Interhemispheric Connectivity during Bimanual Isometric Force Generation

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Abstract

Interhemispheric interactions through the corpus callosum play an important role in the control of bimanual forces. However, the extent to which physiological connections between primary motor cortices are modulated during increasing levels of bimanual force generation in intact humans remain poorly understood. Here, we studied coherence between electroencephalographic (EEG) signals and the ipsilateral cortical silent period (iSP), two well-known measures of interhemispheric connectivity between motor cortices, during unilateral and bilateral 10%, 40%, and 70% of maximal isometric voluntary contraction (MVC) into index finger abduction. We found that EEG-EEG coherence in the alpha frequency band decreased while the iSP area increased during bilateral compared with unilateral 40% and 70% but not 10% of MVC. Decreases in coherence in the alpha frequency band correlated with increases in the iSP area and subjects who showed this inverse relation were able to maintain more steady bilateral muscle contractions. To further examine the relationship between the iSP and coherence we electrically stimulated the ulnar nerve at the wrist at the alpha frequency. Electrical stimulation increased coherence in the alpha frequency band and decreased the iSP area during bilateral 70% of MVC. Altogether, our findings demonstrate an inverse relation between alpha oscillations and the iSP during strong levels of bimanual force generation. We suggest that interactions between neural pathways mediating alpha oscillatory activity and transcortical inhibition between motor cortices might contribute to the steadiness of strong bilateral isometric muscle contractions.
Introduction

Animal studies showed that static bimanual force generation involves activity-dependent adaptations in both primary motor cortices (Murthy and Fetz 1996; Soteropoulos et al. 2011). In agreement, electrophysiological studies in humans using transcranial magnetic stimulation (TMS) demonstrated that isometric bilateral force generation changes the excitability of corticospinal and cortico-cortical projections compared with unilateral force (Sohn et al. 2003; Yedimenko and Perez 2010; Soteropoulos and Perez 2011). Neuroimaging (Theorin and Johansson 2007) and cortex-muscle coherence (Kilner et al. 2003; Perez et al. 2012) studies also revealed that both motor cortices showed distinct changes in activity during bimanual compared with unilateral isometric forces. Although it is well accepted that interhemispheric interactions between motor cortices through the corpus callosum play an important role in the control of bimanual forces (Diedrichsen et al. 2003; Carson 2005; Giovannelli et al. 2009; Tazoe et al. 2013; Perez et al. 2014), the extent to which physiological connections between motor cortices are modulated during increasing levels of bimanual force generation remains poorly understood.

The combination of electroencephalographic (EEG) recordings and physiological circuits tested by TMS has provided a mean to examine activity in overlapping neuronal populations (Paus et al. 2001; Farzan et al. 2013). We tested two well-known measures of interhemispheric connectivity, coherence between EEG signals in sensorimotor cortices (Andrew and Pfurtscheller 1996; Serrien et al. 2003) and the ipsilateral cortical silent period (iSP; Ferbert et al. 1992). EEG-EEG coherence (Pfurtscheller and Lopes da Silva 1999) and the iSP (Boroojerdi et al. 1996) are thought to be mediated by long axons passing through the corpus callosum with a net result coming from coupling between motor cortices and other cortical areas and their interaction with excitatory and inhibitory cortical circuits. EEG-EEG coherence between
sensorimotor cortices in the alpha frequency band and the iSP have been associated with the size and integrity of the corpus callosum (Meyer et al. 1995; Stancak et al. 2002, Teipel et al. 2009; Okumura et al. 2013). Also, studies showed that oscillations between sensorimotor cortices in the alpha frequency band (Svoboda et al. 2002; Abdul-latif et al. 2004) and the iSP (Soteropoulos and Perez 2011; Fling and Seidler 2012) are sensitive to detect changes during strong levels of force generation. Thus, we hypothesized that increasing levels of bilateral isometric forces will change interhemispheric EEG-EEG coherence in the alpha frequency band in association with the iSP. Because alpha oscillatory activity (Haegens et al. 2011; Mehrkanoon et al. 2014) and the iSP (Tazoe and Perez 2013) might play a role in error corrections and in suppressing task-irrelevant activity we also expected that modulation of these physiological interactions will relate to the ability to maintain steady muscle contractions. Evidence showed that interhemispheric interactions between motor cortices can be modulated by somatosensory inputs (Swayne et al. 2006). Therefore, to further examine the relationship between coherence and the iSP during increasing levels of bimanual forces we used electrical stimulation of a peripheral nerve because alpha oscillations (Budini et al. 2014) and interhemispheric inhibition measured by TMS (Tsutsumi et al. 2012) can be modulated by similar afferent inputs.
Methods

Subjects

Sixteen healthy volunteers (8 males, 25.6±1.3 years old, 14 right handed) were included in the study. All subjects gave their informed consent to the experimental procedures, which were approved by the local ethics committee at the University of Pittsburgh. The study was performed according to the guidelines established in the Declaration of Helsinki. Previous studies reported that there is between-subject variance in the magnitude of coherence and iSP measurements (Ushiyama et al. 2011; Mima et al. 2000; Perez et al. 2012, 2014). Consistently, subjects were preselected out of a total of 25 subjects who were screened to ensure that they showed interhemispheric EEG-EEG coherence between sensorimotor cortices at rest and a visible iSP in the first dorsal interosseous (FDI) muscle during unilateral 10\% of maximal voluntary contraction (MVC). This allowed us to measured changes in EEG-EEG coherence and the iSP during our different experimental conditions.

