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Title: Ipsilateral corticomotor excitability is associated with increased gait variability in unilateral transtibial amputees

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Abstract

Ipsilateral primary motor cortex (M1) reorganisation after unilateral lower-limb amputation may degrade function of the amputated limb. We hypothesised unilateral lower-limb amputees would have a bilateral increase in corticomotor excitability and increased excitability of ipsilateral M1 would be associated with increased step-time variability during gait. Twenty transtibial amputees (16-male) aged 60.1 (range 45-80) years, and twenty age- and gender-matched healthy adult controls were recruited. Single-pulse transcranial magnetic stimulation assessed corticomotor excitability. Two indices of corticomotor excitability were calculated. An index of corticospinal excitability determined relative excitability of ipsilateral and contralateral corticomotor projections to alpha-motoneurons innervating the quadriceps muscle of the amputated limb. A laterality index assessed relative excitability of contralateral projections from each hemisphere. Spatial-temporal gait analysis was performed to calculate step-time variability. Amputees had lower index of corticospinal excitability values, indicating relatively greater excitability of ipsilateral corticomotor projections than controls (P=0.04). Lower index of corticospinal excitability values were associated with increased step-time variability for amputated (P=0.04) and non-amputated limbs (P=0.02). This association suggests corticomotor projections from ipsilateral M1 to alpha-motoneurons innervating the amputated limb quadriceps muscle may interfere with gait. Cortical excitability in amputees was not increased bilaterally, contrary to our hypothesis. There was no difference in excitability of contralateral M1 between amputees and controls (P=0.10) and no difference in laterality index (P=0.71). It appears both hemispheres control one quadriceps muscle with predominance of contralateral corticomotor excitability in healthy adults. Following lower-limb amputation, putative ipsilateral corticomotor excitability is relatively increased in some amputees and may negatively impact on function.

Introduction

Bipedal locomotion is the quintessential form of ambulation mastered by humans early in life. Activation of the primary motor cortex (M1) of both cortical hemispheres is essential for lower-limb motor control (Luft *et al.*, 2002; Sahyoun *et al.*, 2004), and amputation presents a unique challenge to the bi-hemispheric control of gait. Interestingly, it appears there is bilateral reorganisation of M1 following amputation (Schwenkreis *et al.*, 2003; Hordacre & Bradnam, 2013). Reorganisation of M1 contralateral to the side of amputation ($M1_{CON}$) increases cortical excitability and is well characterised (Hall et al., 1990; Cohen et al., 1991; Fuhr et al., 1992; Kew et al., 1994; Chen et al., 1998). A concomitant increase in corticomotor excitability ipsilateral to the side of amputation ($M1_{IPSI}$) has not been thoroughly investigated. There is evidence that $M1_{IPSI}$ undergoes reorganisation, likely due to high sensorimotor demand placed on the non-amputated limb early in rehabilitation (Hordacre & Bradnam, 2013). How a bilateral increase in M1 excitability might affect gait in lower-limb amputees is an important question for amputee rehabilitation given the challenges with prosthetic gait and risk of falls (Miller *et al.*, 2001; Pauley *et al.*, 2006). The relationship between M1 excitability and function has not been addressed in studies to date. The spatial-temporal parameter, step-time variability, is a measure indicative of falls risk in older adults (Verghese et al., 2009; Brach et al., 2010) and transtibial amputees (Parker et al., 2013). There is little understanding regarding cortical contributions to gait variability in the healthy population or how amputation affects cortical excitability and gait variability in amputees.

