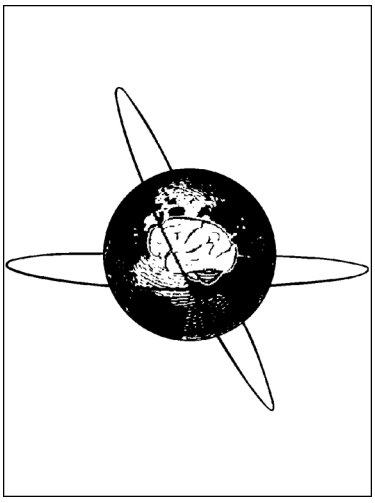

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Novel TMS-EEG indexes to investigate interhemispheric dynamics in humans

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Running title: Interhemispheric TMS-EEG indexes

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Abstract

Objective: To validate two indexes of interhemispheric signal propagation (ISP) and balance (IHB) by combining transcranial magnetic stimulation (TMS) and electroencephalography (EEG).

Methods: We used TMS-EEG to non-invasively stimulate the two hemispheres of 50 healthy volunteers and measured interhemispheric dynamics in terms of ISP and IHB. We repeated our evaluation after three weeks to assess the reliability of our indexes. We also tested whether our TMS-EEG measures were correlated with traditional interhemispheric inhibition (IHI), as measured with motor-evoked potentials (MEPs).

Results: Our main results showed that ISP and IHB (1) have a high reproducibility among all the participants tested; (2) have a high test-retest reliability (3) are linearly correlated with IHI, as measured with MEPs.

Conclusions: The main contribution of this study lies in the proposal of new TMS-EEG cortical measures of interhemispheric dynamics and in their validation in terms of intra- and inter-subject reliability. We also provide the first demonstration of the correlation between ISP and IHI.

Significance: Our results are relevant for the investigation of interhemispheric dynamics in clinical populations where MEPs are not reliable.

Key words: Interhemispheric balance, interhemispheric inhibition, TMS, EEG

Highlights:

- We investigated interhemispheric dynamics by using TMS-EEG in 50 healthy volunteers
- TMS-EEG indexes showed a high inter- and intra-subject reliability when re-tested after 3 weeks
- Our indexes allow investigation interhemispheric dynamics in populations with not reliable MEPs

1. Introduction

In recent years, the investigation of interhemispheric interactions has grown given their crucial role in a number of motor and cognitive functions (Schulte and Müller-Oehring, 2010). In particular, the role of interhemispheric inhibition (IHI) and facilitation (IHF) is fundamental in the production of voluntary unimanual movements (Mayston et al., 1999) but also in situations of semantic (Schulte et al., 2006) and visuospatial competition (Corbetta et al., 2005). In humans, interhemispheric interactions have been investigated in vivo with motor-evoked potentials (MEPs) by non-invasively stimulating the two primary motor cortices (M1) with transcranial magnetic stimulation (TMS). This approach consists in delivering a conditioning stimulus (CS) over one M1 some milliseconds before a test stimulus over the contralateral M1. If there is an influence of the CS over the MEP amplitude evoked by the TS, it can be concluded that the two sites are connected (Rothwell, 2010). The first TMS study using this approach to investigate IHI was conducted by Ferbert and colleagues (1992) and demonstrated that a MEP is inhibited by a pulse applied to the contralateral M1 about 7-13 ms before. This phenomenon is likely produced by the activation of transcallosal outputs from the CS, given that no IHI is observed in patients with no corpus callosum (Meyer et al., 1995). Depending on the interval and the intensity of stimulation, also facilitatory effects can be observed (Hanajima et al., 2001), although most studies report an inhibitory effects, which is likely mediated by at least one inhibitory interneuron in the cortex stimulated with TS (Rothwell et al., 2010). Throughout the years, IHI protocol has been extensively used both in healthy volunteers (e.g. Ridding et al., 2000; Daskalakis et al., 2002) and in patients with neurological disorders (e.g. Shimizu et al., 2002; Duque et al., 2005; Bütefisch et al., 2008). For instance, patients with unilateral cortical stroke showed no IHI in the unaffected hand muscles after TMS of the affected M1, whereas patients with a subcortical stroke caudal to the corpus callosum showed only partial inhibition (Shimizu et al., 2002). In chronic stroke patients, when MEP were obtained during or just before a voluntary movement of the paretic hand, IHI was stronger over the contralateral affected M1, compared to when it was tested in the unaffected M1, contralateral to the non-paretic hand (Duque et al., 2005). Finally, when tested at rest, IHI seems to be abnormally decreased from the affected on the unaffected M1, whereas it is normal from the unaffected to the affected M1 (Bütefisch et al., 2008). Despite the extensive use of this protocol, there is a large variability in the results, due to a number of factors. First, MEPs are not easily evocable in patients with damage of the corticospinal tract, e.g. stroke, motor neuron disease and multiple sclerosis. Second, IHI assessed by paired-pulse TMS shows high intra- and inter-subject variability (De Gennaro et al., 2003). Additionally, MEPs show considerable inter-trial variability mostly due to constant fluctuations in the excitability of corticospinal neurons (Kiers et al., 1993; Darlin et al., 2006). An

