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The effects of paired associative stimulation on knee extensor motor excitability of individuals post-stroke: A pilot study

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Abstract

Objective—Paired associative stimulation (PAS) modulates bilateral distal lower limb motor pathways during walking. We assessed the effects of inhibitory PAS applied to the vastus medialis (VM) motor pathways of chronic stroke patients.

Methods—PAS consisted of 120 electrical stimuli applied to the femoral nerve paired with transcranial magnetic stimulation (TMS) of the lower limb primary motor cortex so that the estimated arrival of the afferent volley occurred 8 ms after delivery of the magnetic stimulus. Stimulus pairs were delivered to the non-paretic VM motor system of 11 chronic stroke patients and the right limb motor system of 11 non-impaired subjects at 0.19 Hz. The effects of PAS on VM motor pathway excitability and muscle activity were assessed during pedaling. TMS-induced motor evoked potential (MEP) amplitudes and the percent of VM activity in the flexion phase of active pedaling (%FLEXVM) was examined before and after PAS.

Results—Inhibitory PAS reduced VM MEP amplitudes in the target limb ($p < 0.05$) of both groups, while post-PAS paretic VM MEP amplitudes increased for some patients and decreased for others. Group mean paretic limb %FLEXVM was not altered by inhibitory PAS.

Conclusions—These results indicate PAS can be used to manipulate motor cortical excitability in proximal lower limb representations, however the sign of induced modulation was unpredictable and cyclic muscle activity was not modified.

Significance—The study has important implications for the development of therapies involving non-invasive brain stimulation to modify abnormal motor behavior following stroke.

Keywords

Stroke; Transcranial magnetic stimulation; Cortical plasticity; Paired associative stimulation; Locomotion; Cycling

1. Introduction

Several non-invasive brain stimulation protocols are being considered as potential tools for modulating cortical excitability and motor behavior. These protocols utilize repetitive transcranial magnetic stimulation (rTMS) (Chen et al., 1997; Pascual-Leone, 2006; Fitzgerald et al., 2006), transcranial direct current stimulation (tDCS) (Hummel et al., 2005; Hummel and Cohen, 2005; Fregni et al., 2005; Reis et al., 2009), and paired associative stimulation (PAS) which is a combination of peripheral nerve electrical stimulation and transcranial magnetic stimulation (TMS) (Stefan et al., 2002; Wolters et al., 2003; Stinear and Hornby, 2005). The effects of these protocols have most often been assessed in the upper limb motor system of healthy individuals, infrequently in the lower limb motor system, and rarely in stroke patients.

Non-invasive brain stimulation holds some promise as a potential therapy for hemiparesis of the upper limb (Fregni et al., 2005, 2006; Williams et al., 2010; Edwards et al., 2009; Schlaug et al., 2008; Boggio et al., 2006; Dafotakis et al., 2008; Kirton et al., 2010; Liepert et al., 2007; Mansur et al., 2005; Nowak et al., 2008; Takeuchi et al., 2008; Khedr et al., 2005; Kim et al., 2006). To the best of our knowledge experiments have not been published that assessed the potential of non-invasive brain stimulation as a therapy, or as an adjuvant to therapy, for proximal paretic lower limb muscles following stroke. One study reported highly variable neurophysiological and functional changes following PAS applied to tibialis anterior (TA) cortical representations in stroke survivors where physiological measures were assessed during tonic contraction (Uy et al., 2003). As a first step to continue this line of research we previously demonstrated that PAS, rTMS and tDCS specifically modulate one lower limb motor cortex despite the close proximity of the two lower limb primary motor cortices either side of the mid-sagittal fissure (Stinear and Hornby, 2005; Jayaram and Stinear, 2008, 2009; Jayaram et al., 2007; Madhavan and Stinear, 2010). However, our research has been largely confined to the motor system of TA assessed during treadmill walking. The present study extended this approach by applying PAS to the proximal lower limb motor system of vastus medialis (VM), the modulation and functional effects of which were assessed while participants pedaled on a cycle ergometer.