Bilateral cortical reorganisation is not unique to amputees. Interhemispheric imbalance from suppression of the ipsilesional and facilitation of the contralesional hemisphere controlling the paretic upper-limb in chronic stroke is associated with poor recovery (Shimizu et al., 2002; Murase et al., 2004; Grefkes et al., 2008). Assessing brain neurophysiology with transcranial magnetic stimulation (TMS) can identify hemispheric imbalance and quantify its impact on function. A laterality index (LI) of contralesional to ipsilesional excitability recorded from the non-paretic and

paretic limbs respectively is one method (Brouwer & Schryburt-Brown, 2006; Wang *et al.*, 2012). A ratio of contralesional to ipsilesional corticomotor projections to the paretic lower-limb, the index corticospinal excitability (ICE) is another. In the latter, upregulation of ipsilateral corticomotor projections from the contralesional hemisphere is associated with poor lower-limb control in stroke (Madhavan et al., 2010; Jayaram et al., 2012). Our rationale for this current study was that a similar upregulation of ipsilateral projections to the amputated limb would degrade gait in lower-limb amputees. The primary aim was to investigate bilateral corticomotor excitability in healthy adults and lower-limb amputees using two neurophysiological indices, the LI and ICE. The secondary aim was to assess if there was an association between neurophysiological indices and gait function. We hypothesised lower-limb amputees would have a bilateral increase in corticomotor excitability and greater excitability of putative ipsilateral corticomotor projections would be associated with increased step-time variability, indicating reduced function.

Materials and Methods

Participants

Twenty unilateral transtibial amputees (16 male), with mean age of 60.1 years (range 45-80), and 21.7 (SD 22.3) years since amputation were recruited. The amputee mobility predictor was used to determine function and categorise K-levels (Gailey *et al.*, 2002). Higher K-level scores indicate greater function (range K1-K4). Description of K-levels is as follows; K1 non-community ambulator, K2 limited community ambulator, K3 unlimited community ambulator, and K4 high functioning ambulator (Gailey *et al.*, 2002). Amputees of K1 level were excluded from the study as they were unable to perform functional gait assessment. A comparator group of 20 age and gender matched healthy adults were purposively recruited as control participants (mean age 59.3 years (range 43-83)). Limb dominance was assessed with the Edinburgh Handedness Inventory (Oldfield, 1971) and in control participants the non-dominant limb was modelled as the amputated limb. Amputee and control participant demographics and clinical characteristics are summarised in table

1. Potential participants with contraindications for TMS, including those with metallic implants, a history of seizures and medications known to alter central nervous system excitability were excluded (Rossi *et al.*, 2009) following screening by a rehabilitation physician. Ethical approval was provided by the Southern Adelaide Clinical Human Research Ethics Committee and all participants provided written informed consent in accordance with the Declaration of Helsinki.

Protocol

Participants attended a single session to assess brain neurophysiology and spatial-temporal gait parameters. During TMS participants were seated comfortably with hip and knee joints flexed to 90°. A seated knee-extension task was used to unilaterally pre-activate the quadriceps muscle (QM) prior to each TMS pulse in response to an auditory cue repeated at 0.2Hz intervals. Consistent muscle activation at 10-15% MVC was achieved by monitoring visual feedback of raw electromyography (EMG) signal from the QM. Transcranial MS pulses were triggered during muscle contractions using Signal software (v5.09).

Electromyography

Surface EMG was recorded from the QM bilaterally using 10mm-diameter Ag/AgCl electrodes (Ambu, Ballerup, Denmark) placed 2cm apart over the muscle bellies, with the distal electrode approximately 12cm superior to the midpoint of the patella. A 20mm-diameter ground Ag/AgCl electrode (3M Health Care, Canada) was placed over the patella. Prior to affixing the electrodes, hair was removed by shaving, and the top layer of skin lightly abraded for optimal contact. Electromyography signals were sampled at 2000Hz (CED 1401; UK), amplified (CED 1902; UK), band-pass filtered (20-1000Hz) and stored for offline analysis (Signal v5.09).

