Stimulation-induced changes in lower limb corticomotor excitability during treadmill walking in humans

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Magnetic stimulation of human primary motor cortex (M1) paired with electrical stimulation of a peripheral motor nerve has been used to produce a lasting modulation of corticomotor (CM) excitability. This 'paired associative stimulation' (PAS) protocol has been used to induce bidirectional changes in excitability in upper limb CM pathways. The present study tested the hypothesis that temporally dependent PAS applied to the common peroneal nerve during the swing phase of walking would induce bidirectional changes in CM excitability consistent with the Hebbian principle of activity-dependent plasticity. Fourteen subjects with no known neurological disorder participated in two data collection sessions each. PAS was delivered as a single block of 120 pairs of stimuli delivered in a 10 min period during treadmill walking at 4.0 km h^{-1}. Changes in CM excitability were assessed by examining the size of motor potentials evoked by transcranial magnetic stimulation prior to and following PAS. Tibialis anterior motor-evoked potentials amplitude increased to 121% over baseline when the magnetic stimulus was delivered over M1 after the estimated arrival time of the afferent volley in sensorimotor cortex and decreased to 83% of baseline when the magnetic stimulus was delivered prior to the estimated afferent volley arrival. This extent of modulation was undiminished following a further 10 min period of walking without stimulation. The temporal nature of the bidirectional effects following PAS, their rapid evolution and subsequent persistence supported the study's hypothesis and were similar to the effects described by others in quiescent muscles of the upper limb.

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Modulation of corticomotor (CM) excitability can be induced in humans using a variety of neural stimulation techniques which invoke principles of activity- (or spike-timing-) dependent neural plasticity. For example, repetitive transcranial magnetic stimulation (rTMS) applied over human primary motor cortex (M1) has been used in a number of protocols to reveal a decrease in CM excitability when the frequency of stimulation was 1 Hz (Muellbacher *et al.* 2000), and an increase in CM excitability when the frequency of stimulation was 5 Hz or more (Peinemann *et al.* 2004). Pharmacological agents have been shown to increase (e.g. D-amphetamine, a noradrenaline (norepinephrine) agonist) or decrease CM excitability (e.g. lorazepam, an allosteric modulator of the $GABA_A$ receptor) (see Ziemann, 2004 for a review). Ischaemic nerve block of the upper limb in combination with rTMS has also been used to demonstrate stimulation-induced alterations in the size of motor-evoked potentials (MEPs) (Ziemann *et al.* 2002). More recently, a technique based on Hebbian principles of synaptic plasticity has been used to increase (Stefan *et al.* 2002; Wolters *et al.* 2003), and decrease the excitability of CM pathways to the upper limb (Wolters *et al.* 2003). This technique, called 'paired associative stimulation' (PAS), follows the association principle first proposed by D. O. Hebb, whereby changes in synaptic weighting are induced by a presynaptic neurone taking part in the repetitive firing of a postsynaptic cell (Hebb, 1949).

Specifically, Wolters *et al.* (2003) observed increases in the size of motor-evoked potentials (MEPs) elicited in the abductor pollicis brevis when suprathreshold TMS was delivered to M1 within 6 ms of sensory-evoked potentials arriving in sensory cortex. In contrast, decreases in MEP size were detected when TMS was delivered within 10 ms prior to the afferent volley arriving at the somatosensory cortex. The effects evolved rapidly, were long lasting, yet reversible.

Studies of PAS involving CM pathways to lower limb motoneurones are limited (Uy *et al.* 2003), and no studies

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have been reported where changes in CM excitability have been induced during muscle activation. The hypothesis adopted for the present experiment predicted that temporally dependent PAS applied to tibialis anterior (TA) pathways during the swing phase of walking would induce bidirectional changes in CM excitability consistent with the Hebbian principle of activity-dependent plasticity. We chose TA as the target muscle because the effects of PAS are likely to be more readily revealed in pathways that are known to have strong CM connections, and the functional importance of the TA motor pool is particularly evident in individuals following neurological injury (Said *et al.* 2001). Studies have revealed the relative strength of CM pathways to TA and soleus (Sol); for example, in response to TMS (Capaday *et al.* 1999) constructed input–output curves of CM pathways to TA and to Sol muscles. Pathways to Sol were less excitable during the stance phase of walking than during a tonic voluntary contraction. However, they found no difference between the excitability of projections to TA during the swing phase of walking and during a voluntary tonic contraction of TA. In a similar study Bawa *et al.* (2002) used surface electromyography (EMG) and single motor unit responses to provide further evidence that CM projections to TA are relatively stronger compared with projections to Sol.

During motor re-training, the inclusion of PAS to manipulate the excitability of injured CM pathways may be of value in the application of walking re-training regimens. Because PAS has been reported previously only in pathways to muscles at rest, and because of our interest in developing adjuncts to walking training protocols, the aim of the present experiment was to determine if bidirectional changes in healthy individuals' TA CM excitability could be induced with PAS applied during walking. If this aim is fulfilled, we intend to investigate the mechanisms of PAS further and assess its value as an adjunct to walking training following neural injury.

Methods

Subjects

Healthy subjects with no known neurological disorder were recruited from the staff of the Rehabilitation Institute of Chicago. The sample size required to reject the null hypothesis was calculated using preliminary data and PS v. 2.1.31 software (Dupont & Plummer, 1990), where the difference in means was 0.25 and the standard deviation was 0.30. The significance level was set at $\alpha = 0.05$ and the power at $1 - \beta = 0.80$, yielding a sample size of 13. Fourteen (6 female, 8 male) subjects volunteered and were recruited for the study. Their ages ranged from 24 to 58 years (mean 31). 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.

Stimulation and recording

M1 was stimulated by a Magstim 200^2 unit (Magstim, Dyfed, Wales, UK) via a Magstim double cone coil (see below for details regarding intensity setting). A tight fitting linen cap was tied on the subject's head with the vertex marked on the cap at the intersection of the inion–nasion and interaural lines. The coil was suspended over the subject's head from an overhead gantry and attached to the cap using Velcro tapes so that the junction of the coils was located in the mid-sagittal plane ~1 cm anterior to the vertex. The large magnetic field generated by the double cone coil and the close proximity of homologous lower limb cortical representations allowed the coil to be fixed in the mid-sagittal plane thus eliminating the need to shift the coil to an optimum site for each TA. Minor adjustments were made to the coil position until optimal-sized MEPs of similar size could be elicited from both TA muscles during the respective limb's late swing phase of treadmill walking. A chin strap attached to the coil and small foam pads were used to increase coil stability. Subjects found this to be a comfortable arrangement, enabling them to adopt their preferred gait pattern, and move their head with ease between trials without causing a shift in the location of the coil. The coil and cap positions were checked repeatedly throughout data collection and coil position did not change in any of the sessions.