PAS is a technique based on Hebbian principles of synaptic plasticity whereby changes in synaptic weighting are induced by a presynaptic neuron taking part in the repetitive firing of a postsynaptic cell (Hebb, 1949). The application of this principle to the human motor system was first applied to corticomotor pathways of a hand muscle, and stimulus timing parameters were set to either increase (Stefan et al., 2002; Wolters et al., 2003) or decrease (Wolters et al., 2003) motor pathway excitability. In these studies, repetitive electrical stimulation of the median nerve was paired with TMS applied over the hand area of the primary motor cortex. Up-regulation of the motor system occurred when the peripheral

volley was timed to arrive in cortex just prior to delivery of the TMS pulse, and down-regulation occurred when the peripheral volley arrived in cortex just after delivery of the TMS pulse. Recently, this predictable modulation has also been revealed in TA corticomotor pathways of neurologically intact individuals (Stinear and Hornby, 2005) and the non-paretic TA of stroke patients (Jayaram and Stinear, 2008), although, during walking the timing of stimulus pairs in relation to the walking cycle phase is critical (Prior and Stinear, 2006). For individuals post-stroke, Jayaram and Stinear (2008) showed that not only was a down-regulation of target (non-paretic) TA corticomotor projections achieved with PAS applied at rest that persisted during walking, but also concurrent up-regulation of the non-target (paretic) TA motor system was evident. It is possible that inhibitory PAS applied to the motor system of the non-paretic limb of stroke patients could serve to reduce a between-hemisphere asymmetry of corticomotor excitability by simultaneously modulating corticomotor excitability in both hemispheres. To date the persistent effects of PAS interventions have only been assessed in lower limb corticomotor projections to TA (Roy et al., 2007; Uy et al., 2003; Prior and Stinear, 2006; Jayaram and Stinear, 2008). The first aim of the present study was therefore to investigate the effects of PAS applied at rest on VM motor pathway modulation assessed during cycling in stroke patients and healthy control subjects. The approach we took is similar to that reported by Jayaram et al. 2007 who compared the effects of PAS applied at rest and assessed pathway modulation during treadmill walking. They found that the effects of applying inhibitory PAS at rest were equivalent to the effects of applying PAS during walking, with a non-significant trend for PAS to be more effective when applied at rest.

The particular importance of shank musculature to walking function (Neptune et al., 2001; Zajac et al., 2003) is task specific, while cycling relies heavily on more proximal muscles. During cycling, the uniarticular knee extensor VM is primarily responsible for extensor force, which is the largest component of propulsive power during pedaling (Kautz and Hull, 1993; Raasch et al., 1997). Our laboratory has shown that paretic VM activity is prolonged into the flexion phase of pedaling and impedes bilateral kinetics and performance (Kautz and Brown, 1998; Schindler-Ivens et al., 2004). These latter authors suggested that abnormal muscle phasing in the paretic limb during pedaling might be due to altered sensory signals and impaired cortical control. Therefore, a second aim of the present study was to determine if PAS has an effect on prolonged paretic VM activity during the flexion phase of pedaling. The present study did not aim to identify where effects of PAS were occurring along the neuroaxis. Instead we assessed PAS as a potential technique for changing muscle phasing in VM during pedaling. We hypothesized that inhibitory PAS applied to the non-lesioned VM motor system will decrease non-lesioned VM motor system excitability and increase lesioned VM motor system excitability. A secondary hypothesis was that reducing the between-hemisphere asymmetry in motor excitability will decrease prolonged paretic VM activity evident during the flexion phase of pedaling. We reasoned that weak descending inputs to paretic limb spinal motor neurons, as a consequence of the between-hemisphere asymmetry in excitability, prevents the appropriate termination of paretic VM activity. This is the first report of the effects of PAS applied to the non-lesioned proximal lower limb motor system of stroke patients.

2. Methods

The study was approved by the Northwestern University Institutional Review Board. All methods conformed to the Declaration of Helsinki and all participants provided written informed consent. All participants were screened for contraindications to TMS (i.e., the presence of metal implants, cardiac pacemakers, unexplained headaches, or history of seizures). Eleven volunteers with persistent hemiparesis due to chronic stroke (P11 months) were recruited for this study (see Table 1) as were eleven neurologically intact controls. The patient group included 3 women and 8 men (mean age 57.4 years, range 37–79) while the non-impaired control group included 4 women and 7 men (mean age 38.7, range 27–68). Inhibitory PAS was applied to the quiescent VM corresponding to the non-lesioned hemisphere for the stroke survivor group, and the right limb of the control group. Participants pedaled a custom cycle ergometer before and after PAS with TMS-induced excitability measures taken from each VM during the middle of the knee extension phase for each limb. In addition, bilateral EMG signals were recorded during continuous pedaling. Post-PAS excitability measures were taken immediately after PAS and every 10 min for 30 min (a total of 4 post-PAS time periods, requiring approximately 6 min of continuous pedaling at each time period). EMG during continuous pedaling was recorded for 90 s intervals starting at 10 min post-PAS, for a total of 3 post-PAS time periods.