additional potential source of bias is that MEPs reflect excitability of the whole corticospinal tract, which can be influenced not only by the excitability of the cortex, but also of the spinal cord (Rösler et al., 2008). On these premises, there is the need of new TMS measures that (1) directly reflect cortical excitability and (2) show a high intra and inter-subject reliability.

In the present study, we combined TMS and electroencephalography (EEG) to directly record cortical activity induced by TMS. Previous studies already used TMS-EEG to investigate interhemispheric dynamics by measuring the propagation of TMS-evoked activity from the stimulated hemisphere to the contralateral one, a measure termed interhemispheric signal propagation (ISP) (Voineskos et al., 2010; Jarczok et al., 2016; Määttä et al., 2017). In their study, Voineskos and colleagues (2010) found an inverse relationship between ISP and microstructural integrity of callosal microfibers, confirming that this measure is mediated by callosal projections. In two recent studies, ISP has been investigated in relation to age both in healthy volunteers (Määttä et al., 2017) and in children with autism spectrum disorder (Jarczok et al., 2016). However, the physiological mechanism underlying this measure remains speculative. In addition, ISP has been previously measured only from one hemisphere; thus, whether this measure could provide some information about interhemispheric balance is still unknown. Finally, there is a lack of evidence of its reliability and sensitivity. In the present study, our objective was to find reliable and sensitive measures of interhemispheric dynamics in terms of transmission and balance. To this aim, we applied TMS-EEG over M1 of the left (LH) or right hemisphere (RH) of a large sample of healthy volunteers (50) and assessed the propagation from the stimulated hemisphere to the contralateral one. To assess inter-session reliability of our measures, we repeated the evaluation of a subset of participants (33) after three weeks. Additionally, to investigate whether our cortical TMS-EEG measures were related to corticospinal TMS-EMG measure, we measured IHI with MEPs and investigated correlations between the different measures. Finally, as an exploratory analysis, we tested whether there were differences related to age.

2. Methods

2.1 Ethical approval

Fifty healthy volunteers (29 females; 2 left-handed; 37.5±18.6 years) were enrolled for the study. All participants had to sign a written informed consent. Handedness was assessed with the Edinburgh Handedness Inventory Test (Oldfield, 1971). Only participants not presenting TMS exclusion criteria were recruited (Rossi et al., 2009). The experimental procedure was approved by the Local Ethical Committee and performed in accordance with the Declaration of Helsinki (Sixth revision, 2008).

2.2 Procedure

Each participant underwent a TMS-EEG session to evaluate interhemispheric propagation; 33 participants repeated the TMS-EEG session after three weeks. A subset of participants (17) underwent an additional TMS-EMG session to evaluate IHI with MEPs, using a paired-pulse TMS protocol (see below). During the experiment, participants were seated on an armchair in front of a PC screen at 80 cm of distance. They were asked to keep their arms in a relaxed position and to fixate on a white cross (6×6 cm) to limit eye movements. To avoid possible auditory ERP responses related to the TMS click, participants wore in-ear plugs that played a masking noise reproducing the specific time-varying frequencies of the TMS click (Massimini et al., 2005). For each participant, we adjusted the intensity of the white noise by increasing the volume (always below 90 dB) until s/he could no longer hear the click (Paus et al., 2001).