Transcranial Magnetic Stimulation

Single-pulse TMS was delivered using a Magstim 200 stimulator (Magstim Company, Dyfed, UK). A flat 70mm wing diameter, figure eight coil was held tangentially over the scalp with the handle pointing 30° posterior-medially in the transverse plane. This coil orientation was determined from extensive piloting (Hordacre & Bradnam, 2013). As a guide, the coil was initially positioned 1cm posterior, 1.5cm lateral to the vertex (Madhavan *et al.*, 2010; Hordacre & Bradnam, 2013). The 'hotspot' for evoking maximal responses in the contralateral active QM was then determined for each M1 by systematically moving the coil over a 1cm grid from this location and marked on the scalp. Active motor threshold (AMT) was determined separately for each M1 as the minimum stimulus intensity eliciting a 100μV motor evoked potential (MEP) in five of ten stimuli in the contralateral QM (Rossini *et al.*, 1994). The stimulus intensity evoking a maximal MEP response (MEP_{MAX}) in the contralateral QM was determined for each M1. Three stimulus-response (S-R) curves were constructed from MEPs recorded at equally spaced intensities between AMT and MEP_{MAX} (inclusive). Two S-R curves were constructed from MEPs at six different intensities in each QM following stimulation of the respective contralateral M1 (contralateral S-R curves). A third S-R curve was constructed from MEPs recorded in QM of the amputated limb following stimulation of $M1_{IPSI}$ (ipsilateral S-R curve). The ipsilateral S-R curve was constructed with one additional stimulus intensity above MEP_{MAX} to account for higher thresholds to evoke ipsilateral MEPs (Ziemann *et al.*, 1999), which usually equated to 90-95% MSO. For each intensity of the S-R curve, 14 MEPs were collected in random order.

Responses where pre-stimulus root mean square EMG (rmsEMG) were 2 SD above or below the mean were removed prior to averaging (range 0-2) to ensure consistency of MEP responses. From the retained traces, MEPs were measured peak-to-peak, averaged and plotted against stimulus intensity. The slope of the S-R curve was determined from the linear portion by linear regression. A computerised mathematical algorithm was used to determine the steepest section of the S-R curve. The algorithm used a sliding window of different combinations of consecutive points along the S-R

curve (minimum 3, maximum all) to systematically select those that made up the steepest slope (SR_{SLOPE}) (figure 1). SR_{SLOPE} was used to calculate two indices of corticomotor excitability for each participant. First, ICE assessed excitability of contralateral and ipsilateral corticomotor projections to the amputated limb (Madhavan *et al.*, 2010). Negative ICE values indicate relatively greater excitability of ipsilateral, compared to contralateral, descending corticomotor projections. The equation to calculate ICE was;

$$ICE = \frac{(\text{contralateral } SR_{SLOPE} - \text{ipsilateral } SR_{SLOPE})}{(\text{contralateral } SR_{SLOPE} + \text{ipsilateral } SR_{SLOPE})}$$

Second, a LI was determined to assess excitability of contralateral corticomotor projections innervating the amputated and non-amputated limb respectively. Negative LI values indicate relative greater excitability of contralateral projections to alpha-motoneurons innervating QM of the non-amputated limb. Positive LI values indicate relative greater excitability of contralateral projections to alpha-motoneurons innervating QM of the amputated limb. The equation to calculate LI was;

$$LI = \frac{(\text{contralateral } SR_{SLOPE}\ M1_{CON} - \text{contralateral } SR_{SLOPE}\ M1_{IPSI})}{(\text{contralateral } SR_{SLOPE}\ M1_{CON} + \text{contralateral } SR_{SLOPE}\ M1_{IPSI})}$$

The ipsilateral silent period (ISP) was used to assess interhemispheric inhibition from stimulation of $M1_{IPSI}$ at 80%MSO (Chen *et al.*, 2003; Trompetto *et al.*, 2004; Avanzino *et al.*, 2007). This intensity was chosen because at higher intensities the onset of the ISP was often masked by the MEP. Data were rectified and averaged and the ISP measured from this average. Ipsilateral SP onset was defined when post-stimulus EMG fell below the mean of the pre-stimulus EMG for a continuous period of 10ms in a window 20-80ms after the stimulus. Ipsilateral SP offset was

defined when EMG returned to baseline levels (Chen *et al.*, 2003; Trompetto *et al.*, 2004; Avanzino *et al.*, 2007). The ISP was calculated as the area between onset and offset points relative to the mean of the prestimulus rmsEMG (ISP$_{AREA}$), expressed in mV•ms (see figure 2).

