectified EMG traces from adductor longus and tibialis anterior during a successful hip adduction torque matching task of 40% maximum voluntary contraction. The green traces indicate responses from lesioned hemisphere coil placement. Purple traces indicate responses from contralesional hemisphere coil placement. **Insets:** Broken horizontal lines indicated three standard deviations above the mean 100ms pre-TMS EMG activity for each trace. Broken vertical lines mark the MEP onset defined when five consecutive points in the EMG trace remain above three standard deviations of the mean pre-TMS EMG activity. Arrows indicate the first of 5 consecutive points to cross the pre-TMS threshold. Note that here we detect a 0 ms delay between lesioned and contralesional adductor MEPs whereas a 2ms was detected for TA.

Figure 6. Interhemispheric Onset Latency Distribution

Distribution of latency differences between MEPs elicited with contralesional and lesion coil placements. Bars indicate MEP responses from all muscles for each group. Percentage of responses for each cohort are recorded on top of the bars. Note that a large proportion of responses from both groups are < 3ms, limiting certain neurophysiological interpretations.

Table 1**Stroke and control participant information**

The clinical assessments were performed by a licensed physical therapist. All subjects had no cognitive deficits. Subjects were not excluded due to sensory or proprioceptive impairments. Increases in stimulator output (percentage of max stimulation) required to match ADD MEPS following contralesional hemisphere coil placement are tabulated. Abbreviations: H – hemorrhagic; I – ischemic; LMFM – Lower Motor Fugl-Meyer. Note that some stroke participant characteristics were not available.

Subject	Stroke	Gender	Age	Post (years)	Stroke Details	LMFM	CON-H Stimulation Increase
S1	NA	M	43	8	Right	26/34	7
S2	I	M	64	7	Left insula adjacent frontal, parietal, anterior temporal	NA	5
S3	I	M	60	11	Left	26/34	8
S4	I	M	63	8	Left inferior parietal, anterior temporal	30/34	5
S5	H	M	61	7	Right	NA	5
S6	H	F	53	4	Left basal ganglia	18/34	3
S7	H	M	56	6	Left thalamic hemorrhage	NA	5
S8	I	M	50	5	Right internal capsule	NA	8
S9	I	M	67	2	Left middle cerebral artery	16/34	3
S10	H	F	60	2	Right corona radiata, basal ganglia, internal capsule	NA	7
S11	I	M	59	9	Left Subcortical	22/34	5
Stroke Mean (SD)		M = 9	58 (7)	6(3)			5.5(1.75)
Control (n=10)		M = 8	37(11)				5.8(2.97)