Recordings

Electromyographic (EMG) activity was recorded bilaterally from FDI muscles by surface electrodes (Ag-AgCl; 10 mm diameter) secured to the skin over the belly of each muscle. For measurements of coherence, EEG activity was recorded from sensorimotor cortices bilaterally, using pairs of adhesive electrodes (AMBU Neurolne 720, Wet Gel Snap Electrode) positioned 3 cm lateral and 2 cm anterior or posterior to the vertex (Perez et al. 2012; Fig. 1B). EEG from each side was derived from a differential recording between the electrode pair on that side; the anterior electrode was connected to the non-inverting input of the amplifier. Signals were amplified and filtered (EMG: gain 500 to 2000, bandpass 30 Hz – 2 kHz; EEG: gain 50K, bandpass 3 Hz – 2 kHz). EMG together with force signals were sampled at 1000 Hz, while EEG
was sampled with 5000 Hz (Spike2 and Signal software, CED Ltd.). We examined motor output steadiness by measuring the coefficient of variation of the rectified EMG and force amplitude (Graziadio et al. 2010) in the FDI muscle during unilateral and bilateral 10%, 40% and 70% of MVC. The stability index of EMG (sEMG) and force (sForce) was estimated as follows:

\[
s_{EMG} = 1 - \frac{SD(EMG_{Rectified})}{mean(EMG_{Rectified})}
\]

\[
s_{Force} = 1 - \frac{SD(Force)}{mean(Force)}
\]

**Experimental paradigm**

Subjects were seated with both arms flexed at the elbow by 90° with the forearm pronated and the wrist restrained by straps. The left and right index fingers were attached to custom two-axis load cells, which measured the forces exerted by the subject (Fig. 1A). At the start of the experiment, subjects performed 2-3 brief MVCs (3-5 s) with the index finger into abduction, separated by 60 s. The maximal forces were used to set targets for subsequent submaximal contractions. During maximal contractions subjects were verbally encouraged to perform maximally and visual feedback was provided (Gandevia 2001). All subjects participated in two testing sessions. In one session we assessed EEG-EEG coherence between sensorimotor cortices and in the other session we assessed the iSP at rest and during unilateral and bilateral index finger abduction at 10%, 40% and 70% of MVC in a randomized order (Figs. 1C and D). Custom software was written to acquire signals from load cells to display visual feedback corresponding to 10%, 40% and 70% of MVC in real time (LabView, California). Subjects were instructed to perform unilateral or bilateral forces by controlling one or two cursors on a computer monitor to a target line displaying the force target. Additional verbal feedback was provided to the subjects to ensure that both arms performed the correct task at all times. Note that
unilateral index finger abduction was performed with the non-dominant hand. Four sets were tested with 5 to 10 min intervals. Each set consisted of 10 trials per-condition, each trial lasted 4 s with 15 s of rest between trials. Thus, subjects performed 40 trials in each condition in a randomized order.

**EEG-EEG coherence**

As the iSP measures interhemispheric inhibition with directionality, we examine functional coupling between sensorimotor cortices by using EEG-EEG directed coherence (Kaminski and Blinowska 1991). We measured EEG-EEG directed coherence from the dominant to the non-dominant hemisphere in the alpha (8–13 Hz) and beta (13–30 Hz) frequency band across conditions. EEG activity was recorded from sensorimotor cortices bilaterally, using pairs of adhesive electrodes (AMBU Neurol ine 720, Wet Gel Snap Electrode) positioned 3 cm lateral and 2 cm anterior or posterior to the vertex (Perez et al. 2012; Fig. 1B). These locations correspond to regions between C3 and C4 areas and Cz in the 10-20 system. At rest, EEG-EEG coherence in the alpha frequency band (mean across subjects=0.13±0.01, range across subjects: 0.049 to 0.23) was larger than coherence in the beta frequency band (mean across subjects=0.067±0.005, range across subjects: 0.038 to 0.11; p<0.001). Similarly, normalized resting EEG power in the non-dominant (p<0.01) and dominant (p<0.01) hemisphere was larger in the alpha compared with the beta frequency band. Since individuals performed isometric unilateral and bilateral index finger abduction for 4 s we confined the analysis to the last 2 s of the hold phase of the task (see shaded area in Fig. 2A). The EEG data were also visually inspected to reject trials with eye movements or excessive muscle artifacts and data were down-sampled to 500 Hz. A total of 5.4±4.7 trials in which eye movements or excessive muscle artifacts were detected in EEG recording were excluded from further analysis.
directed coherence was also measured from the dominant to the non-dominant side to make
comparisons across hemispheres as needed.

For EEG-EEG directed coherence (DC) calculation, two non-overlapping 500 time point
segments (corresponding to a time period of 2 s extending back from the end of trial) were taken
from each trial and processed by an autoregressive model (AR) with its order selected by the
Bayesian information criterion (Schwarz 1978). As in Witham et al. (2011), directed coherence
was calculated by using the averaged AR model coefficient and normalized as suggested by
Geweke (1982):

\[
\text{DC}_{d \rightarrow n}(f) = \frac{
\left| H_{nd}(f)H_{nd}^*(f)C_{dd} \right|}{H_{nd}(f)H_{nd}^*C_{dd} + H_{nn}(f)H_{nn}^*(f)C_{nn}}
\]

(2)

Where \( H_{nd} \) and \( H_{nn} \) are the directional transfer function representing the causal
influence of signal \( d \) on signal \( n \) and signal \( n \) on itself respectively, \( C_{kk} \) (i.e., \( k = d \) or \( n \)) is
the covariance of the noise innovations of signal \( k \) in the AR model, and \( \ast \) denotes complex
conjugation. Directed coherence was estimated for each subject in each condition at frequency
bands between 8-13 Hz and 13-30 Hz. The significance level for the directed coherence was
calculated as follow (Baker et al. 2006):

\[
Z = 1 - 0.05^{(1/L^{-1})}
\]

where \( L \) is the total number of non-overlapping sections and the directed coherence was
considered significant (p<0.05) if it was greater than \( Z \). Several bins would be expected to be
above the significance limit by chance. Therefore, the significant effect of the directed coherence
was examined by using the binominal distribution to estimate the minimum number of frequency
bins required to above the significance level (Witham et al. 2011). In this case, the directed
coherence was considered significant if the total number of points above the significance level
was more than 2 and 4 in the alpha and beta frequency bands separately. EEG-EEG coherence
was not present at lower 1-2 Hz (coherence was lower than Z, p<0.05) and higher 500-510 Hz
(coherence was lower than Z, p<0.05) frequency bands. EEG power was normalized to the total
power (summed over the alpha and beta frequencies) obtained at rest. This allowed an estimate
of the proportion of power contributed by a given frequency band; fixing the reference as the rest
condition made changes in power easier to interpret. Mean EEG-EEG directed coherence at each
frequency band at each condition was expressed as % of the coherence measured at rest in each
subject.