2.3 TMS-EEG session

Analysis of interhemispheric signal propagation (ISP) and balance (IHB) was performed with TMS-EEG. For the TMS-EEG session, we used a Magstim R^2 stimulator with a 70 mm figure-of-eight coil (Magstim Company Limited, Whitland, UK), able to produce a biphasic waveform with a pulse width of ~0.1 ms. Coil positioning was the same used for corticospinal evaluation. Intensity of stimulation was set at 90% of the RMT, this was defined as the lowest TMS intensity able to evoke at least five out of ten MEPs with a >50 μV peak-to-peak amplitude in the relaxed contralateral FDI (Rossini et al., 1994). Throughout the entire experiment, EMG was constantly monitored to ensure that participants were relaxed and that not MEPs were evoked during the TMS-EEG sessions. Each session consisted of two blocks of 120 TMS single-pulses applied at a random ISI of 1.8-2.2 s applied over FDI hotspot of the LH and RH. The order of stimulation of the two hemispheres was counterbalanced across patients. EEG activity was recorded using a TMS-compatible DC amplifier (BrainAmp, BrainProducts GmbH, Munich, Germany) with 64 TMS-compatible Ag/AgCl pellet

electrodes positioned according to the 10-20 International System. The reference was positioned on the nose tip, the ground on AFz electrode. Skin/electrode impedance was kept under 5 kΩ. Sampling rate of EEG recordings was 5 kHz.

To off line analyse TMS-EEG data we used Brain Vision Analyzer (Brain Products GmbH, Munich, Germany) and EEGLAB toolbox running in a MATLAB environment (MathWorks Inc., Natick, USA). As a first step, data were segmented into epochs from 1 s before TMS to 1 s after it. Then, we removed and replaced data, using a cubic interpolation, from 1 ms before to 10 ms after the TMS pulse from each trial. Afterwards, data were downsampled to 1000 Hz and band-pass filtered between 1 and 80 Hz (Butterworth zero phase filters). To reduce noise from electrical sources we applied a 50-Hz notch filter. Prior to analysis, all the epochs were visually inspected and those with excessively noisy EEG were excluded (resulting in less than 5% for each dataset). Basing on previously established criteria (Casula et al., 2017), we identify and remove components reflecting muscle activity, eye movements, blink-related activity, and residual TMS-related artifacts by means of independent component analysis (INFOMAX-ICA). Finally, the signal was re-referenced to the average signal of all the electrodes. For the two left-handed participants, we collapsed data from the dominant RH on the left one and vice versa, so that the LH was considered dominant for the entire sample.

TMS-evoked activity was analyzed in the temporal, spatial and oscillatory domain. First, we rectified the TMS-evoked activity recorded over three electrodes surrounding the two M1s, i.e. C3, CP3, CP5 for the left M1 and C4, CP4, CP6 for the right M1. These electrodes were chosen basing on previous TMS-EEG studies assessing M1 local excitability (e.g. Jarczok et al., 2016; Casula et al., 2016; 2018; Määttä et al., 2017). We then averaged the amplitude of the rectified TMS-evoked activity from 20 to 150 ms after the TMS pulse for the stimulated M1 and from 30 to 160 ms for the M1 contralateral to the stimulation. These time windows were chosen based on (1) the mean duration of the GABA-receptor-mediated inhibitory neurotransmission, i.e. ~150 ms (Fitzgerald et al., 2009; Voineskos et al., 2010; Jarczok et al., 2016; Määttä et al., 2017; Casula et al., 2018) and (2) on the transcallosal interhemispheric latency, i.e. ~10 ms (Ferbert et al., 1992; Jarczok et al., 2016). Finally, we computed the ISP both from the LH (ISP_{LH}) and from the RH (ISP_{RH}) with the following formula:

$$ISP = \frac{TMS\ evoked\ activity\ (non-stimulated\ M1)}{TMS\ evoked\ activity\ (stimulated\ M1)}$$

To assess the ISP balance between the two hemispheres, we computed the IHB as follows:

$$IHB = \frac{ISP_{LH}}{ISP_{RH}}$$

To evaluate the TMS-evoked response in terms of cortical oscillations, we performed a time-frequency decomposition based on a complex Morlet wavelet (cycles=3.5), then we computed the TMS-related spectral perturbation (Delorme and Makeig, 2004; Casula et al., 2016), over the left and right M1 cluster of electrodes, in the theta (4-7 Hz), alpha (8-13 Hz), beta (14-30 Hz) and gamma (31-45 Hz) frequency.