Peripheral nerve stimulation was delivered from a Model DS7A constant current stimulator (Digitimer Ltd, Hertfordshire, UK) via a Meditrace 133 surface Ag–AgCl electrode (Tyco Healthcare, Ludlow, Chicopee, MD, USA) used as a cathode placed over the deep peroneal nerve (PN) just distal and anterior to the fibula head at a position where maximum TA muscle contraction and minimum peroneal muscle contraction was elicited. The anode was a 25 cm^2 Dura-Soft (Pain Management Technologies, Inc., Akron, OH, USA) electrode placed over the ipsilateral patella.

The timing of stimulator trigger pulses was achieved using custom-built heel switches and a two channel electronic delay circuit. The cycle phase was established using trigger pulses and EMG bursts displayed on an oscilloscope. The target cycle phase was the middle of the large TA burst that occurs in late swing as the ankle is dorsi-flexed prior to heel strike. The interstimulus interval (ISI) between Digitimer and Magstim units was set using a second component of the delay circuit.

Surface EMG was recorded from TA and Sol via Ag–AgCl EMG electrodes (ConMed SureTrace, Utica, NY, USA) following standard skin preparation. Signals were amplified ($\times$ 1000), sampled at 2000 Hz and band-pass filtered (10–500 Hz) using a MyoSystem 1400 (Noraxon, USA, Inc., Scottsdale, AZ). A Tektronix TDS 2014 storage

oscilloscope (Tektronix Inc., Beaverton, OR, USA) was used to monitor EMG in real time. Data were stored to computer disk for later analysis.

Experimental protocol

An approximate motor threshold for PN stimulation was determined by finding the lowest current level at which a twitch in TA could be detected by inspection and palpation. The current level was then set at 120% of this threshold to ensure a modest but distinct twitch was elicited. PN stimulation was always delivered to the self-reported 'dominant' lower limb, i.e. the limb each subject said they preferred to use to kick a ball. This group of subjects all reported their right lower limb as being dominant. The TMS intensity that was used for the PAS intervention and for measures of CM excitability was set to give MEP amplitudes of 1.0–1.5 mV peak to peak in the TA during late swing. The ISI for the pairs of electrical and magnetic stimuli was determined by first estimating the latency of the MEPs recorded from the stimulated limb's TA. The ISI was then set as follows. To achieve PAS-induced facilitation (PAS_{fac}) of CM pathways, the ISI was set so that the TMS was delivered at an ISI equivalent to the estimated MEP latency plus 5 ms. This interval was consistent with the intervals selected by others for achieving facilitation using PAS applied to upper limb pathways (Stefan *et al.* 2002; Wolters *et al.* 2003). To achieve PAS-induced inhibition (PAS_{inhib}) of CM pathways, the ISI was set so that the TMS was delivered at an ISI that was 10 ms shorter than the estimated MEP latency. Previous work in the upper extremity (Stefan *et al.* 2002; Wolters *et al.* 2003) and preliminary experiments in this laboratory confirmed that this process reliably achieved facilitation and inhibition, respectively.

Upon recruitment, subjects were assigned consecutive Roman numerals (to XIV) for identification. To control