2.1. Cycle ergometer

As depicted in Fig. 1, participants were positioned on a custom cycle ergometer equipped with an electric motor to control pedaling velocity. A rigid backboard and a seat attached to the ergometer supported the trunk and head. Participants were secured to the backboard with nylon straps, and shoulder supports were used to minimize trunk movement. The entire ergometer was attached to a hydraulic tilt which was used to position the backboard at a comfortable incline of 12° from horizontal during PAS and at 30° from horizontal during active pedaling. Force transducers coupled to each pedal measured the applied force in three dimensions (Delta 660, ATI-IA Inc., Garner, NC) and allowed online monitoring of pedaling effort throughout the experimental session. Three optical encoders (BEI Model EX116-1024-2), one at each pedal spindle and one coupled to the crank, provided measurements of the crank and pedal angles with an accuracy of 0.3°. Pedaling speed was controlled by an electric motor (8:1 gear reducer, 10 HP with flux vector drive; model SV3000, Seco Electronics, Lancaster, SC). For a desired pedaling speed of 40 rpm, actual performance of 40.5 ± 0.8 rpm can be achieved under extreme applied forces (Rogers et al. 2004). The motor provides mechanical isolation of the two limbs such that the force applied to one pedal during active pedaling does not affect the motion of, or force applied to, the contralateral pedal. Therefore pedaling kinematics were equivalent for all target and non-target limbs examined, regardless of the level of impairment for individuals post-stroke.

2.2. Electromyography

Bipolar silver surface electrodes (DelSys, 10 mm length, 1 mm width, 1 cm inter-electrode distance) were placed over the muscle belly of both VMs. In preparation for placement of the EMG electrodes, the skin over each muscle was cleaned and gently abraded with an alcohol swab. A common reference electrode was placed over the tibia on the anterior aspect

of the leg. Electrodes were secured with adhesive tape and with tensor bandages to prevent electrode movement during the experiment. EMG signals were amplified with a gain of 10 at the electrode site before remote differential amplification (common mode rejection ratio: 92 dB, gain range: 100–10,000 times, frequency response: 20–450 Hz) and low-pass filtering (500 Hz, custom-designed filter). The digital optical encoder and force transducer signals were converted to analog with a digital-to-analog converter module before sampling. All data were sampled online at 2000 Hz via a 12-bit analog-to-digital converter (National Instruments) and Labview software.

2.3. Transcranial magnetic stimulation

A Magstim 200 (Magstim, Dyfed, Wales, UK) stimulator was triggered via custom Labview software to deliver magnetic stimuli to the lower limb motor cortex. A linen cap was tied tightly on the subject's head and taped to the skin of the forehead. The vertex (taken as the intersection of the inter-aural and nasion-inion lines) was marked on the cap. A Magstim double cone coil was then placed onto the cap at a position on the mid-sagittal plane where the intersection of the two embedded coils would be approximately 2 cm posterior to the vertex. The coil was oriented to induce a posterior–anterior current flow in the cortex. Slight adjustments to the coil position were made until MEP amplitudes of 2–3x background EMG amplitude were elicited from VM with minimal TMS intensity at the crank position corresponding to the middle of the VM burst during pedaling. This resulted in pre-PAS VM MEP amplitudes for control subjects of (mean $\pm$ SD) 254 μ V $\pm$ 166 (left) and 252 μ V $\pm$ 149 (right), and in persons post-stroke 386 μ V $\pm$ 228 (non-paretic) and 216 μ V $\pm$ 132 (paretic). The coil cable was supported with an adjustable rigid frame attached to the cycle ergometer, and once the appropriate coil position was determined, the cable was affixed to the frame with tape. In addition, Velcro® tapes between the coil casing and the top of the linen cap further secured the coil position. A chin-strap was then attached to the coil, and foam pads were inserted between the head and the lateral aspects of the coils to increase the stability of the coil placement. This comfortable arrangement, along with nylon straps securing the participant's trunk to the ergometer backboard, allowed individuals to pedal in their natural style with minimal trunk and head movement. The position of the coil was checked frequently during data collection.