2.4 TMS-EMG session

Analysis of interhemispheric inhibition (IHI) was performed with TMS-EMG. We used a Magstim 200 stimulator with a 70 mm figure-of-eight coil (Magstim Company Limited, Whitland, UK), which produces a monophasic pulse of ~80 μs length. Coil positioning was functionally defined as the M1 spot in which TMS evoked the largest MEPs in the contralateral relaxed FDI muscle. The coil was oriented tangentially to the scalp at about 45° angle away from the midline, thus inducing a posterior-anterior current in the brain. The intensity of stimulation for single-pulse TMS was adjusted to evoke an MEP of ~1mV peak-to-peak amplitude. Paired-pulse TMS was carried out with two Magstim 200 stimulators connected by a Bistim module and two 70 mm figure-of-eight coils. To test interhemispheric inhibition (IHI), we delivered a conditioning stimulus (CS) at 1 mV MEP intensity over one M1, which preceded a test stimulus (TS) delivered at 1 mV MEP intensity over the contralateral M1 by 10 ms. Ten TMS paired pulses were delivered for each M1 (Ferbert et al., 1992). IHI was then computed by peak-to-peak MEP amplitude as follows:

$$IHI = \frac{MEP_{conditioned}}{MEP_{test}}$$

MEPs were recorded from the FDI muscle contralateral to TMS by using 9-mm-diameter Ag–AgCl surface cup electrodes. The active and reference electrodes were placed over the belly muscle and over the metacarpophalangeal joint of the index finger, respectively. Responses were filtered at 5 Hz and 2 kHz with a sampling rate of 5 kHz and amplified using a Digitimer D360 amplifier. EMG recordings were performed with SIGNAL software (Cambridge Electronic Devices).

2.5 Statistics

All data were analyzed using SPSS statistics (SPSS Inc., Chicago, USA). Prior to undergoing ANCOVA procedures, we assessed normal distribution of neurophysiological data with Shapiro-Wilks' test. Significance level was set at $\alpha=0.05$. To test for data sphericity we used Mauchly's test; when sphericity was violated (i.e. Mauchly's test < 0.05), we used the Huynh–Feldt ε correction. Pairwise comparisons were corrected by the Bonferroni method.

TMS-evoked cortical activity was analyzed by means of a two-way ANCOVA with within-subject factors “stimulation” (left, right) and “hemisphere” (stimulated vs. contralateral). RMT, IHI and ISP were separately analyzed by means of one-way ANCOVAs with a within-subject factor “stimulation”. To test for age-related differences, all the ANCOVAs were also performed with “age” as a covariance. For the same reason, we tested linear relationship between IHB and age by means of Pearson’s coefficient. All the results were reported distinguishing a “young” group, in which participants have ≤35 years (36 participants; 19 females; mean age 26±3 years; range 22-25 years) and an “adult” group, in which participants have >45 years (14 participants; 10 females; mean age 64±13 years; range 45-65). Test-retest reliability of ISP and IHB was assessed by means of intra-class correlation coefficient (ICC). In order to investigate linear relationships between cortical, i.e. ISP and IHB, and corticospinal measures, i.e. IHI, we used Pearson's coefficient since we found that data were normally distributed.

3. Results

The entire procedure was well tolerated and no significant side effects were reported. Three participants (younger) were excluded due to excessive EEG artefacts. Analysis of RMT showed a significant main effect of stimulation [$F(1,46)=9.975$; $p=0.003$; $\varepsilon=.178$] revealing that the RMT of the left dominant hemisphere was significantly lower compared to the non-dominant right one (66.82±0.23 *vs.* 68.74±0.24) with no difference related to the two groups ($p>0.05$). By including age as a covariate in our general linear model, the main effect of stimulation remained significant ($p=0.007$).