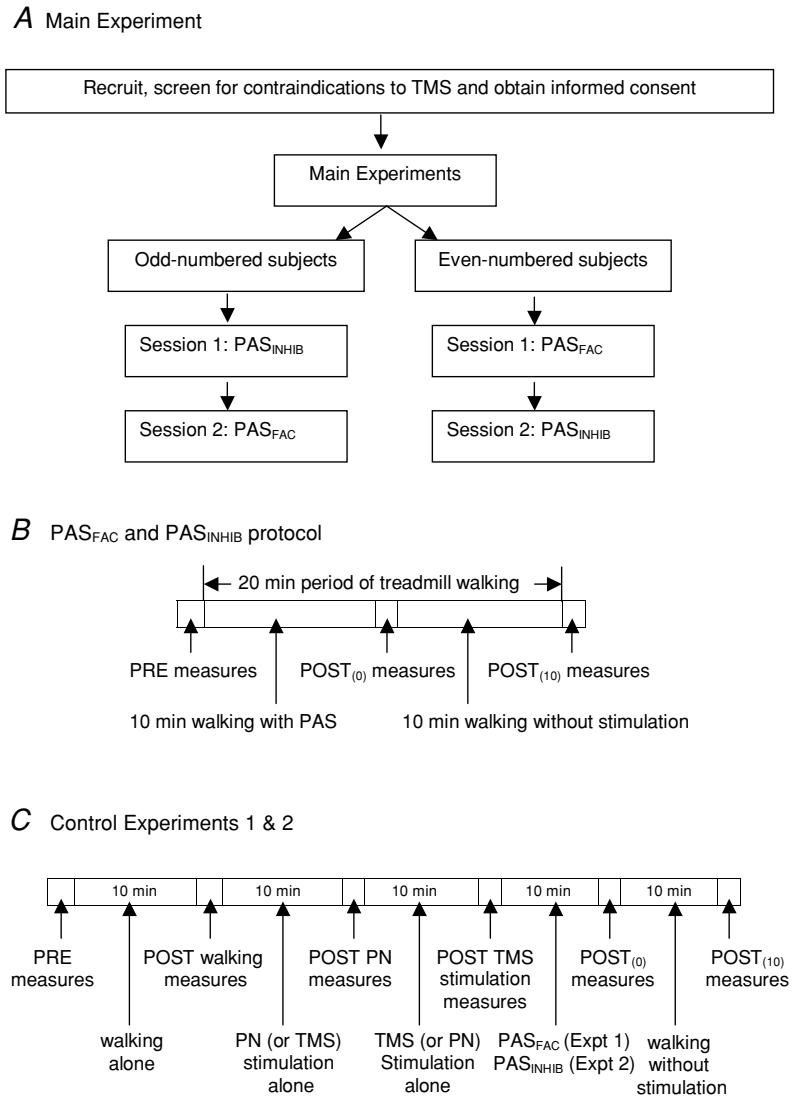

Figure 1. Schematics of experimental protocols
The main experimental sessions are illustrated in *A*, where in the first session, odd-numbered subjects received PAS_{inhib} and even-numbered subjects received PAS_{fac}. In the second session odd-numbered subjects received PAS_{fac} and even-numbered subjects received PAS_{inhib}. Time points when Pre, $Post_0$ and $Post_{10}$ measures were made during the 20 min period of treadmill walking are illustrated in *B*. Similarly, the time points when measures were made during the 50 min period of walking for the control experiments are illustrated in *C*. After Pre measures were made, control sessions always began with a 10 min period of walking without stimulation, followed immediately by a 10 min period of PN stimulation only for even-numbered subjects and TMS only for odd-numbered subjects. The type of stimulation was reversed for the third 10 min period. During the fourth and fifth 10 min periods, PAS was applied in the same manner as the main experiment. In the first control session even-numbered subjects received PAS_{fac} and odd-numbered subjects received PAS_{inhib}. In the second control session even-numbered subjects received PAS_{inhib} and odd-numbered subjects received PAS_{fac}.

for order effects, even-numbered subjects received PAS_{fac} in the first of two sessions and odd-numbered subjects received PAS_{inhib}. The two sessions were scheduled at least 1 week apart. In the second session the subject-stimulation order was reversed. In each session subjects walked on the treadmill at 4.0 km h^{-1} for a period of 20 min during which 120 pairs of stimuli were applied during the first 10 min. The schematics in Fig. 1 illustrate the protocols used in the main experiments and the control experiments.

Measures of CM excitability were taken during walking prior to the intervention, at the end of the 10 min period of intervention and at the end of the 20 min period of walking from EMG data collected from the TA and Sol of both limbs. A set of 12 TMS-evoked responses were collected (1 every 5 s) during late swing of each limb. The mean MEP size was taken as a pre-intervention (Pre) measure of CM excitability. After the initial 10 min period of PAS, the mean MEP size of a further set of 12 TMS-evoked responses were taken as a measure of post-intervention CM excitability ($Post_0$). This process was repeated after a further 10 min period of walking during which no stimulation was delivered. The mean MEP size of this set of responses was taken as a 10 min post-intervention ($Post_{10}$) measure of CM excitability.

Control experiments

Four subjects from the main experiment (VI, VII, IX and XIII) were subsequently recruited to participate in three control experiments. Two of these experiments assessed the effects of walking alone, electrical stimulation of the peroneal nerve during walking alone, or TMS during walking alone, on MEP amplitude recorded from TA only (see Fig. 1). In one experiment, PAS_{fac} was applied immediately following the three control conditions. In another experiment, PAS_{inhib} was applied immediately following the three control conditions. All data were collected during one continuous period of walking. The order of PN stimulation alone and TMS alone was alternated to control for order effects. In a third experiment, PAS_{fac} was applied as subjects voluntarily held a tonic contraction of TA. Subjects were sitting on a chair and weights were added to the dorsum of the right foot so that dorsiflexion produced a level of EMG similar to that generated by the individual during the late swing phase of walking. The PAS_{fac} protocol for this control experiment was the same as in the main experiment.

Analysis

Peak-to-peak MEP amplitude, rectified MEP area, and root mean square (r.m.s.) pre-MEP EMG amplitudes were calculated. Up to 20% of responses were discarded to bring the background EMG r.m.s. amplitude means for the three conditions for each subject to within ±2%. The remaining MEP values were retained for analysis. Subject means of Pre, $Post_0$ and $Post_{10}$ MEP amplitude and area were then calculated from individual response values. The $Post_0$ means and the $Post_{10}$ means were expressed separately as a percentage of Pre means. Group means were tested for differences from 100% (unity) using one-tailed *t* tests, and also tested using separate two-way repeated measures ANOVAs. The factors were 'pathway', stimulated and non-stimulated (Stim, Non-Stim), and 'time' ($Post_0$, $Post_{10}$) as factors. A significance level of 0.05 was adopted for all statistical analyses.

Data from the first two control sessions were treated in a similar manner. Mean MEP size following walking alone ($Post_{walk}$), following PN stimulation during walking ($Post_{PN}$), and following TMS alone ($Post_{TMS}$) were expressed as a percentage of pre-intervention mean MEP size (C_{Pre}). For statistical purposes post-PAS unsigned changes in MEP amplitude were pooled from the four PAS_{fac} and four PAS_{inhib} trials. Means of MEP amplitude at $Post_0$ and $Post_{10}$ from the third control experiment (tonic contraction) were pooled and the differences between means derived during walking and tonic contraction were tested with a paired *t* test. For all control experiments differences between means and unity were tested for significance as conducted in the main experiment.

Results

An example of a 4 s simultaneous recording of EMG from right TA and left Sol is shown in Fig. 2 during late swing and late stance, respectively, from Subject V during treadmill walking. The MEPs have been extracted from the EMG to illustrate the typically triphasic TA MEP, the typically polyphasic Sol MEP, and post-MEP EMG silent periods.

Temporally dependent and persistent modulations of MEP size were evident

Figure 3 illustrates individual subject's mean MEP amplitudes in stimulated TA CM pathways following PAS_{fac} and PAS_{inhib}. MEP amplitude at $Post_0$ and $Post_{10}$ is expressed as a percentage of Pre. The main result was that group mean facilitation and inhibition was 121% and 83%, respectively, and that no reduction in these means was evident between $Post_0$ and $Post_{10}$. The figure illustrates the general effects and variability of PAS on stimulated pathways. Twelve subjects demonstrated facilitation and 12 subjects demonstrated inhibition. Two subjects (II, XII) failed to demonstrate facilitation, and two failed to demonstrate inhibition (XI, XIV).

Opposite sign modulation was evident in non-stimulated pathways

Of particular interest was that inhibition was evident in non-stimulated (opposite limb) TA pathways when facilitation was induced in stimulated limb TA pathways. Figure 4 illustrates subjects' mean MEP amplitudes in stimulated and non-stimulated TA CM pathways following PAS_{fac}. Note the four subjects highlighted with heavy dashed lines (I, III, IV, VIII) where *inhibition* was evident in their contralateral non-stimulated TA pathways. Eight subjects revealed an increase in CM excitability in stimulated *and* non-stimulated pathways, with greater facilitation on the stimulated side except for subject VII who demonstrated greater facilitation in non-stimulated pathways.