2.4. Femoral nerve stimulation

Electrical stimulation was delivered in the form of a 1-ms square wave pulse via a Digitimer Constant Current stimulator (DS7A, Digitimer Ltd., Hertfordshire, UK) via Ag/AgCl stimulating electrodes (Blue Sensor NF-50, Ambu Inc., Glen Burie, MD, USA) pressed into the femoral triangle medial to both the inguinal fold and point of palpated femoral pulse, longitudinal to the estimated path of the femoral nerve, with the anode distal. The electrode position was adjusted to achieve VM contraction with minimal adductor muscle contraction at a minimal current setting.

2.5. Paired associative stimulation

Femoral nerve stimulation was delivered at 120% of motor threshold for VM, where motor threshold is defined as the minimum current level where a visible and palpable twitch in VM can be detected. One experimenter determined threshold for all subjects and sessions. Active

TMS motor threshold (AMT) was determined (at the crank position corresponding to the middle of the VM burst for each limb) to be the stimulator intensity that resulted in 5 of 10 MEP responses just distinguishable from the background EMG. The stimulator output corresponding to maximum MEP amplitude (MEPmax) was then determined, defined as lowest stimulus intensity beyond which increasing stimulus intensity does not increase MEP amplitude. To achieve consistent and repeatable MEPs at 2–3x background EMG amplitude, cortical stimulation throughout the experiment was achieved using a TMS intensity midway between VM AMT and VM MEPmax [healthy subjects $50.6 \pm 2.4\%$ maximum stimulator output (MSO)], determined at the crank position corresponding to the middle of the VM burst. This intensity in the non-paretic leg was used for PAS in stroke subjects (paretic leg $53.8 \pm 2.0\%$ MSO, non-paretic leg $49.1 \pm 2.0\%$ MSO). PAS in all sessions was applied at rest per Jayaram et al. (2007) who demonstrated that the effects of PAS assessed during walking were evident when the intervention was applied to the lower limb cortex at rest. Consistent with previous studies (Jayaram et al., 2007; Prior and Stinear, 2006; Stinear and Hornby, 2005) the inter-stimulus intervals (ISI) were individualized for each subject based on their estimated MEP latency. Specifically, to achieve PAS-induced inhibition of the targeted motor system, the ISI was set so that TMS was delivered at an ISI that was 8 ms shorter than the estimated MEP latency of the targeted VM (healthy $26.2 \text{ ms} \pm 3.1$, stroke $28.8 \text{ ms} \pm 2.5$). A Bistim module was used to achieve the correct ISI between the femoral nerve stimulation and TMS. One hundred and twenty (120) stimulus pairs at a frequency of 0.19 Hz were delivered while the subject lay reclined to a backboard position of 12° from horizontal on the cycle ergometer. A low level of attention was maintained by having subjects count the number of TMS discharges.

2.6. Assessing corticomotor excitability with motor evoked potentials (MEPs)

The same intensity of TMS used for PAS was also delivered to the targeted motor cortex. With the motor driven ergometer system moving at 40 rpm, participants were asked to “pedal along with moderate effort, using both legs,” while 20 TMS stimuli were delivered at the crank angle corresponding to the middle of the VM burst for each limb. This was done in five 2.5 min periods for each VM. Bilateral pedal forces were monitored to ensure that participants were pedaling with both legs, and were maintaining the same effort level throughout the experimental session. While pedaling, stimulation delivery was triggered at the appropriate crank position by signals from the crank coupled optical encoder using custom Labview software. Prior to PAS the data were designated PRE and following PAS they were designated POST 0, 10, 20, 30 min. For one control individual and one person post-stroke, data could only be taken for the first two POST periods, while an additional 2 persons in each group needed to terminate the session at 20 min. Following data collection, pre-trigger root mean square EMG amplitude (mV r.m.s) was calculated for the 40 ms of data just prior to the stimulus artifact and MEP amplitude (mV peak-to-peak) was calculated using custom Matlab code. To match for the level of background EMG across time periods, responses were discarded if they were associated with high or low amplitude pre-trigger EMG to bring the range and mean pre-trigger EMG amplitudes for each subject and muscle to within $\pm 5\%$. After this process, typically 10 responses remained for each condition. For each subject, POST means were expressed as a percentage of PRE means. This process

ensures MEP amplitudes are estimated across conditions with equivalent levels of motoneuron firing.