Spatial-temporal gait variability

Spatial-temporal gait was assessed using an instrumented GAITRite walkway (CIR-Systems Inc., NJ, USA) with embedded pressure sensors to capture individual footfall data over an active area 4.9m x 0.6m. Participants completed 10 consecutive passes over the GAITRite at their self-selected comfortable walking speed. Data were sampled at 120Hz and analysed using GAITRite software (version 4.5.5). Variability of spatial-temporal parameters was assessed by the coefficient of variation (CoV), calculated as SD divided by the mean expressed as a percentage. The primary gait outcome measure, step-time variability, is a velocity dependent parameter and greater variability can occur in raw data with variations in walking speed between trials (Beauchet *et al.*, 2009). Individual gait trial parameters were therefore normalised to a walking speed of 1m/s for all participants prior to calculation of CoV in accordance with similar previous studies (Hof, 1996).

Data Analysis

The normality of data was checked with a Shapiro-Wilk test. Post-hoc tests explored significant effects and were corrected for multiple comparisons using a modified Bonferroni correction (Rom, 1990). Significance level was set at $P \leq 0.05$ and SPSS software was used for all statistical analyses (IBM corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0).

Corticomotor excitability

Differences between amputee and control ICE, LI and ISP$_{AREA}$ were tested with separate independent t-tests. To further understand fundamental contributions of each hemisphere to ICE ratios, SR$_{SLOPE}$ and MEP latencies were separately analysed using one-way ANOVAs. The four

independent variables were; amputee contralateral, amputee ipsilateral, control contralateral, control ipsilateral. To understand how the contralateral and ipsilateral SRSLOPE contributed to ICE in amputees, correlation analysis was performed between ICE and ipsilateral SRSLOPE, ICE and contralateral SRSLOPE, and between the ipsilateral and contralateral SRSLOPE used in the ICE calculations. To further understand fundamental contributions of each hemisphere to LI ratios, SRSLOPE and MEP latencies were separately analysed using one-way ANOVAs. The four independent variables were; amputee amputated and non-amputated limb, control dominant and non-dominant limb. Background EMG was compared across the two contralateral S-R curves with a 2 group (amputee, control) x 6 condition (S-R curve intensities) ANOVA. Similarly a 2 group (amputee, control) x 7 condition (S-R curve intensity) ANOVA was used for the ipsilateral S-R curve.

Functional gait variability

Step-time variability normalised to a walking speed of 1m/s was assessed with a one-way ANOVA. The four independent variables were; amputee amputated and non-amputated limb, and control dominant and non-dominant limb.

Corticomotor excitability and gait function

Linear regression models analysed association between the primary corticomotor excitability measures (ICE, LI) and step-time variability (normalised with log(10) transformation) and were controlled for factors known to influence gait (age, time since amputation, stump length and indication for amputation) (Gonzalez *et al.*, 1974; Gailey *et al.*, 1994; Kang & Dingwell, 2008; Callisaya *et al.*, 2010; Mâaref *et al.*, 2010).

Results

No adverse events were experienced during TMS or gait analysis.