Table 1 sets out the group means derived from $Post_0$ and $Post_{10}$ TA MEP amplitude and MEP area data following PAS_{fac} and PAS_{inhib} expressed as a percentage of Pre. The P values indicate whether the means differed from unity (100%).

Persistent modulation was evident in stimulated pathways

Persistent stimulated pathway modulation was revealed in the analysis of TA MEP *amplitude* following PAS_{fac} using a 2-way repeated measures ANOVA. This analysis revealed an effect of 'pathway' (Stim *versus* Non-Stim, $F_{1,13} = 20.83$, $P < 0.001$), but no effect of 'time' ($P > 0.3$) (see Fig. 5*A*). Similarly, the analysis of TA MEP *area* following PAS_{fac} revealed an effect of 'pathway' (Stim *versus* Non-Stim, $F_{1,13} = 14.54$, $P = 0.002$), but no effect of 'time' ($P > 0.5$) (see Fig. 5*B*). The analysis of TA MEP amplitude and area following PAS_{inhib} using a 2-way, repeated measures ANOVA did not reveal an effect of 'pathway' or of 'time' (see Fig. 6)

Some subjects revealed robust bidirectional modulation

An inspection of the extent of facilitation and inhibition in each subject's stimulated TA pathways (see Fig. 7) suggested there was a correlation between the extent of facilitation and inhibition. While a regression analysis did not reveal a significant correlation, when the analysis was restricted to the seven 'best modulators' (I, V, VI, VIII, IX, XI, XIII) who demonstrated the largest percentage differences between facilitation and inhibition (> 40), a significant positive correlation emerged ($r^2 = 0.88$, $P = 0.005$). This analysis indicated that although these subjects demonstrated robust modulation in both directions, greater facilitation was associated with less inhibition.

Modulation was also revealed in soleus pathways

Following PAS_{fac} applied to TA pathways, no effects were revealed by the ANOVA of Sol responses, although group mean Sol MEP area differed from 100% in the stimulated limb at $Post_{10}$ (Fig. 8). Sol responses were collected during

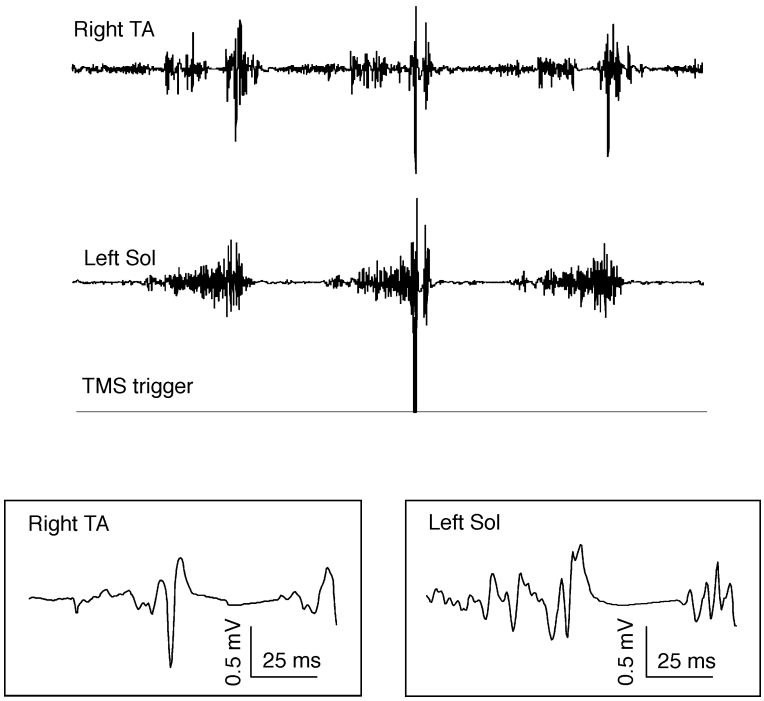

Figure 2. Sample EMG recorded simultaneously from the right TA and the left Sol during late swing and late stance, respectively
Four seconds of EMG from Subject V showing bursts in right TA (top trace) and left Sol (middle trace) during treadmill walking at 4.0 km h^{-1}. The bottom trace indicates when the TMS trigger occurred during late swing and late stance, respectively. Simultaneous MEPs (including the post-MEP silent period) can be distinguished in each trace at the time of the trigger. These MEPs are illustrated on a larger scale in the boxes below. Left box, TA MEP; right box, Sol MEP. The TA MEP is typically triphasic and the Sol MEP is typically polyphasic.

late stance. Data from four subjects were omitted from the following analyses because either motor threshold for Sol MEPs was not reached (recall that TA was the target muscle and parameters were established to optimize TA responses), or background Sol EMG could not be matched within an individual across the three trials. 'Stim' refers to means of Sol responses recorded from the muscle ipsilateral to stimulated TA pathways, 'Non-Stim' refers to means of Sol responses recorded from the muscle contralateral to stimulated TA pathways. Of the eight PAS_{fac} Sol means (amplitude, area; 'Stim', 'Non-Stim'; $Post_0$, $Post_{10}$), only 'Stim' $Post_{10}$ area differed from 100% (mean, 112; $P = 0.044$) (see Fig. 8*B*). The remaining means ranged from 94 to 107. The ANOVA for amplitude and area did not reveal effects of 'pathway' or 'time' (all $P > 0.1$). Following PAS_{inhib} (not illustrated), two of the eight Sol means differed from 100%: the 'Stim' amplitude mean (122%) at $Post_{10}$ ($P = 0.029$) and the 'Stim' area mean (116%) at $Post_0$ ($P = 0.041$). The ANOVA for amplitude revealed an effect of 'time' where 'Stim' $Post_{10}$ (mean, 121%) was greater than $Post_0$ (mean, 109%), $F_{1,10} = 5.7$, $P = 0.038$).