TMS

TMS pulses were delivered from a Magstim Rapid\textsuperscript{2} stimulator (Magstim Company)
through a figure-eight coil with its handle pointing backward approximately 45° away from the
midline. During testing the TMS coil was held to the head of the subject with a custom coil
holder with the head secured with straps against a headrest to restrict movements. TMS
measurements included: resting motor threshold (RMT) and the iSP. The RMT was defined as
the minimum intensity which evoked MEPs of at least 50 µV in peak-to-peak amplitude in at
least half of 10 consecutive trials in the relaxed FDI (Rothwell et al. 1999).

iSP

The iSP was measured following a previously standardized method (Trompetto et al.
2004). It was recorded in the non-dominant FDI while stimulating the dominant motor cortex
when subjects performed unilateral and bilateral index finger abduction at 10%, 40%, and 70%
of MVC. At the start of each experiment, the intensity of TMS was adjusted to produce a visible
iSP without a previous facilitation. Based on previous literature, we started the test with
intensities 10% or 20% above the RMT. If the iSP was unclear, the stimulus intensity was increased in small steps until the iSP was present without evoking a short-latency facilitation. TMS was applied at the same intensity in all conditions tested in each subject (118.7±4.3% of RMT) over the dominant motor cortex. iSP onset and offset were defined as the time-point when the EMG dropped below the mean (minimal duration of 10 ms) and the time-point when the EMG returned through this level, respectively. The area of the iSP was calculated using the following formula: \[ \text{iSP area} = (\text{mean EMG}) \times (\text{iSP duration}) – (\text{au}_{\text{iSP}}) \], where mean EMG is the mean amplitude of rectified EMG for 100 ms of prestimulus period, and \text{au}_{\text{iSP}} is the area under the rectified iSP. The iSP area was normalized against the level of contraction \[ \text{iSP area normalized to contraction} = \text{iSP area} / (\text{mean EMG} \times \text{mean duration of iSP}) \]. Mean duration of iSP was obtained from all conditions in all subjects. During each contraction, TMS was delivered three times at 1 s interval to give a total of 30 trials of each condition. During unilateral contractions, the iSP latency (10%=33.5±3.7 ms, 40%=34.6±4.2 ms, and 70%=33.6±3.1 ms; \( p=0.5 \)) and duration (10%=26.9±5.1 ms, 40%=27.4±5.2 ms, and 70%=28.6±6.6 ms; \( p=0.6 \)) were similar across force levels. Whereas the iSP area during unilateral 70% was increased compared with 40% and 10% of MVC (10%=40.8±17.8%, 40%=42.5±12.7%, and 70%=50.0±14.3%; \( p=0.01 \)). In additional experiments \( n=7 \), the iSP was measured in the dominant index finger while stimulating the non-dominant motor cortex (TMS intensity 116.7±15.8% of RMT) when subjects performed unilateral and bilateral index finger abduction at 10%, 40%, and 70% of MVC.

**Effects of peripheral nerve stimulation on EEG-EEG coherence and the iSP**

EEG-EEG coherence between sensorimotor cortices and the iSP in the non-dominant FDI muscle were measured with and without a preceding train of electrical pulses given to the
dominant ulnar nerve at the wrist at 8 Hz and 30 Hz at rest and during bilateral isometric contraction at 70% of MVC (n=10). This force level was chosen since the modulation of coherence in the alpha frequency band and the iSP area was stronger at this force level. The intensity used for electrical stimulation was defined as the minimum intensity needed to evoke a motor response of at least 50 µv in peak-to-peak amplitude in at least 5 of 10 consecutive trials in the relaxed FDI (4.8±1.2 mA). The last electrical pulse was given 25 ms before the TMS pulse given to elicit the iSP. First, we tested the effect of stimulation at 8 Hz and 30 Hz at rest on EEG-EEG coherence in both frequency bands. For this, we applied 8 or 27 pulses in the last sec of each frame in a total of 30 frames (with 240 pulses in total at 8 Hz and 810 pulses in total at 30 Hz). Note that EEG-EEG coherence was measured at intervals between the electrical pulses stimulus artifact. EEG traces were visually inspected and data 5 ms before and after each stimulus artifact was removed from the analysis. A total of 925 ms were extracted when 8 pulses were applied per frame and a total of 735 ms were extracted when 27 pulses were applied per frame. Time points for coherence analysis were matched by analyzing coherence during 735 ms in each frame at each frequency band. Later, the iSP was tested, using the same methodology described above, during bilateral 70% of MVC alone or preceded by a train of electrical pulses at 8 Hz (240 pulses), 30 Hz (810 pulses) in a randomized manner. We also stimulated the ulnar nerve at 8 Hz but increased the number of pulses (8 pulses per frame in 101 frames, total 808 pulses) to match the number of pulses given during 30 Hz.