2.7. VM EMG activity during cycling

Prior to the PAS block and at the end of each POST epoch, 90 s of bilateral VM EMG data were recorded as 3×30 s trials while the participant continued to “pedal along with moderate effort, using both legs.” To characterize changes in motor function and allow comparisons between limbs and subject groups this study focused on relative muscle activity phasing; and therefore an EMG processing technique was used that normalized EMG amplitude for each task. To this end, rectified EMG signals were normalized throughout every pedaling cycle as a percentage of activity produced during that cycle (Schindler-Ivens et al., 2004). To ensure only cycles where both limbs were actually producing pedal force were analyzed, only the thirty cycles with the greatest non-normalized total integrated EMG amplitude (iEMG) in the paretic (non-target) limb were used for subsequent analysis. EMG data collected during continuous pedaling at the end of the experiment were not included in the cycle phase analyses for six individuals because three individuals were too fatigued and three data sets contained artifacts that invalidated the estimate of EMG amplitude of the cycle phase of interest. Therefore continuous pedaling data were analyzed for the remaining six persons in each group.

2.8. Data analyses: MEPS

Student's *t*-tests were used to determine if means at each time period and also means averaged across time periods for each muscle differed from 100% (i.e. there was significant modulation). A three-way mixed ANOVA was conducted on the post-PAS MEP amplitude expressed as a percentage of pre-PAS MEP amplitude to assess for effects of group (control, stroke) side (target, non-target), and time (POST 0, 10, 20, 30). A significance level of 0.05 was adopted.

2.9. Data analyses: VM EMG activity during cycling

We sought to determine whether PAS induced changes in cortical excitability altered VM muscle activity during its presumed OFF phase during the pedaling cycle. Published experimental and modeling data have established muscle activation timings appropriate to maintain smooth pedaling, maximize energetic efficiency, and maximize net positive work (Raasch and Zajac, 1999; Hakansson and Hull, 2005). Under this framework, the pedaling cycle was split into overlapping phases, defined by the functional grouping and activation of muscles. Raasch and Zajac (1999) defined four such phases, of which flexion (FLEX) and extension (EXT) have been shown to act as alternating pairs, so that a muscle maximizing its contribution to EXT should be inactive during FLEX. Therefore the uniarticular knee extensor VM should be OFF during FLEX (150–315 deg past top-dead-center). This region of the pedaling cycle was used for subsequent analysis. The normalized and averaged VM muscle %activity curves were integrated over the FLEX phase for each epoch (%FLEXVM). Integrated %activity across a 90-degree subset of the FLEX phase has been shown previously to be related to locomotor impairment following stroke (Kautz and Brown, 1998), and a maximum appropriate amount of VM activity across this phase has

been established at 5%. Extrapolating this value across the full FLEX phase yields a suggested maximum %activity of 10%. Students *t*-tests were used to determine mean %FLEXVM at each time period and also mean %FLEXVM averaged across time periods for each muscle differed from 10%. POST %FLEXVM means were then expressed as a percentage of PRE %FLEXVM means. A two-way repeated measures ANOVA model was then used on post-PAS %FLEXVM expressed as a percentage of pre-PAS %FLEXVM for each subject group (CONTROL or STROKE) to test for a significant effect of TIME (POST 10, 20, 30) and LIMB (target, non-target). A significance level of 0.05 was adopted for these comparisons.

2.10. Relationship between MEP amplitude and %FLEXVM post-PAS

We sought to determine if the degree of PAS induced corticomotor excitability was related to post-PAS changes in VM muscle activity for persons post-stroke. As there was large between-subject time course of variability in both MEP amplitude and %FLEXVM we used regression analyses to examine the relationship between these two parameters in the paretic limb of persons post-stroke, collapsed over all post-PAS epochs. A significance level of 0.05 was adopted for this comparison.

3. Results

3.1. Changes in MEP amplitude post-PAS

Post-PAS MEP amplitude changes in Fig. 2 indicate that the effect of PAS on the targeted limb (top panels) was to reduce MEP amplitudes across both neurologically intact and post-stroke subjects, although individuals showed different time courses of modulation. The mixed ANOVA of MEP amplitude revealed a main effect of limb (target vs. non-target) where $F_{(1,20)} = 9.49$, $p = 0.006$, and no effect of group ($F_{(1,20)} = 0.056$, $p = 0.81$) or time ($F_{(3,60)} = 1.4$, $p = 0.25$), and no interactions (all $p > 0.15$).