Figure 1 depicts the local and global cortical response following stimulation of M1 in healthy younger volunteers. Analysis of local M1 TMS-evoked activity (figure 1A) revealed a sustained cortical response lasting ≈250 ms, with a maximum activation at ≈100-150 ms; the same temporal dynamic was observable in the oscillatory domain with a maximum activation at ≈100-150 ms in the alpha frequency. Pattern of activation was similar, in terms of waveform and amplitude, between the stimulations of two hemispheres, with a strong reduction of activity in the hemisphere contralateral to the stimulation. Analysis of global TMS-evoked cortical activity (figure 1B) revealed a well-known sequence of positive and negative deflections lasting ≈250 ms, as usually observed after M1 stimulation (Casula et al., 2016; 2018a; 2018b). A first activation was focused over the stimulated M1 (20-40 ms) with an immediate spread over ipsilateral posterior areas and frontal areas (100 ms). At 150 ms, we observed a prominent bilateral distribution over both the hemispheres. This pattern was observable in a similar way in the two hemispheres. Figure 2 depicted the TMS-evoked activity in the two hemispheres (stimulated and contralateral) for each participant. In the young group, approximately 80% of the participants showed an inhibition of TMS-evoked activity in the hemisphere contralateral to the stimulation: 26 out to 33 when stimulating LH (3.06±0.33 μV *vs.* 1.99±0.2 μV); 32 out to 33 when stimulating RH (3.02±0.36 μV *vs.* 1.94±0.27 μV). The adult group showed the same trend with more than 85% of participants showing an inhibition of TMS-evoked activity in the hemisphere contralateral to the stimulation: 12 out to 14 when stimulating LH (2.79±0.33 μV *vs.* 1.18±0.1 μV) and RH (2.32±0.29 μV *vs.* 1.06±0.11 μV). The analysis of TMS-evoked activity revealed a significant stimulus×hemisphere interaction [$F(1,46)=78.134$; $p<0.001$; $\varepsilon=.629$]. By including age as a covariate in our general linear model, the stimulus×hemisphere interaction remained significant ($p=0.004$). Post-hoc analysis comparing the two hemispheres showed that TMS-evoked activity was inhibited in the hemisphere contralateral to the stimulation, both when stimulating LH (2.98±0.25 μV *vs.* 1.75±0.15 μV; $p<0.001$) and RH (2.81±0.27 μV *vs.* 1.68±0.2 μV; $p<0.001$). Figure 3 (panel A) shows ISP for the entire sample and separately for young and adult after LH and RH stimulation. We observed a

consistent inhibition, i.e. ISP<1, both after LH stimulation (total: 0.68±0.05; young: 0.76±0.05; adult: 0.49±0.07) and RH stimulation (total: 0.67±0.05; young: 0.70±0.05; adult: 0.59±0.10). The analysis of ISP did not reveal any significant differences between the two hemispheres [$F(1,46)=0.43$; $p=0.836$; $\varepsilon=.001$], the results did not change by adding age as a covariate ($p=0.163$). Figure 3 (panel B) showed IHB for the entire sample (1.17±0.09) and for the two groups (young: 1.17±0.08; adult: 1.18±0.23). The analysis of the linear relationship between IHB and age did not reveal any significant correlation ($r=.016$; $p=0.913$). Figure 3 (panel C) shows IHI from the two hemispheres, we observed a consistent inhibition when tested from the left hemisphere (48.54±18.04) and for 14 participants out to 17 when tested from the right hemisphere (60.94±32.22). Analysis of IHI reveal no difference related to the side of stimulation [$F(1,45)=3.233$; $p=0.091$; $\varepsilon=.168$].

Analysis of test-retest reliability revealed a high reliability for IHB (0.82; $p<0.001$), ISP_{LH} (0.76; $p<0.001$) and ISP_{RH} (0.72; $p<0.001$). Analysis of linear relationship between cortical (ISP) and corticospinal (IHI) measures showed significant positive correlations both when inhibition was tested from LH ($r=.558$; $p=0.010$; figure 3D) and from RH ($r=.432$; $p=0.042$; figure 3E).