Statistical analysis

Two-way repeated-measures ANOVAs were performed to determine the effect of FORCE (10%, 40%, 70% of MVC) and CONDITION (unilateral, bilateral) on mean alpha and beta EEG-EEG coherence and mean normalized EEG power. The same analysis was performed
to determine the effect of HAND (dominant, non-dominant) and FORCE on mean rectified EMG activity, sEMG, and sForce using the resting condition in the comparisons as needed. One-way repeated-measures ANOVAs were completed to examine the effect of FORCE on mean alpha and beta EEG-EEG coherence and mean normalized EEG power during unilateral contractions and at each contraction level, and also to examine the effect of FORCE on the onset, duration, and iSP area. The same analysis was used to determine the effect of STIMULATION (8 Hz, 30 Hz, no stimulation) on mean alpha and beta Hz EEG-EEG coherence at rest and the iSP area during bilateral index finger abduction at 70% of MVC. A post hoc Tukey test was used to test for significant comparisons. In addition, two-way repeated-measures ANOVAs were performed to determine the effect of FORCE and SIDE (dominant to non-dominant, non-dominant to dominant) during bilateral trials on alpha and beta Hz EEG-EEG coherence and the iSP. Pearson correlation analysis was used as needed, Bonferroni corrected for multiple comparisons. To further examine the relationship between physiological measures and motor performance, multiple regression analyses were conducted. At each force level, we used changes in sEMG across conditions as the dependent variable and changes in iSP and EEG-EEG coherence across conditions as independent variables. Significant predictions on estimated regression were determined only when variance inflation factors were less than 5. Significance was set at P<0.05 and group data are presented as the mean±SD in the text.

**Results**

**EMG**

Figures 2A and 2B illustrate data from a single representative subject during bilateral index finger voluntary contraction of the FDI muscle. In this subject, the mean rectified EMG
activity increased in the non-dominant (Fig. 2A) and dominant (Fig. 2B) hand while performing 10%, 40%, and 70% of MVC. The gray bars show the region over which the coherence analysis was completed. During unilateral contractions, we found an effect of FORCE (F(2,15)=188.2, p<0.001) on mean rectified FDI EMG activity. Post hoc testing showed that mean rectified EMG activity increased during 40% and 70% compared to 10% of MVC (10%=13.9±6.7, 40%=34.7±11.1, 70%=53.5±11.3; p<0.001). Mean rectified EMG activity was also increased at 70% compared with 40% of MVC (p<0.001). We also found an effect of FORCE (F(2,15)=146.2, p<0.001) but not HAND (F(1,15)=0.3, p=0.6) or in their interaction (F(2,30)=0.9, p=0.4) on mean rectified FDI EMG activity during bilateral contractions (non-dominant hand: 10%=12.9±7.3, 40%=33.9±11.4, 70%=54.5±16.7% of MVC, p<0.001; Fig. 2C and dominant hand: 10%=15.9±7.9, 40%=36.8±11.6, 70%=55.8±12.8% of MVC, p<0.001; Fig. 2D) hand. EMG activity was also larger during 70% compared with 40% of MVC in both hands (p<0.001).

To examine motor output steadiness we measured the sEMG and sForce across conditions. We found an effect of FORCE (F(2,15)=3.4, p=0.04), CONDITION (F(1,15)=13.4, p=0.002) and in their interaction (F(2,30)=3.7, p=0.03) on sEMG during bilateral compared with unilateral contractions. Here, the sEMG decreased during bilateral (10%=0.77±0.006, 40%=0.79±0.006, 70%=0.81±0.005) compared with unilateral (10%=0.77±0.007, 40%=0.76±0.01, 70%=0.78±0.006) contractions at 40% and 70% (p<0.001), but not at 10% of MVC (p=0.2). No changes in sEMG we observed between 70% and 40% of MVC (p=0.3).

Similarly, sForce decreased during bilateral (10%=0.86±0.02, 40%=0.88±0.01, 70%=0.91±0.01) compared with unilateral (10%=0.87±0.012, 40%=0.91±0.01, 70%=0.94±0.01) contractions at 40% (p=0.01) and 70% (p=0.002), but not at 10% of MVC (p=0.6).
Figures 3A-C illustrate the population mean EEG-EEG coherence measured from dominant to non-dominant sensorimotor cortices in all subjects tested. Note that coherence in the alpha frequency band decreased to a larger extent during bilateral compared with unilateral contraction at 40% and 70% of MVC. Whereas coherence in the beta frequency band remained similar during bilateral and unilateral contractions at all force levels. See results for EEG-EEG coherence during unilateral contractions on Table 1.

Repeated-measures ANOVA showed an effect of FORCE ($F_{(2,15)}=12.4,$ $p<0.001$), CONDITION ($F_{(1,15)}=8.4,$ $p=0.01$) and in their interaction ($F_{(2,30)}=5.1,$ $p=0.01$) on normalized EEG-EEG coherence from the dominant to the non-dominant hemisphere in the alpha band. Post hoc testing showed a decrease in coherence during bilateral compared with unilateral contraction at 40% (unilateral=93.8±5.5, bilateral=77.8±6.0, $p<0.001$; Fig. 3B-E) and 70% (unilateral=86.9±3.7, bilateral=64.1±5.1, $p<0.001$; Fig. 3C-F) but not at 10% of MVC (unilateral=97.5±7.1, bilateral=95.6±5.1, $p=0.7$; Fig. 3A-D). We found no differences in EEG-EEG coherence in the alpha band between 70% and 40% of MVC ($p=0.2$). The normalized EEG power decreased to a similar extent during 40% and 70% of MVC ($p<0.01$; Fig. 4A) in the non-dominant hemisphere and also decreased during 70% of MVC ($p<0.01$; Fig. 4C) in the dominant hemisphere. In contrast, we found no effect of FORCE ($F_{(2,15)}=1.8,$ $p=0.1$), TASK ($F_{(1,15)}=1.4,$ $p=0.3$) nor in their interaction ($F_{(2,30)}=0.3,$ $p=0.7$) on normalized EEG-EEG coherence in the beta frequency band (Fig. 3). However, note that EEG-EEG coherence in the beta band decreased to a larger extent during unilateral and bilateral contractions at 40% ($p<0.001$) and 70% ($p<0.001$) compared with 10% of MVC. No changes were observed in the normalized EEG power decreased in the beta frequency band (Fig. 4B-D).
We also examined EEG-EEG coherence from the non-dominant to the dominant hemisphere during the same motor tasks. See results for EEG-EEG coherence in this direction during unilateral contractions on Table 1. Repeated-measures ANOVA showed an effect of FORCE ($F(2,15)=12.7, p<0.001$), not CONDITION ($F(1,15)=1.3, p=0.2$) but in their interaction ($F(2,30)=3.5, p=0.04$) on normalized EEG-EEG coherence in the alpha frequency band. Post hoc testing showed a significant decrease in coherence during bilateral (10%=93.3±5.7, 40%=85.9±5.3, and 70%=69.9±3.0) compared with unilateral (10%=94.5±5.1, 40%=85.9±3.2, and 70%=83.4±2.9) contraction at 70% (p=0.02), but not at 10% (p=0.6) and 40% of MVC (p=0.1). Note that as before, we also found no changes in beta band coherence across force levels ($F(2,15)=1.4, p=0.2$) and conditions ($F(1,15)=3.1, p=0.1$). In addition, we compared directional differences in coherence across hemispheres during increasing levels of MVC in bilateral trials. Repeated-measures ANOVA showed an effect of FORCE (alpha: $F(2,15)=38.2, p<0.001$; beta: $F(2,15)=1.5, p=0.2$), but not SIDE (alpha: $F(1,15)=0.4, p=0.5$; beta: $F(1,15)=0.6, p=0.4$) nor in their interaction (alpha: $F(2,30)=3.5, p=0.2$; beta: $F(2,30)=0.2, p=0.8$) on normalized EEG-EEG coherence in the alpha and beta frequency band, suggesting that the magnitude of coherence from non-dominant to dominant and from dominant to non-dominant sensorimotor cortices was similar across increasing levels of force.