Non-paretic limb VM MEP amplitude change differed significantly from baseline (100%) at all four time periods, while the target limb VM MEP amplitude changes for controls were significantly different only for the first two time points. However, the percent change in MEP amplitude averaged across all time periods for each subject revealed that the target VM MEP amplitudes ($\pm$ SEM) decreased to 85% (4.6) of baseline ($p = 0.009$) for healthy controls (Fig. 3), and 79% (4.1) of baseline ($p = 0.001$) for the non-paretic limb. In contrast, the non-target VM MEP amplitudes varied in both modulation direction and time course across subjects in both groups (Fig. 2, bottom panels). As such, non-target VM MEP amplitudes did not differ significantly from 100% at any of the four post-PAS time periods nor was the average percent change in MEP amplitude across time periods significant for either subject group ($p = 0.781$ post-stroke; $p = 0.700$ control) (Fig. 3).

3.2. %VM muscle activity

Group mean muscle activity for bilateral VM revealed little systematic change in VM activity patterns following PAS for either limb of either subject group. Integrated %activity across the first 90-degree subset of the FLEX phase (coincident with early to mid knee flexion) has been shown in previous studies to be related to locomotor impairment following

stroke (Brown et al., 1997; Kautz and Brown, 1998; Brown and Kautz, 1998; Kautz and Patten, 2005; Schindler-Ivens et al., 2004), and %VM activity across this phase is typically below 5% for non-impaired controls. Extrapolating this value across the full FLEX phase yields a suggested maximum %FLEXVM of 10%. Pre-PAS %FLEXVM in neurologically intact controls was 7.4 (1.6) for the non-target limb, and 7.4 (1.3) for the target %FLEXVM ($p > 0.05$) (Fig. 4). For persons post-stroke, pre-PAS %FLEXVM in the paretic (non-target) limb was 15.7 (2.9) and for the non-paretic (target) limb it was 8.0 (1.4). The difference between the non-paretic and paretic limb pre-PAS %FLEXVM was significant ($p = 0.017$) indicating that the paretic limb %FLEXVM was impaired relative to the non-paretic limb. There was no difference in pre- to post-PAS %FLEXVM means ($p > 0.05$). Regression analysis revealed no significant correlation between post-PAS MEP amplitude changes and %FLEXVM during cycling for the paretic VM ($R^2 = 0.123$, $p = 0.141$).

4. Discussion

Here we report, for the first time, a PAS induced down-regulation of motor excitability in proximal lower limb musculature assessed during a cyclical locomotor task. The PAS induced modulation was evident in the targeted non-paretic limb of stroke patients and the targeted right limb of neurologically intact control subjects. This down-regulation of the targeted motor system supported the first part of our hypothesis. The modulation persisted for several minutes during cycling and its time course varied between participants. These findings are similar to those previously reported by Jayaram and Stinear (2008) for the TA motor system assessed during treadmill walking. Interestingly, one important feature of our VM data was not consistent with the TA data of Jayaram and Stinear. In their study motor excitability was up-regulated for the paretic limb, whereas in the present study the paretic VM motor system revealed a range of modulation: from strong inhibition to strong facilitation. The up-regulation of the paretic VM and the non-target VM of healthy subjects that was evident in half of all our participants was consistent with the up-regulation revealed by Jayaram and Stinear (2008) and partially supported the second part of our hypothesis. However, contrary to the hypothesis, in the present study *down regulation* of the paretic and non-target VM was observed in half of all participants. Also contrary to the hypothesis, group mean VM activity during paretic limb flexion (%FLEXVM) was unchanged following PAS. Individual patients exhibited patterns of flexion activity that ranged from strong decreases, to strong increases. These changes were not correlated with the sign of paretic VM motor system modulation.

Three important questions arise from the present excitability measures. First, what gave rise to the high variability in modulation of the target motor system? Second, why was this variability more evident in non-target paretic limb responses? Third, what implications do our preliminary data have for the development of non-invasive brain stimulation as an adjuvant to lower limb motor therapy following stroke? In regard to the first question, while all participants demonstrated the expected down-regulation in response to inhibitory PAS, the weak modulation in some participants and strong modulation in others suggests that subjects vary in their susceptibility to PAS as we have previously noted for PAS of the lower limb motor system (Stinear and Hornby, 2005). It is unlikely the range of modulation

resulted from imprecise afferent conduction time estimates which might have been an issue had these parameters been set for the paretic VM.