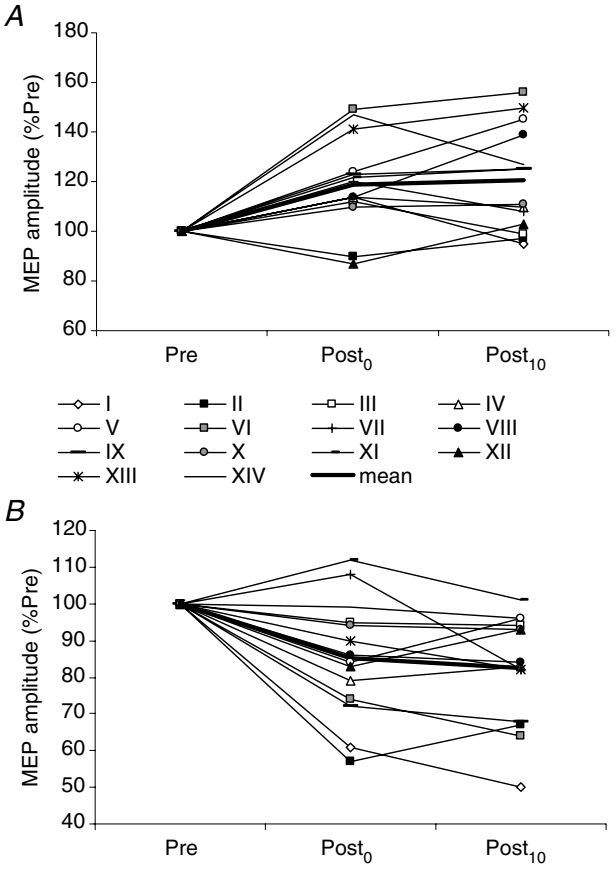

Figure 3. Individual and group means of MEP amplitude showing facilitation following PAS_{fac} and inhibition following PAS_{inhib}

Individual responses for fourteen subjects in stimulated TA CM pathways following PAS_{fac} (*A*) and PAS_{inhib} (*B*) showing persistent modulation in group means illustrated by the thick continuous line. All values are expressed as a percentage of Pre.

Robust modulation was not evident with peripheral and cortical stimulation alone

Only PAS induced a significant modulation in MEP amplitude. Analysis of control data recorded from TA during the eight sessions conducted on four subjects did not reveal an effect of walking alone, PN stimulation alone, or TMS alone, but did reveal an effect of PAS. The $Post_{PN}$ and $Post_{TMS}$ means (101 and 105%, respectively) did not differ from 100% using one-tailed *t* tests (all $P > 0.3$). The $CPost_0$, and $CPost_{10}$ conditions (equivalent to the intervention in the main experiment) differed from 100%, where the $CPost_0$ mean was 114% ($P = 0.034$), and the $CPost_{10}$ mean was 110% ($P = 0.011$). A one-way ANOVA revealed an effect of condition ($F_4 = 3.0$, $P = 0.033$), and *post hoc* Tukey HSD tests (to detect 'honestly significant differences') revealed an effect of PAS where the $CPost_0$ mean (114%) was greater than the $Post_{walk}$ mean (96%) ($P < 0.05$). For illustrative purposes Fig. 9 separates the control condition means of the four control sessions that were concluded with PAS_{fac} from the four that were concluded with PAS_{inhib}.

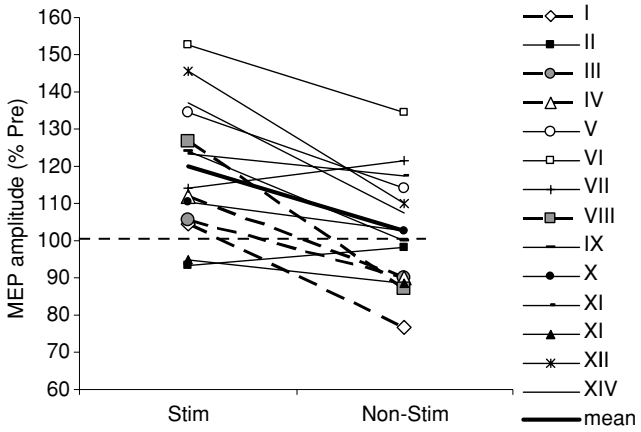

Figure 4. A comparison of TA MEP amplitude for individual subjects following PAS_{fac} in stimulated and non-stimulated TA CM pathways

Means for individual subjects are illustrated in stimulated limb and non-stimulated limb TA CM pathways. Stimulated (Stim) and non-stimulated (Non-Stim) values are subject's means of responses recorded immediately after a 10 min period of stimulation ($Post_0$) and at the end of a further 10 min of walking without stimulation ($Post_{10}$). The group means are illustrated by the thick continuous line. The horizontal thin dashed line represents Pre values. Values for four subjects (I, III, IV, VIII) that are highlighted with thick dashed lines and large symbols revealed inhibition in non-stimulated TA CM pathways.

Table 1. Differences from 100 for group means of TA MEP size expressed as a percentage of Pre

		MEP amplitude				MEP area			
		Stim		Non-Stim		Stim		Non-Stim	
		Mean (%)	*P*	Mean (%)	*P*	Mean (%)	*P*	Mean (%)	*P*
PAS_{fac}									
	$Post_0$	119	0.002	104	0.569	123	0.002	106	0.106
	$Post_{10}$	121	0.002	102	0.408	119	0.011	104	0.057
PAS_{inhib}									
	$Post_0$	85	0.004	92	0.155	88	0.013	98	0.569
	$Post_{10}$	82	< 0.001	97	0.514	85	0.007	100	0.913

PAS applied during tonic contraction also induced facilitation

In the last control experiment, the extent of facilitation of TA pathways was similar to that derived from the main experiment (123%). The $Post_0$ and $Post_{10}$ MEP amplitudes for the four control subjects (% Pre) were pooled ($n = 8$) separately for data collected during walking and during tonic contraction. The tonic contraction mean (118%) differed from unity ($P = 0.002$). The tonic contraction mean did not differ from the walking mean (120%) using a paired *t* test ($P = 0.79$).