**iSP**

Figure 5A illustrates examples of the iSP, measured from the non-dominant to dominant motor cortex, elicited in the FDI muscle during unilateral and bilateral contractions in a representative participant. Note that the area of the iSP was increased during bilateral compared with unilateral contraction at 40% and 70% of MVC.
Repeated-measures ANOVA showed an effect of FORCE (F(2,15)=13.8, p<0.001), CONDITION (F(1,15)=8.8, p=0.01) and in their interaction (F(2,30)=10.4, p<0.001) on the iSP area (Fig. 5B). Post hoc testing showed that the iSP area increased during bilateral compared with unilateral contraction at 40% (unilateral=42.5±12.7%, bilateral=50.8±16.1%; p<0.001) and 70% (unilateral=50.0±14.3%, bilateral=57.1±16.4%; p<0.001) of MVC. No differences were found in the iSP area between 40% and 70% of MVC (p=0.7). When considering individual subjects, 12 out of 16 showed an increase in the area of the iSP during bilateral compared with unilateral contraction at 70% of MVC and 12 out of 16 subjects showed an increase in the area of the iSP at 40% of MVC. No differences were found in the iSP area during unilateral and bilateral 10% of MVC (p=0.4). When the iSP was tested from the non-dominant to the dominant motor cortex (but now subjects completed the unilateral task with the dominant hand) we found an effect of FORCE (F(2,6)=27.3, p<0.001), CONDITION (F(1,6)=30.5, p=0.001) and in their interaction (F(2,12)=7.2, p=0.009) on iSP area. As before, the iSP area increased during bilateral compared with unilateral contraction at 40% (unilateral=36.5±11.1%, bilateral=41.9±13.2%; p<0.001) and 70% (unilateral=40.3±12.8%, bilateral=46.2±11.9%; p<0.001) of MVC. No differences were found in the iSP area during unilateral and bilateral 10% of MVC (unilateral=35.4±8.4%, bilateral=37.1±10.5%; p=0.2). Furthermore, we found an effect of FORCE (F(2,6)=17.3, p<0.001), but not SIDE (F(1,6)=0.03, p=0.8) nor in their interaction (F(2,12)=1.7, p=0.2) on iSP area.

A negative correlation was found between changes in the iSP and coherence in the alpha band during bilateral compared to unilateral contractions at 40% (r=-0.65, p=0.006; Fig. 6B) and 70% (r=-0.67, p=0.005; Fig. 6C) but not at 10% (r=-0.04, p=0.9; Fig. 6A) of MVC. Also, a positive correlation was found between changes in the iSP area and measures of motor output steadiness at 40% [sEMG: r=0.60, p=0.03 (Fig. 6E); sForce: r=0.61, p=0.03] and 70% (sEMG:
Effects of peripheral nerve stimulation on EEG-EEG coherence and the iSP

Figures 7A illustrates raw traces of EEG signals, the iSP, and MEPs elicited in the FDI muscle during bilateral 70% of MVC in a representative participant. Note that the iSP area decreased during stimulation at 8 Hz compared with 30 Hz and no stimulation.

When tested at rest, repeated-measures ANOVA showed an effect of STIMULATION on the EEG-EEG coherence in the alpha ($F(2,4)=28.6$, $p<0.001$; Fig. 7B) and beta ($F(2,4)=7.8$, $p=0.01$; Fig. 7B) frequency band. We found that stimulation at 8 Hz increased coherence in the alpha band by $30.1\pm10.3\%$ compared to no stimulation without changing coherence in the beta band. Whereas, stimulation at 30 Hz increased coherence in the beta band by $15.4\pm5.9\%$ compared to no stimulation without changing coherence in the alpha band. Repeated-measures ANOVA showed an effect of STIMULATION ($F(2,9)=9.7$, $p<0.001$; Fig. 7C-D) on the iSP area during bilateral 70% of MVC. Here, our results revealed that the iSP area decreased during stimulation at 8 Hz ($8\ Hz=37.4\pm4.7\%$, $30\ Hz=42.5\pm5.5\%$, no stimulation=$43.7\pm7.8\%$, $p=0.002$) but not at 30 Hz compared with no stimulation ($p=0.7$). No effects of the stimulation at 8 Hz and 30 Hz were found on the FDI MEP size on the dominant FDI during bilateral abduction of 70% of MVC compared with no stimulation ($F(2,9)=1.4$, $p=0.3$; Fig. 7E-F; Table 2).
Discussion