Corticomotor excitability measures

Corticomotor excitability measures are summarised in table 2. Amputees had smaller ICE values than controls ($t_{(38)} = 2.07$, $P = 0.04$). Five amputees had negative ICE values, while no controls had negative ICE values. There were no significant differences between amputees and controls for LI ($P = 0.71$) or ISP$_{AREA}$ ($P = 0.42$). The one-way ANOVA to elucidate fundamental contributions to the ICE ratio found a difference in SR$_{SLOPE}$ ($F_{(3,76)} = 4.55$, $P = 0.006$). Post-hoc analysis revealed amputees had steeper ipsilateral SR$_{SLOPE}$ ($t_{(38)} = 1.96$, $P = 0.03$) , but not contralateral SR$_{SLOPE}$ ($P = 0.10$) compared to control subjects. As expected, contralateral SR$_{SLOPE}$ was steeper than ipsilateral SR$_{SLOPE}$ for both amputees ($t_{(19)} = 3.07$, $P = 0.006$) and controls ($t_{(19)} = 4.52$, $P = 0.001$). There were differences in MEP latency ($F_{(3,76)} = 6.05$, $P = 0.001$), with post-hoc analysis revealing ipsilateral MEP onset latency was 1.5ms longer than contralateral MEP onset latency for both amputees ($t_{(19)} = 3.03$, $P = 0.004$) and controls ($t_{(19)} = 2.98$, $P = 0.005$). Relative contributions of SRslope to smaller ICE ratios in amputees were analysed. There was no correlation between ICE and contralateral SR$_{SLOPE}$ ($P = 0.17$) or ICE and ipsilateral SR$_{SLOPE}$ ($P = 0.48$). There was a significant positive correlation between ipsilateral SR$_{SLOPE}$ and contralateral SR$_{SLOPE}$ ($r = 0.76$, $P = 0.001$) (see figure 3). The one-way ANOVA to elucidate fundamental contributions to the LI ratio showed a difference in SR$_{SLOPE}$ ($F_{(3,76)} = 2.60$, $P = 0.05$). Post-hoc analysis revealed contralateral SR$_{SLOPE}$ evoked from stimulation of M1$_{IPSI}$ to be steeper in amputees than controls ($t_{(38)} = 1.70$, $P = 0.04$). SR$_{SLOPE}$ evoked from stimulation of M1$_{CON}$ was no different between amputees and controls ($P = 0.10$). There was a difference in MEP latency ($F_{(3,76)} = 3.43$, $P = 0.02$), with post-hoc analysis revealing amputee MEP latency measured on the amputated limb to be shorter than the non-amputated limb ($t_{(38)} = 2.46$, $P = 0.02$). There was no difference in MEP latency between sides for control subjects ($P = 0.98$). For amputees average background EMG for the amputated limb contralateral S-R curve was 0.04mV (SD 0.03), non-amputated limb contralateral S-R curve was 0.06mV (SD 0.05), and ipsilateral S-R curve was 0.04mV (SD 0.02). For controls average

background EMG for the non-dominant limb contralateral S-R curve was 0.05mV (SD 0.02), dominant limb contralateral S-R curve was 0.04mV (SD 0.02), and ipsilateral S-R curve was 0.04mV (SD 0.02). There were no main effects of group (all $P > 0.12$) or intensity (all $P > 0.99$) for background EMG.

Functional gait variability

Normalised step-time variability for amputees was 5.15% (SD 2.7) on the non-amputated limb and 5.15% (SD 3.4) on the amputated limb. For control participants normalised step-time variability was 4.77% (SD 1.4) on the dominant limb and 4.61% (SD 1.7) on the non-dominant limb. There were no significant differences in step-time variability ($P = 0.86$).

Corticomotor excitability and gait function

For amputees, linear regression models controlling for age, time since amputation, stump length and indication for amputation demonstrated a negative relationship between ICE and step-time variability on the amputated ($R^2 = 0.64$, $P = 0.04$) and non-amputated limb ($R^2 = 0.64$, $P = 0.02$) (see figure 4). No other independent variables controlled for were significant ($P > 0.12$). There was no relationship between LI and step-time variability for amputees on the amputated ($P = 0.55$) and non-amputated limb ($P = 0.79$). For control subjects there were no relationships between ICE and normalised step-time variability on the dominant ($P = 0.36$) or non-dominant limb ($P = 0.86$), or LI and normalised step-time variability on the dominant ($P = 0.95$) or non-dominant limb ($P = 0.75$).

Discussion

This study investigated bilateral corticomotor excitability and step-time variability in healthy adults and unilateral transtibial amputees. There were several findings with relevance for corticomotor control of the lower-limb and the effect of lower-limb amputation. In healthy adults there was bilateral cortical control of one quadriceps muscle, with predominance of contralateral over

ipsilateral excitability. In some amputees there was a change in the relative excitability between the hemispheres whereby greater ipsilateral, relative to contralateral excitability lead to smaller ICE values. Smaller ICE values in amputees were associated with increased step-time variability on both the amputated and non-amputated limb. Contrary to our hypothesis, there was no bilateral increase in corticomotor excitability in amputees as contralateral M1 excitability was no different to controls. The LI was no different between amputees and controls. The results of this study and their putative implications for amputee rehabilitation are discussed below.