Discussion

The main finding was that PAS induced changes in dorsiflexor CM excitability of healthy subjects during walking. Bidirectional changes in lower limb CM excitability induced by PAS during walking have not previously been reported. Four criteria characterizing the physiological profile of PAS-induced phenomena in the human motor system have previously been described by Stefan *et al.* (2000, 2002); and Wolters *et al.* (2003). Three of these were evident in the present data. The first is the temporal rule, in which the same group of subjects demonstrated an increase in CM excitability when the paired ISI was 5 ms longer than the estimated MEP latency, and also demonstrated a decrease in CM excitability when the paired ISI was 10 ms shorter than the estimated MEP latency. The second criterion is rapid evolution, also demonstrated in the present experiment, where 120 pairs of stimuli were delivered over a 10 min period (cf. 90 pairs delivered over a 30 min period: Stefan *et al.* 2000, 2002; Wolters *et al.* 2003). While the relationship between stimulation period and the number of pairs of stimuli has yet to be examined, the present data clearly illustrate a rapid evolution of bidirectional changes in CM excitability. The third criterion is persistence of effects following paired stimulation. The present group data revealed no reduction in facilitation or inhibition at $Post_{10}$ compared with $Post_0$ (see Figs 5 and 6). The fourth criterion is reversibility which was not assessed in the present experiment.

Stefan *et al.* (2000, 2002) and Wolters *et al.* (2003) referred to PAS-induced phenomena as 'long-term potentiation-like' (LTP-like) and 'long-term depression-like' (LTD-like). Their choice of terminology was based on the characteristics of the temporal

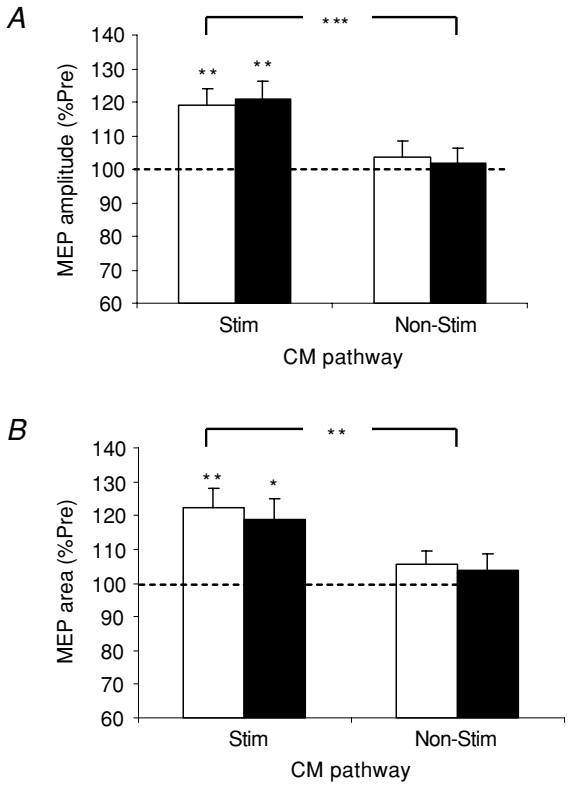

Figure 5. Group means of MEP amplitude and MEP area reveal a persistent effect of PAS_{fac} in stimulated TA pathways but no effect in non-stimulated TA pathways
The comparison of mean MEP size (*A*, amplitude; *B*, rectified area) during late swing recorded from stimulated TA pathways and during late swing recorded from non-stimulated TA pathways (contralateral). MEP size is expressed as a percentage of pre-intervention (Pre). Open bars represent the means of $Post_0$, and filled bars represent the means of $Post_{10}$ responses. The horizontal dashed line represents the Pre mean. Non-bracketed stars indicate the significance of the difference of the means from 100% (one-tailed *t* tests). Bracketed stars indicate the significance of the difference between Stim and Non-Stim means (2-way repeated measures ANOVA). $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Error bars represent 1 S.E.M.

nature of the phenomena, its rapid evolution, persistence, and reversibility. Further, these authors found that PAS-induced facilitation was blocked by dextromethorphan, an *N*-methyl-D-aspartate receptor antagonist known to block long-term potentiation in animal tissue slice preparations. Despite these similarities, a clear relationship between the effects of PAS and the *in vitro* induction of LTP has not been established.

Inspection of Fig. 3 reveals that during PAS_{fac} four subjects continued to increase MEP amplitude (Fig. 3*A*), and during PAS_{inhib} five subjects continued to decrease MEP amplitude (Fig. 3*B*) in the 10 min period of walking immediately following PAS termination. This finding and the between-subject variability is consistent with the previous results from Stefan *et al.* (2000). Further, it was also noted that PAS failed to elicit MEP changes in a few subjects during either conditioning protocol (Fig. 3), indicating that some subjects were amenable to both PAS_{fac} and PAS_{inhib} and others were not amenable to either. This suggestion is also supported by the regression analysis of the seven 'best modulators' (Fig. 7*B*). If the difference in the extent of modulation between subjects had been merely the result of inappropriate setting of the inter-stimulus interval between stimulus-pairs no correlation between robust facilitation and robust inhibition would have been evident. However, inappropriate setting of parameters as the cause of the between-subject variability in the extent of modulation across the two sessions cannot be entirely ruled out. More robust effects of PAS may be evident if afferent conduction time was estimated from sensory-evoked potentials (SEP) recorded from the scalp in response to electrical stimulation of the peroneal nerve. There are no reports of PAS where specific interstimulus

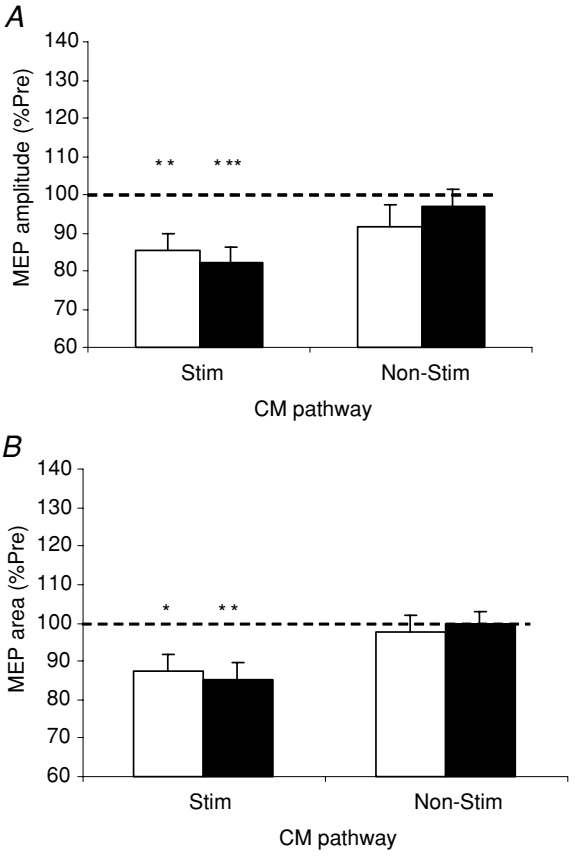

Figure 6. Group means of MEP amplitude and MEP area reveal a persistent effect of PAS_{inhib} in stimulated TA pathways but no effect in non-stimulated TA pathways
The comparison of mean MEP size (*A*, amplitude; *B*, rectified area) during late swing recorded from stimulated (Stim) TA pathways and during late swing recorded from non-stimulated (Non-Stim) TA pathways (contralateral). MEP size is expressed as a percentage of pre-intervention (Pre). Open bars represent the means of $Post_0$, and filled bars represent the means of $Post_{10}$ responses. The horizontal dashed line represents the Pre mean. Stars indicate the significance of the difference of the means from 100% (one-tailed *t* tests). $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, d.f. 13. Error bars represent 1 S.E.M.