Our results demonstrate an inverse relation between alpha oscillations and the iSP during strong levels of bimanual force generation in intact humans. Specifically, we found that EEG-EEG coherence between sensorimotor cortices in the alpha frequency band decreased during bilateral compared to unilateral 40% and 70% but not 10% of MVC. Whereas the iSP area increased during bilateral compared to unilateral 40% and 70% but not 10% of MVC. Notably, decreases in coherence in the alpha band were associated with increases in the iSP area during high force levels and subjects who showed this inverse relation were able to maintain more steady bilateral muscle contractions. Electrical stimulation of the ulnar nerve at the wrist at the alpha frequency increased coherence in the alpha band and decreased the iSP area during 70% of MVC. We propose that inverse interactions between neural pathways mediating alpha oscillatory activity and transcallosal inhibition between motor cortices might contribute to the steadiness of strong bilateral isometric muscle contractions.

Interhemispheric communication during bimanual force generation

It is well accepted that interactions between motor cortices during bimanual force generation take place, at least in part, through the corpus callosum (Diedrichsen et al. 2003; Carson 2005; Giovannelli et al. 2009; Yedimenko and Perez 2010; Soteropoulos and Perez 2011; Tazoe et al. 2013; Perez et al. 2014). Thus, we measured EEG-EEG coherence between sensorimotor cortices to examine interhemispheric communication (Andrew and Pfurtscheller 1996; Serrien et al. 2003; 2004) during increasing levels of bimanual force. Our findings that EEG-EEG coherence decreased during bilateral compared with unilateral force at 40% and 70% of MVC in the alpha but not in the beta frequency band agree with evidence suggesting that interhemispheric interactions at these frequency bands serve distinct functions (Brinkman et al. 2013; Perez et al. 2014).
and are related to separate functional networks (Hari and Salmelin 1997). This also agrees with previous studies showing that coherence between sensorimotor cortices in the alpha band is sensitive to detect changes during strong levels of force generation (Svoboda et al. 2002; Abdulalatif et al. 2004). The decrease in coherence in the alpha but not in the beta band might be related to a lesser synchronization between motor cortical networks during strong voluntary contractions (Kristeva et al. 2007; Perez et al. 2012). This is supported by the decrease in EEG spectral power that we observed in the alpha but not in the beta band in both hemispheres during high force levels. Since the beta rhythm is associated with motor cortical function (Brown 2000) it is intriguing that beta coherence did not change during increasing levels of bilateral compared with unilateral force. However, it is important to consider that most associations of the beta rhythm with motor cortical function has been demonstrated for EEG-EMG coherence (Baker 2007). Indeed, some differences have been reported between EEG-EEG and EEG-EMG coherence at these different frequency bands. For example, alpha and beta EEG oscillations are largely detected from the hand post-Rolandic somatosensory area and the pre-Rolandic motor area, respectively (Pfurtscheller and Lopes da Silva 1999). Whereas EEG-EMG coherence is usually absent in the alpha (Baker et al. 2003) and present in the beta (Baker 2007) frequency band when electrodes are positioned at similar locations. Although the magnitude of beta band coherence was similar during unilateral and bilateral increasing force levels the overall magnitude of beta band coherence decreased during 40% and 70% compared to 10% of MVC. This is consistent with previous evidence showing a progressive reduction in beta band coherence in the sensorimotor cortex contralateral to a hand performing increasing levels of force (Perez et al. 2012) and agrees with evidence showing that EEG-EEG coherence in the beta band change with increasing task demands (Serrien et al. 2004).
We also measured the iSP to examine interhemispheric communication between motor cortices during increasing levels of bimanual force. It is thought that the iSP measures transcallosal inhibition from the stimulated to the contralateral motor cortex (Ferbert et al. 1992; Meyer et al. 1995; Trompetto et al. 2004) and a transcallosal route of the iSP has been supported by an absent or delayed iSP in patients with agenesis or surgical lesions of the corpus callosum (Meyer et al. 1995). We found that the iSP area increased during bilateral compared with unilateral forces at 40% and 70% of MVC. This agree with previous findings showing that the magnitude of iSP increased during bilateral compared with unilateral voluntary contractions (Giovannelli et al. 2009; Yedimenko and Perez 2010; Soteropoulos and Perez 2011; Perez et al. 2014). We also found that the iSP also remained similar during unilateral and bilateral contractions at 10% of MVC in agreement with previous results (Fling and Seidler 2012). Indeed, some studies reported that stronger contractions by one arm are needed to detect differences in the magnitude of the iSP during bilateral forces (Yedimenko and Perez 2010; Soteropoulos and Perez 2011; Perez et al. 2014). Altogether, our results show an inverse modulation of alpha oscillations and transcallosal inhibition between motor cortices during strong levels of bilateral isometric muscle contractions.