In regards to the second question, for the patients where unpredicted inhibition of the paretic limb VM was evident, it is possible that ipsilateral projections from the non-lesioned hemisphere may have contributed to the inhibition. This idea is supported by a study using fMRI during unilateral passive extension of the knee where Luft et al. (2005) found a similar extent of BOLD activity in both hemispheres of stroke patients, and weak lateralization even amongst some non-impaired control subjects. In addition, large variability in lateralization was evident across subjects in both groups. The Luft et al. (2005) lateralization scores for knee movement in their control group were weaker than those reported by Sahyoun et al. (2005) for passive ankle movement. If both hemispheres are strongly connected to proximal lower limb muscles in some stroke patients, it is not surprising that the suppressive effects of inhibitory PAS targeted to one hemisphere would be evident in both limbs. Another possible explanation for the range of facilitation and suppression of paretic VM cortical excitability is lesion location. However, an inspection of our results revealed no relationship. Most participants had mixed location lesions (Table 1) and these individuals revealed robust facilitation through robust suppression (Fig. 2). Another explanation for the range of paretic VM motor system suppression and facilitation is that patients' inter-hemispheric neural control of motor system excitability may have varied greatly. For the upper limb, asymmetrical inter-hemispheric inhibition post-stroke is thought to result in an asymmetry of between-hemisphere excitability with the non-lesioned motor cortex being hyper-excitable (Williams et al., 2010; Kirton et al., 2010; Murase et al., 2004; Cicinelli et al., 2003). But there is no research on inter-hemispheric inhibition for the lower limb because of the technical limitations imposed by the close proximity of lower limb motor cortices and the low spatial resolution of TMS. If inter-hemispheric interactions exist for the lower limb similar to those reported in the upper limb literature, reducing inter-hemispheric inhibition by down-regulating the target cortex should have up-regulated non-target limb motor excitability. However, this doesn't explain a bilateral down-regulation, because an absence of inter-hemispheric inhibition should have resulted in an absence of modulation, not a robust suppression. Another possibility is that the low rate of TMS may have down-regulated the non-target motor system excitability. This is unlikely however, because it would mean that a remarkably high up-regulation must have occurred for those patients who demonstrated increased excitability above 150% of baseline. Finally, it is possible that day-to-day (or time-of-day) variation in susceptibility to PAS may have influenced our results (Sale et al., 2008), although it is difficult to explain a range of suppression through facilitation on day-to-day effects. We therefore favor the hypothesis that a variable extent of between-subject ipsilateral connectivity to paretic lower limb motoneurons explains our results. Assessing ipsilateral connectivity suffers the same technical limitations as the assessment of interhemispheric inhibition. Up until recently, ipsilateral activity has been inferred from the poor lateralization of BOLD signal as described above. However, we have recently described large between-patient variability in an indirect measure of ipsilateral connectivity to lower limb motoneurons that supports our present favored hypothesis (Madhavan et al., 2010).

Addressing the third question, the finding that PAS increased the excitability of the non-target paretic VM in some patients and decreased the excitability in others has important implications for the development of non-invasive brain stimulation as an adjuvant to lower limb motor therapy. Our present data, albeit from a small sample, suggest a simple relationship does not exist for between-hemisphere opposite sign modulation using non-invasive brain stimulation for the proximal lower limb motor system as previously reported for the distal lower limb motor system (Jayaram and Stinear, 2008). To explore this notion further, future research needs to determine if both hemispheres are engaged in the control of proximal lower limb function following stroke. For a patient who is controlling their paretic limb from the non-lesioned motor cortex, it would be counter-intuitive to suppress the excitability of motor projections from that hemisphere to paretic limb motor neurons. Indeed, up-regulating the non-lesioned motor system may be helpful. For a patient who is controlling their paretic limb from the lesioned hemisphere, up-regulating the non-lesioned motor system may be helpful.