4. Discussion

In the present manuscript, we provide the first detailed characterization of novel TMS-EEG indexes of interhemispheric dynamics, in terms of reliability and specificity. To this aim, we tested two different TMS-EEG measures, i.e. ISP and IHB, in a large sample of healthy volunteers including both young and adult people to test possible age-related differences; we repeated our evaluation after three weeks and we tested whether our TMS-EEG indexes correlated with traditional TMS-EMG measures. Our main results showed that ISP and IHB (1) showed a highly consistent trend among the 50 participants tested, i.e. low inter-subject variability; (2) had a high test-retest reliability, i.e. low intra-subject variability; (3) showed a positive correlation with IHI, as measured with TMS-EMG.

To test interhemispheric transmission, we first computed the TMS-evoked activity over the stimulated hemisphere and over the contralateral one. We found that ≈85% of the entire sample showed a consistent pattern of inhibition, i.e. less activity over the non-stimulated hemisphere. This effect was highly reproducible among young and adult participants with no differences related to age. Previous studies found a difference in TMS-evoked EEG response in relation to maturation of motor system (Jarczok et al., 2016; Määttä et al., 2017). In their studies, Määttä and colleagues (2017) and Jarczok and coworkers (2016) found substantial differences, both in cortical excitability and in interhemispheric transmission, between children, adolescences and adults. In our study, we did not find any age-related differences; this is probably due to the fact that the age range of our sample was restricted to young (between 22 and 35 years) and adults (between 45 and 65 years). Thus, we can conclude that the loss of neurones and white matter fibers due to age between 22 and 65 years do not produce relevant changes in cortical excitability nor in interhemispheric transmission. When tested with MEPs, ≈80% of participants showed a consistent inhibition, i.e. conditioned MEPs were lower in amplitude, with no differences related to the side of stimulation. To further characterize the interhemispheric transmission, we computed the ISP, which is the percentage of activity that propagates from the stimulated hemisphere to the contralateral one. We found a consistent reduction of contralateral TMS-evoked activity, i.e. ISP<1, in both young and adult volunteers with no differences related to the side of stimulation. Previous studies suggested that ISP reflects the transcallosal interhemispheric transmission given that it correlates with the fractional anisotropy of the corpus callosum in healthy adults (Voineskos et al., 2010). Although this study suggested a relation between ISP and IHI, no one previously investigated whether the suppression of TMS-evoked cortical and corticospinal activity (i.e. MEPs) were correlated. In our study, 17 participants were tested with the traditional IHI protocol with two coils positioned over the two motor cortices. The two coils delivered two pulses, i.e. conditioning and test, at an ISI of 10

ms, which was the same interval used for the ISP computation. Notably, this interval was chosen being an optimal interval for a prominent inhibition (Ferbert et al., 1992), which has been previously used in TMS-EEG studies computing ISP (e.g. Voineskos et al., 2010; Jarczok et al., 2016; Määttä et al., 2017). Our IHI protocol showed that both the hemispheres significantly produced an inhibition of MEPs evoked from the contralateral hemisphere, as expected. More importantly, we found that ISP was significantly correlated with IHI from both sides, i.e. participants who showed a higher inhibition of MEP amplitude also showed less interhemispheric propagation of TMS-evoked activity. The relation between corticospinal and cortical TMS-evoked measures has not been fully elucidated so far. Previous works reported a positive correlation between the amplitude of MEPs and TEP peaks (e.g. Paus et al., 2001; Huber et al., 2008); however, most of the studies in TMS-EEG literature did not find any significant correlations between the two measures (e.g. Bender et al., 2005; Bonato et al., 2006; Pellicciari et al., 2013; Casula et al., 2014; Rocchi et al., 2018). The absence of strong correlations has been explained with the different physiological origin of MEPs and TEPs. Indeed, MEPs reflect the excitability of the pyramidal tract, which is affected by a combination of spinal, cortical and subcortical mechanisms (Rossini et al., 1994); whereas TEPs results from the activation of inhibitory and excitatory post-synaptic potentials (Ilmoniemi et al., 1997). However, when MEPs and TEPs are analyzed as IHI and ISP respectively, seem to reflect a similar interhemispheric dynamic. From a physiological point of view, interhemispheric inhibition is known to be mediated through callosal fibers. Animal studies showed that callosal projections act on GABAergic interneurons, which are known to be responsible for IHI (Daskalakis et al., 2002; Chen et al., 2004; Irlbacher et al., 2007). Specifically, GABAa- and GABAb-ergic interneurons seem to be responsible for the early and later IHI, although in humans the mechanism has not completely clarified (Irlbacher et al., 2007; Müller-Dahlhaus et al., 2008). Our results suggest that ISP reflects, at least to some extent, the transcallosal-mediated interhemispheric inhibition, which so far has been only measured with indirect corticospinal indexes, i.e. MEPs. From a clinical point of view, this result is particularly relevant considering that ISP can be computed even in populations where MEPs are not reliable or not easily evocable, as we recently observed in stroke patients (Koch et al., 2018).