An intriguing question is if changes in coherence in the alpha frequency band and the iSP interact, at least to some extent, during strong levels of bilateral isometric force. Several of our results support this possibility. First, we found that changes in the iSP area were negatively correlated with changes in coherence in the alpha band at 40% and 70% of MVC. The correlations found between EEG-EEG coherence in the alpha frequency band and the iSP at stronger levels of force suggest that changes in EEG-EEG coherence reflect changes in cortical interactions. It has been shown that TMS has direct access to the circuitry in the motor cortex.
involved in the generation of oscillations of corticospinal cells (Hansen and Nielsen 2004). Thus, our results as previous findings (Baker and Baker 2003) indicate that inhibitory cortical circuits might have an effect on the modulation of cortical oscillations. Second, we found that both the magnitude of the iSP and EEG-EEG coherence in the alpha band remained similar during 40% and 70% of MVC. Evidence showed that the firing rate of motor cortical cells (Evarts et al. 1983; Maier et al. 1993) and BOLD signal from motor cortex (Dettmers et al. 1995) saturated at high force levels. Thus, a lack of modulation in both measurements at similar levels of force might be in part related to a ceiling effect during bilateral contractions, suggesting that these processes might undergo parallel changes. The lack of changes in EEG-EEG coherence at both frequencies during bilateral compared with unilateral 10% of MVC agrees with previous findings showing no changes in coherence (Andres et al. 1999; Serrien et al. 2004) and oscillatory activity (Murthy and Fetz 1996) between sensorimotor cortices at similar low force levels. The lack of changes in the iSP at this low force level support the view that changes in coherence between sensorimotor cortices possible involve changes in cortical inhibitory circuits. Third, we found that the iSP area measured during bilateral 70% of MVC increased with electrical stimulation at 8 Hz but not at 30 Hz. We used electrical stimulation of afferent fibers as a source modulator since alpha oscillations (Budini et al. 2014) and interhemispheric inhibition measured by TMS (Tsutsumi et al. 2012) are changed by similar peripheral afferent inputs. The fact that the same stimulation paradigm increased alpha coherence and decreased the iSP also supports the view of possible interactions between neural pathways mediating these effects. It is important to consider that coherence between EEG channels spaced less than 10-12 cm apart might contain contributions due to volume conduction (Mima and Hallett 1999; Nunez et al. 1997). Although in our study to decrease this effect, EEG signals were recorded using bipolar electrodes 4 cm
apart (Nunez et al. 1997) and EEG-EEG coherence was absent at lower (1-2 Hz) and higher
(500-510 Hz) frequencies (Winter et al. 2007), we cannot completely exclude the possibility that
changes in volume conduction affected our results.

*Functional significance*

Despite the undoubted importance of callosal pathways in interhemispheric communication, their functional role during bimanual force generation remains largely unknown. A possibility is that strong transcallosal inhibition between motor cortices could represent a mechanism to suppress neural cross-talk (Rokni et al. 2003) in EEG signals when executing simultaneous movements with both hands. Another possibility is that during strong bilateral forces more motoneurones are active and an increase in the iSP could help to prevent unwanted muscle activity to match the desired level of effort. On one hand, studies proposed that changes in oscillations in the alpha frequency band might have a role in error corrections (Mehrkanoon et al. 2014) and suppressing task-irrelevant neuronal processing (Haegens et al. 2011). On the other hand, it has been proposed that changes in interhemispheric inhibition between motor cortices might contribute to suppress unwanted EMG activity (Cincotta and Ziemann 2008) and to suppress task-irrelevant activity during specific types of finger movements (Tazoe and Perez 2013). Thus, it is tempting to speculate that both measurements could be linked in a functional manner since subjects who showed decreases in coherence in the alpha band and increases in the iSP were able to maintain more steady strong muscle contractions. However, caution must be taken in extrapolating these results to bilateral functions since correlations do not imply causality and previous evidence showed that interactions between actively moving arms and those obtained during isometric contractions differ (Carson et al. 1994; Carson 1995). Although some studies found during bimanual actions directional differences in the magnitude of coherence
between sensorimotor cortices (Serrien et al. 2003) others have found some discrepancies (Pollock 2005). Nevertheless, the lack of directional differences in the magnitude of alpha band coherence and the iSP in our study favors the view that these mechanisms might contribute to general aspects of bimanual force generation.


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Figure legends

**Figure 1. Experimental setup.** A, schematic of the experimental setup showing the posture of both hands during testing. B, electrode positions for electroencephalographic (EEG) recordings. C, D: diagrams showing the visual display presented to all subjects during testing unilateral and bilateral isometric index finger abduction. Subjects were instructed to perform 10%, 40%, and 70% of maximal voluntary contraction (MVC) with the index finger into abduction with the non-dominant hand while the contralateral dominant hand remained at rest (C, unilateral trials) or performed 10%, 40%, and 70% of MVC (D, bilateral trials). The colored bars represent the targets to which subjects needed to move a cursor. The distance between the bars represent the magnitude of force required to accomplish on each task, normalized to the index finger abduction MVC determined in each participant.

**Figure 2. Electromyography (EMG) recordings.** In a single representative subject, mean rectified EMG activity in the non-dominant (A) and dominant (B) hand performing bilateral fingers abduction of 10% (red), 40% (green) and 70% (blue) of MVC is shown. The gray bars mark the region over which coherence analysis was performed. C and D: group data (n=16) showing mean rectified EMG activity (expressed as a % of MVC) during bilateral index finger abduction in the non-dominant hand (C) and the dominant hand (D). Error bars indicate SEs; *p<0.05.

**Figure 3. EEG-EEG coherence.** A, Traces show EEG-EEG coherence from the dominant to the non-dominant motor cortex averaged across 16 subjects performing unilateral (Uni.) and bilateral (Bil.) index finger abduction at 10% (A), 40% (B) and 70% (C) of MVC. Graphs show the normalized mean EEG-EEG coherence in all conditions tested. The abscissa shows the
frequency band tested (alpha=8-13 Hz and beta=13-30 Hz). The ordinate show the normalized mean EEG-EEG coherence expressed as a % of mean EEG-EEG coherence at each frequency band tested at rest. Error bars indicate SEs; *p<0.05.

Figure 4. EEG power. Graphs show the normalized mean EEG-EEG power in the left (non-dom., A-C) and dominant (dom., B-D) hemisphere in all conditions tested in the alpha (8-13 Hz, left column graphs) and beta (13-30 Hz, right column graphs) frequency band. The abscissa shows the MVC tested (10%, 40%, and 70% of MVC). The ordinate shows the normalized mean EEG-EEG power expressed as a % of mean EEG-EEG power at each frequency band tested rest. The broken horizontal black line shows the EEG power at each frequency band at rest. Error bars indicate SEs; *p<0.05.