Another aim of the present study was to determine if PAS could influence VM muscle activity during the portion of the pedaling cycle when VM activity is prolonged and adversely affects performance. Group means of %FLEXVM confirmed previous studies by revealing more FLEXVM in the paretic than the non-paretic leg. Following PAS some patients exhibited patterns of strong increases and others strong decreases in FLEXVM activity. These differences caused the group mean of %FLEXVM to remain unchanged following PAS. A regression analysis failed to reveal an association between lesioned motor system modulation and prolonged VM activity. Contrary to the second part of our hypothesis, down-regulating the non-lesioned motor system did not decrease paretic limb %FLEXVM in our group of patients. One explanation is that changes in motor system excitability have an unpredictable or minimal affect on the timing of muscle activity. Another is that motor excitability per se has nothing to do with the prolonged muscle activity as demonstrated during the employed pedaling task. The relationship between motor system excitability and lower limb behavior has not previously been reported. Those patients who did demonstrate an increase in %FLEXVM may have done so because an up-regulation of lesioned motor system excitability may simply increase descending drive and further prolong VM activity. While inspection of data supports this suggestion, the lack of a consistent up-regulation of lesioned VM excitability, and lack of a correlation between modulation sign and changes in %FLEXVM prevents any firm conclusions from being made, especially for these preliminary data. If this lack of a relationship is supported in future experiments with larger sample sizes where same sign modulation is achieved, the implication is that paired associative stimulation is not a candidate adjuvant for motor therapy designed to suppress prolonged and abnormal knee extensor activity during locomotion.

In conclusion, this preliminary study reports for the first time the effects of PAS applied to the motor system of a proximal lower limb muscle of healthy individuals and stroke survivors. The variability in the magnitude and sign of modulation in our data indicates that investigation of the factors underling individual patient responses to PAS must first be conducted before the ability of PAS to modify lower limb motor behavior is examined.

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HIGHLIGHTS

- The ability of non-invasive brain stimulation to modulate motor cortical excitability holds great promise for the study and treatment of motor disorders such as hemiparesis.
- Here we report for the first time a paired associative stimulation protocol that down-regulated the motor excitability of non-paretic proximal lower limb musculature during a cyclic locomotor task for individuals with unilateral stroke.
- Of particular importance was the finding that motor excitability of paretic proximal lower limb musculature as up-regulated (as predicted) in 6 to 11 participants, yet, surprisingly was down-regulated in the remaining 5.

Fig. 1.

Cycle ergometer. Subjects were positioned on a custom designed, motor driven cycle ergometer. The servomotor maintained the pedaling speed at 40-rpm during movement, and held the crank arms static during PAS. During pedaling the backboard was tilted to the 30° position shown, while during PAS and rest breaks it was tilted back to 12° for participant comfort. Force sensors at the pedals provided real-time feedback of pedaling effort during trials.

Fig. 2.

Time course for individual subject's MEP amplitude change during pedaling following PAS. Top plots show MEP amplitude change in the limb targeted with inhibitory PAS for each subject group (left or non-paretic limb, for controls and stroke group, respectively). Bottom plots show the simultaneous MEP amplitude change in the opposite limb (right or paretic limb, for controls and stroke group, respectively). Each value is the post-PAS mean expressed as a percentage of the pre-PAS mean, where 100% represents no change from baseline pre-PAS.

Fig. 3.

MEP amplitude change following PAS for the target and non-target legs for stroke and control subjects. Each bar is the post-PAS mean amplitude expressed as a percentage of the pre-PAS mean amplitude for the VM muscle averaged across all time periods. The horizontal dashed line represents the pre-PAS mean amplitude. Stars indicate the significant difference from 100%. Control target $p = 0.009$, Stroke target $p = 0.001$.

Fig. 4.

Bilateral pre and post-PAS %FLEXVM during pedaling. Each bar is the mean normalized muscle activity during FLEX. The dashed line represents maximum expected %FLEXVM for optimal pedaling function. The non-target limb is the paretic limb in the Stroke group and the left leg for controls. Paired *t*-tests within group and limb indicate no statistical significance between pre and post-PAS %FLEXVM. However, the difference between non-paretic and paretic limb %FLEXVM was also significant ($p = 0.017$).

Table 1

Characteristics of persons post-stroke.

Subject	Age (years)	Gender	Years post-stroke	Plegic side	FM (34 max)	Lesion Location
I	62	M	15.7	L	21	SC/C
II	56	F	11.9	R	23	SC
III	59	M	18.0	L	23	SC/C
IV	53	M	16.3	R	24	NA
V	64	M	21.9	L	23	NA
VI	58	F	5.3	R	20	SC
VII	79	M	13.9	R	NA	SC
VIII	37	M	9.9	L	30	SC/C
IX	61	M	5.4	L	NA	SC/C
X	51	M	4.3	L	20	SC/C
XI	52	F	5.6	R	24	SC
	57 ± 10	8M, 3F	11.7 ± 6.0	6L, 5R	23 ± 3	5 SC/C, 4 SC

FM, Fugl Meyer lower limb motor score; NA, not available; SC/C, sub-cortical and cortical; SC, sub-cortical.