In the current study there was an increase in the ipsilateral SR$_{SLOPE}$ recorded in the quadriceps muscle of the amputated limb, suggesting greater ipsilateral corticomotor excitability. This conclusion must be interpreted conservatively however. A limitation of the TMS method used in this study is the 'hotspot' for lower-limb muscle representations is adjacent to the interhemispheric fissure, raising the question of whether the coil inadvertently stimulated both hemispheres. Certainly, ipsilateral responses to TMS in the lower-limb are a mix of ipsilateral and contralateral descending inputs as acknowledged previously (Madhavan *et al.*, 2010; Jayaram *et al.*, 2012). We cannot know what proportion of the responses used to calcuate SR$_{SLOPE}$ in the current study were ipsilateral in origin. However, our results indirectly suggest that a good proportion of the ipsilateral response was mediated from projections other than the contralateral corticospinal tract. Ipsilateral responses were greater in amputees while contralateral responses recorded in the same quadriceps muscle were no different to controls. If the offset coil location for evoking ipsilateral responses was mostly stimulating the contralateral hemisphere, there should have been no difference between groups. Nonetheless, we cannot be sure and refer to ipsilateral responses as 'putative' in the following discussion.

Motor control of the quadriceps muscle depends upon both ipsilateral and contralateral hemispheres in humans, although it appears predominance of the contralateral hemisphere is normal. Functional

magnetic resonance imaging studies demonstrate that isolated knee flexion/extension and ankle dorsiflexion/plantar flexion movements activate both ipsilateral and contralateral M1 (Luft *et al.*, 2002; Sahyoun *et al.*, 2004). Our TMS findings progress this understanding by demonstrating a contralateral predominance of cortical excitability, as observed in healthy adults was not associated with gait variability. If following lower-limb amputation putative ipsilateral corticomotor excitability dominated over contralateral, there was an association with increased gait variability. While both hemispheres contribute to the control of human gait, it is evident that the balance of excitability between the M1's is critical for normal function. Unexpectedly there was no bilateral increase in corticomotor excitability in amputees as there was no difference in M1CON excitability between amputees and controls. This was surprising as previous lower-limb amputee studies have reported reorganisation of M1CON in the forms of lower thresholds for TMS (Chen et al., 1998), larger MEPs (Fuhr et al., 1992), reorganisation of motor maps (Schwenkreis *et al.*, 2003), or a reduction in intracortical inhibition (Chen et al., 1998). It is not obvious why M1CON excitability was unaffected in this group of amputees, but might relate to factors such as length of time since amputation and their relatively high ambulatory function.While contralateral corticomotor excitability over the non-amputated limb was greater in amputees this did not influence the LI, which did not discriminate between amputees and control participants.

Amputees had smaller ICE values than controls indicating relatively greater putative ipsilateral to contralateral corticomotor excitability. Smaller ICE values could potentially result from a steeper ipsilateral SRSLOPE, or flatter contralateral SRSLOPE. To elucidate which hemisphere contributed to negative ICE values, we separately correlated each M1 SRSLOPE with ICE. There was no relationship for either hemisphere, indicating negative ICE was not an absolute increase in M1IPSI or decrease in M1CON excitability. Instead it was those amputees with a *relatively* greater putative ipsilateral to contralateral M1 excitability that demonstrated smaller ICE values. It is also likely that smaller ICE values indicate greater M1IPSI excitability than the suppression of M1CON because the

ISP, assessing the degree of interhemispheric inhibition from M1$_{IPSI}$ to M1$_{CON}$, was similar for amputees and controls. Ipsilateral SP's evoked in the lower-limb are considered to be mediated by transcallosal pathways similar to the upper-limb (Lo & Fook-Chong, 2004). The ISP results, at least in part, from activation of interhemispheric projections across the corpus collosum from the stimulated hemisphere that inhibit the homologous area of the contralateral hemisphere (Chen *et al.*, 2003; Trompetto *et al.*, 2004). The net result of an increase in interhemispheric inhibition is suppression of corticomotor output from contralateral M1 (Ferbert *et al.*, 1992; Di Lazzaro *et al.*, 1999), evoking a longer ISP, which was not seen in the current study. Finally, increased interhemispheric inhibition would result in a negative LI value which was also not observed. The findings of the present study indicate smaller ICE values in amputees compared to controls likely result from increased excitability of putative ipsilateral descending projections to the spinal cord.