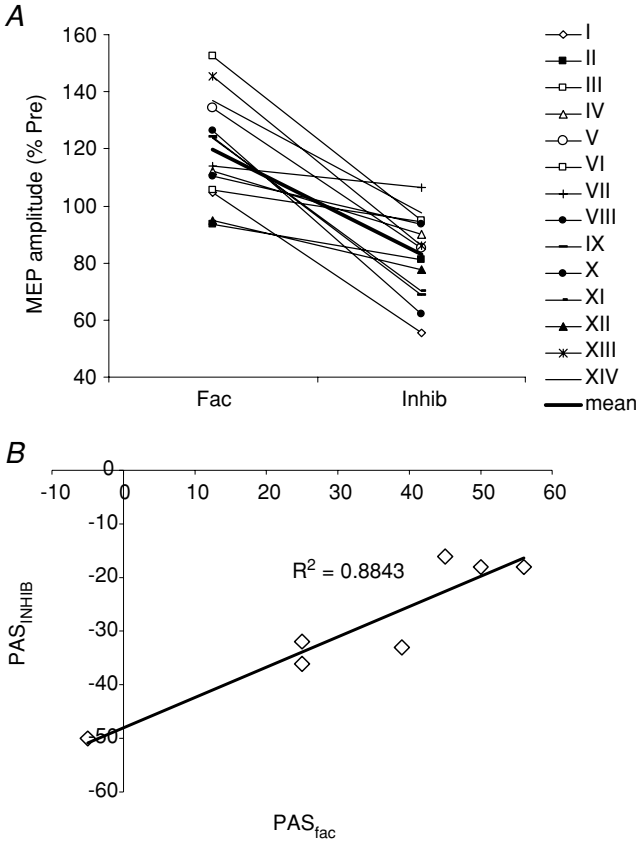

Figure 7. Subjects who revealed robust facilitation in response to PAS_{fac} also revealed robust inhibition in response to PAS_{inhib}
A comparison of the effects of PAS_{fac} and PAS_{inhib} in each subject. *A*, averaged $Post_0$ and $Post_{10}$ values of individual subject's means. The thick line represents the group mean. *B*, a scatter plot of means from subjects I, V, VI, VIII, IX, XI and XIII with fac-inhib differences of > 40 revealing that although greater facilitation was associated with less inhibition, subjects who demonstrated robust facilitation in one session also demonstrated robust inhibition in the other session ($P = 0.005$).

intervals have been based on SEP latency of individual subjects.

An intriguing observation made from comparing the extent of facilitation during PAS_{fac} in stimulated pathways compared with non-stimulated pathways (Fig. 4) was that four subjects who demonstrated robust facilitation also demonstrated robust inhibition in non-stimulated pathways. Although data from these four subjects in the present experiment cannot provide an explanation for this observation, if group data in a future experiment were to reveal a similar opposite-side-effect of PAS, the observation would warrant further investigation.

In the present experiment CM pathways to TA were chosen as the target. All stimulation parameters for probing CM excitability and for PAS were set for the target pathways. Therefore Sol data may be disregarded or interpreted with great caution. Notwithstanding this caveat, it is interesting to note that Sol MEP amplitude *increased* to 122% in the $Post_{10}$ condition of the stimulated limb when PAS_{inhib} was being applied to the target TA pathway. The finding may indicate a reciprocal inhibitory mechanism for this agonist–antagonist pair of ankle effectors. However, during PAS_{fac}, a reciprocal effect was not evident and a modest 112% facilitation (not inhibition) was evident at $Post_{10}$ in the stimulated limb Sol (Fig. 8*B*). At best, these data might indicate a non-specific facilitation of Sol pathways. The data warrant the design of an experiment that would examine the specificity of PAS on CM pathways to TA and to Sol separately. Changes in CM excitability in pathways to muscles of the leg as well as muscles of the shank could be included and optimal stimulation parameters could be established for each muscle pathway to enhance the validity of observed changes in non-target CM excitability.

An important issue addressed in the present experiment is whether any of the components of the intervention applied during walking modulate CM excitability on their own. The eight sessions conducted with four subjects revealed no statistically significant effects on MEP amplitude of walking alone, electrical stimulation of the peroneal nerve during walking alone, or TMS during walking alone. Significant effects were only revealed following PAS applied at the end of the control data collection sessions that involved walking. An inspection of Fig. 9 reveals that some modulation of MEP amplitude was taking place over the course of the control sessions; however, the extent of the modulation was small and failed to reach statistical significance compared with the

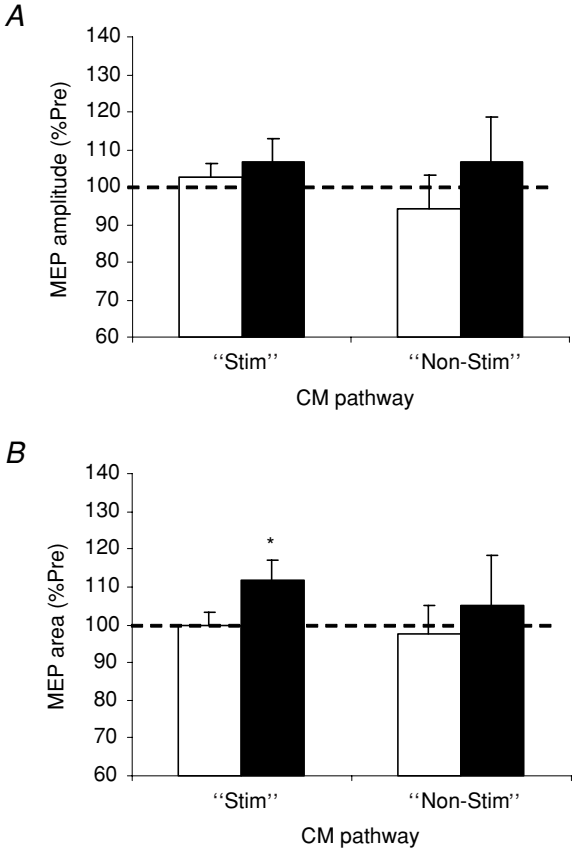

Figure 8. Only the group mean of MEP area recorded from Sol in the stimulated limb during late stance following PAS_{fac} of TA pathways differed from unity