Figure 5. Ipsilateral cortical silent period (iSP). A, Traces show the rectified EMG activity in a representative subject during iSP testing during the unilateral (Uni., gray traces) and bilateral (Bil., black traces) index finger abduction at 10%, 40%, and 70% of MVC. Each trace shows the average of 30 trials. The broken horizontal black lines shows the mean EMG activity over 100 ms before TMS. The vertical broken black lines shows the time of TMS during testing. B, The graph shows the iSP group data (n=16) during unilateral and bilateral contractions. The abscissa shows the force levels tested (10%, 40% and 70% of MVC). The ordinate show the normalized iSP area. Error bars indicate SEs; *p<0.05.

Figure 6. Correlation between EEG-EEG coherence, iSP, and sEMG. Graphs show a correlation analysis between changes in EEG-EEG coherence in the alpha frequency band and the iSP area (left column) and between changes in sEMG signals and the iSP area (right column) during bilateral compared with unilateral contractions at 10% (A, D), 40% (B, E), and 70% (C,
F) of MVC. In all graphs the abscissa shows normalized iSP area (difference in the iSP area during bilateral versus unilateral contractions). The ordinate shows the normalized EEG-EEG coherence in the alpha frequency band (difference in the EEG-EEG coherence during bilateral versus unilateral contractions, A-C) and the normalized sEMG (difference in the sEMG during bilateral versus unilateral contractions, D-F). Error bars indicate SEs. *p< 0.05.

Figure 7. Effects of peripheral nerve stimulation on EEG-EEG coherence and the iSP.

Traces show EEG signals at rest (A) and rectified EMG activity during iSP testing (C) and motor evoked potentials (MEPs) in the FDI muscle (E) in a representative subject tested during bilateral index finger abduction at 70% of MVC without electrical stimulation (No stim., black traces), with peripheral nerve stimulation of the ulnar nerve at the wrist at 8 Hz (blue traces) and 30 Hz (gray traces). The broken horizontal black line (C) shows the mean EMG activity over 100 ms before TMS. Graphs show the group data (n=10). The abscissa shows all conditions tested (No stim., 8 Hz, and 30 Hz). The ordinate shows the normalized EEG-EEG coherence from the dominant to the non-dominant motor cortex in the alpha and beta frequency band (B), the normalized iSP area (D) and the size of MEPs elicited in the dominant hand during iSP testing (F). Note that at rest electrical stimulation at 8 Hz increased EEG-EEG coherence in the alpha frequency band whereas stimulation at 30 Hz increased EEG-EEG coherence in the beta frequency band. Also note, that during 70% of MVC stimulation at 8 Hz decreased the iSP area while MEP size elicited by the TMS stimulation were maintained constant across conditions. Error bars indicate SEs; *p<0.05.
Figure 1

A. Non-dominant hand and Dominant hand

B. Diagram showing electrode placement with labels 2 cm, 3 cm, and Gz.

C. UNILATERAL TASK:
- 70% Rest
- 40% Rest
- 10% Rest

D. BILATERAL TASK:
- 70% Rest
- 40% Rest
- 10% Rest
Figure 2

A 10% of MVC

40% of MVC

70% of MVC

Time relative to end holds (s)

B 10% of MVC

40% of MVC

70% of MVC

Time relative to end holds (s)

C

EMG (% of MVC)

100

80

60

40

20

0

10% 40% 70%

Contraction strength (% of MVC)

D

EMG (% of MVC)

100

80

60

40

20

0

10% 40% 70%

Contraction strength (% of MVC)
Figure 3

A

B

C

D

E

F
Figure 4

A. 8-13 Hz

B. 13-30 Hz

C. 8-13 Hz

D. 13-30 Hz

Norm. non-domin. EEG power

Contraction strength (% of MVC)

10% 40% 70%
Figure 5

A

10% of MVC

Uni. contraction

Bil. contraction

Mean EMG

40% of MVC

TMS

20 ms

0.1 mV

70% of MVC

B

Normalized SP area (%)

10% 40% 70%

Contraction strength (% of MVC)
Figure 6

A. 10% of MVC

B. 40% of MVC

C. 70% of MVC

D. 10% of MVC

E. 40% of MVC

F. 70% of MVC

Coherence change (%) vs. iSP area change (%)

- A: r = -0.04, p = 0.89
- B: r = -0.65, p = 0.006
- C: r = -0.67, p = 0.005
- D: r = 0.47, p = 0.19
- E: r = 0.60, p = 0.03
- F: r = 0.61, p = 0.03
Table 1

Table 1. Coherence and power during unilateral contractions (mean±SD)

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<th></th>
<th>Rest</th>
<th>10%</th>
<th>40%</th>
<th>70%</th>
<th>( P )</th>
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<td><strong>Coherence</strong></td>
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<td>8-13 Hz</td>
<td>0.13±0.01</td>
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<tr>
<td>13-30 Hz</td>
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<td>0.061±0.004</td>
<td>0.057±0.004</td>
<td>0.056±0.004</td>
<td>&lt;0.001</td>
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| **Power in**   |          |          |          |          |          |
| **dominant M1**|          |          |          |          |          |
| 8-13 Hz        | 12.4±0.76| 11.2±0.74| 10.9±0.83| 10.1±0.79| <0.001   |
| 13-30 Hz       | 1.45±0.29| 1.21±0.24| 1.04±0.17| 1.02±0.18| <0.001   |

| **Power in**   |          |          |          |          |          |
| **non-dominant M1** |      |          |          |          |          |
| 8-13 Hz        | 12.2±0.75| 11.4±0.84| 11.1±0.88| 10.5±0.81| <0.001   |
| 13-30 Hz       | 1.31±0.25| 1.11±0.11| 0.96±0.13| 0.94±0.14| <0.001   |
Table 2

<table>
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<th>P</th>
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<td>Onset (ms)</td>
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<td>Duration (ms)</td>
<td>25.4±5.2</td>
<td>26.8±6.8</td>
<td>27.5±5.4</td>
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<td>MEP (mV)</td>
<td>6.5±1.9</td>
<td>6.1±1.8</td>
<td>6.2±2.3</td>
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<td>Norm. area (%)</td>
<td>43.7±7.8</td>
<td>37.4±4.7</td>
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