Smaller ICE values in amputees were associated with increased step-time variability for both the amputated and non-amputated limb. Increased step-time variability is a predictor of higher falls risk in amputees (Verghese *et al.*, 2009; Brach *et al.*, 2010; Parker *et al.*, 2013). A causal relationship was not tested in the current study. However, the strong association between smaller ICE values and functional measures may indicate that upregulated putative ipsilateral projections degrades function, or alternatively compensatory gait patterns may increase excitability of putative ipsilateral corticomotor projections. Further studies are required to demonstrate cause and effect, and may prove ICE is a valid neurophysiological marker of reduced function in lower-limb amputees. It is unlikely that smaller ICE values in amputees were a direct result of the clinical characteristics or pathology leading to the amputation as those with negative ICE were a disparate group. This indicates changes in corticomotor excitability were independent of the clinical characteristics or pathology. The pathways responsible for the putative ipsilateral MEPs also cannot be determined from this study, but may involve reticulospinal projections descending to the spinal cord as suggested for the upper-limb following stroke (Ellis *et al.*, 2007; Schwerin *et al.*, 2008; Ellis *et al.*,

2012). Reticulospinal pathways bilaterally innervate axial and proximal alpha-motoneurons important for the control and postural support of muscles subserving locomotion (Drew *et al.*, 2004). Reticulospinal projections branch extensively as they terminate in the spinal gray matter (Peterson & Abzug, 1975; Matsuyama *et al.*, 1999), to link widely separated sections of the spinal cord (Lemon, 2008). Increased activity in the reticulospinal tract would lead to non-specific activation of muscles producing motor conflict that may degrade prosthetic gait (Kagerer *et al.*, 2003). Evidence of abnormal EMG patterns during amputee gait (Huang & Ferris, 2012) support the idea of motor conflict, and should be further investigated in gait variability studies.

There are two potential limitations of this study to consider. First, amputee participants were higher functioning and may not be representative of the community. Despite this, there were smaller ICE values in amputees with relatively poorer function. Second, there was a lack of homogeneity for indications for amputation across participants. In particular vascular amputations have associated peripheral neuropathies and general deconditioning compared to trauma amputations. However, of the amputees with negative ICE, only one was a vascular amputee arguing against this confounding factor (see table 1). The indication for amputation was controlled for in our ICE and step-time variability regression analysis and was a non-significant factor.

In conclusion, control of the quadriceps muscle during normal human gait may depend upon the relative corticomotor excitability between hemispheres; with a predominance of contralateral control. Following amputation, a change in the balance of cortical excitability might affect gait function. In the current study, amputees had smaller ICE values compared to controls, and smaller ICE values were associated with increased step-time variability. This indicates an increase in putative ipsilateral to contralateral excitability may increase step-time variability, which may in turn lead to greater risk of falls in amputees. Future studies should seek to demonstrate causal relationships between measures of cortical neurophysiology, such as ICE, and gait function. This

understanding would have the potential to improve amputee clinical practice.

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Abbreviations: AMT, active motor threshold; ANOVA, analysis of variance; EMG, electromyography; ICE, index of corticospinal excitability; ISP, ipsilateral silent period; LI, laterality index; M1, primary motor cortex; $M1_{CON}$, primary motor cortex contralateral to the amputated limb; $M1_{IPSI}$, primary motor cortex ipsilateral to the amputated limb; MEP, motor evoked potential; QM, quadriceps muscle; TMS, transcranial magnetic stimulation.

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