The comparison of mean MEP size (*A*, amplitude; *B*, rectified area) during late stance recorded from the Sol ipsilateral to the stimulated TA pathways ('Stim') and during late stance recorded from the Sol ipsilateral to the non-stimulated TA pathways ('Non-Stim'). MEP size expressed as a percentage of pre-intervention (Pre). Open bars represent the means of $Post_0$, and filled bars represent the means of $Post_{10}$ responses. The horizontal dashed line represents the Pre mean. The star indicates that the mean differed from 100% (one-tailed *t* test), $P < 0.05$. Error bars represent 1 S.E.M.

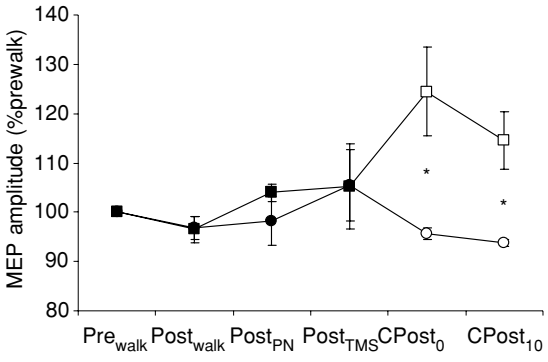

Figure 9. Control conditions during walking did not significantly modulate MEP size

■, the means of the four control subjects (VI, VII, IX, XIII) during the session that culminated in PAS_{fac}. ●, the means of the four control subjects during the session that culminated in PAS_{inhib}. Filled symbols represent means expressed as a percentage of Pre_{walk}. Open symbols represent means expressed as a percentage of either $Post_{TMS}$ or $Post_{PN}$. Stars indicate that means of unsigned changes in MEP amplitude following PAS_{fac} and PAS_{inhib} that were grouped for statistical analysis differed from 100% (see text for details). Error bars are 1 S.E.M.

modulation that followed PAS. These findings suggest that components of the PAS protocol as used in the present experiment do not significantly modulate CM excitability on their own. Finally, the results of the third control condition where subjects held a tonic TA contraction during the application of PAS_{fac} were consistent with the findings of Capaday *et al.* (1999) and Bawa *et al.* (2002) who showed that CM projections to TA are relatively stronger compared with projections to Sol. Capaday *et al.* (1999) found no difference between the extent of TA pathway excitability during walking and during a voluntary tonic contraction, and we found no difference between these two tasks in the extent of modulation induced by PAS.

The level of the neuroaxis at which the effects of PAS are occurring is assumed to be the M1 region because the temporal relationship between the paired stimuli is set to occur when the induced afferent volleys are estimated to have arrived in the cortex. This hypothesis has previously been supported by Stefan *et al.* (2000) who provided evidence of motoneurone stability despite significant increases in MEP amplitude following PAS. They reported no significant change in F-wave or M-wave amplitude or in the amplitude of potentials evoked by electrical stimulation at the level of the brainstem as a result of PAS. They also found that when they applied PAS to increase MEP amplitude there was an increase in the length of the post-MEP EMG silent period, suggesting the cortical component of the silent period had increased in length due to an increase in cortical input to motoneurone pools. Furthermore, Wolters *et al.* (2003) demonstrated that significant decreases in MEP amplitude induced by PAS were not evident in F-wave amplitude or the amplitude of potentials evoked by electrical stimulation at the level of the brainstem. In the present experiment silent periods were not examined because silent periods are difficult to assess in an EMG burst during walking where EMG amplitude is changing as a function of time. Since the stimuli were delivered in the present experiment toward the end of the swing phase, the return of voluntary EMG occurred at the offset of the EMG burst. A demonstration of where on the neuroaxis the effects of PAS were taking place was not attempted because the present experiment was primarily designed to assess the ability of PAS to modulate the excitability of the CM pathway as a whole during walking. However, the time window in which paired stimuli were delivered (-10 ms to 5 ms), and the lack of an effect of peripheral nerve stimulation alone suggests a supraspinal site. Future experiments to assess intracortical excitability and the excitability of spinal circuitry may provide further evidence of the cortex as the site where effects of PAS occur during walking.

Although PAS used to increase CM excitability during walking is unique to the present study, other authors have reported the use of functional electrical stimulation to increase CM excitability. For example, Knash *et al.* (2003) demonstrated increased MEP size in the TA with repetitive electrical stimulation of the common peroneal nerve. Significant facilitation was observed within the first 10 min of stimulation, and lasted up to 30 min following cessation of stimulation. Further, Khaslavskaia *et al.* (2002) delivered repetitive electrical stimulation to the peroneal nerve of healthy subjects and found that CM excitability was increased to a greater extent when stimulation was combined with voluntary dorsiflexor activation than when the muscle was at rest. Such augmented effects of repetitive electrical stimulation on CM excitability with combined voluntary effort may play a role in the restoration of motor behaviours following neurological injury (Field-Fote & Tepavac, 2002; Yan *et al.* 2005).

In conclusion, the hypothesis adopted for the present experiment was supported by evidence that PAS applied to ankle dorsiflexor CM pathways during the swing phase of walking induced temporally dependent bidirectional changes in CM excitability consistent with the Hebbian principle of activity-dependent plasticity. Similarities between these data derived from the lower limb, and data derived from CM pathways to hand muscles (Stefan *et al.* 2000, 2002; Wolters *et al.* 2003) suggest that the same neural mechanisms were involved. Of particular importance was the demonstration of these phenomena during walking which has not previously been reported. Walking training protocols applied to neurologically impaired individuals where CM excitability is suppressed, may benefit from the addition of a training adjunct that increases CM excitability.

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