To test the balance between the two hemispheres, i.e. the difference on the amount of interhemispheric transmission from the two hemispheres, we computed IHB. This measure offers a novel and direct measure of the balance between the two hemispheres and, to our knowledge, has never been used before. In the present study, we found the same IHB value for adult volunteers (1.18) and a very similar IHB for the young group (1.17), although they showed a lower variability. Such difference can be ascribed to a more efficient inhibitory mechanism in younger people, as

demonstrated in previous studies using a motor task (e.g. Talelli et al., 2008). On the other hand, in line with our results, there is no evidence of age-related differences in interhemispheric inhibitory mechanism when tested at rest (Hinder et al., 2012). Finally, to ensure the reliability of our measures we tested their repeatability after three weeks from the first evaluation. Both ISP and IHB showed a high reproducibility as assessed from ICC (Brown et al., 2017), a result that supports their use for clinical and research purposes, especially in light of the high variability usually observed with MEPs.

There are some limitations in the present study. First, the different stimulation paradigms, i.e. single-pulse for ISP and paired-pulse for IHI, made the two measures not directly comparable. This could account for the weak (0.432), but still significant (0.042), correlation we found between the two measures when tested from the non-dominant hemisphere, whereas this correlation was stronger (0.558) and highly significant (0.01) when tested from the dominant hemisphere. This result is in line with previous studies that found higher RMT and MEP variability when tested from the non-dominant hemisphere. In addition, it might be possible that suppression of TMS-evoked activity results, at least to some extent, from a degradation of the TMS-evoked activity spreading through biological tissue (Määttä et al., 2017). However, we tend to exclude this factor for several reasons: (1) ISP is higher when tested in adults who have larger heads and thus longer distance between cortical areas, compared to children (Jarczok et al., 2016); (2) when tested in the same hemisphere, i.e. intrahemisperical signal propagation, the ISP is greater than when tested interhemispherically; and (3) ISP is not dependent on the intensity of stimulation. It is also important to consider that our conclusions are limited to M1-M1 interactions. We focused on this area because one of our aims was to verify if our cortical measures were related to previous MEP measures of interhemispheric interactions, but from our study we cannot be sure whether ISP measured in different areas could reflect pure interhemispheric dynamics. Thus, further studies investigating interhemispheric interactions of associative areas such as frontal and parietal cortices, are needed. Finally, we chose to focus on one ISI, i.e. 10 ms, because it was already investigated in previous TMS-EEG (e.g. Voineskos et al., 2010; Määttä et al., 2017; Jarczok et al., 2016) and IHI studies (e.g. Ferbert et al., 1992) but it is possible that the same, or stronger, inhibitory interhemispheric interactions can be observable at larger ISIs. In principle, we could have expected to observe also a facilitatory effect, given that callosal fibers are mostly excitatory in nature (Chen, 2004). In literature, most studies reported inhibitory effects after stimulation of the two M1, whereas facilitatory effects have been observed less consistently (e.g. Hanajima et al., 2001). This is likely due to the fact that excitatory callosal projections are very focal and they require a very weak stimulation intensity to be explored (Asanuma and Okuda, 1962). Thus, even if we used an intensity

just above the RMT (90%), it is likely that inhibitory effects outweigh the facilitatory (Irlbacher et al., 2007).

In conclusion, the main contribution of this study lies in the proposal of new TMS-EEG measures of interhemispheric dynamics, and in their validation in terms of intra- and inter-subject reliability. We also provide the first demonstration of the linear relationship between ISP and IHI. This result is particularly important to test interhemispheric dynamics in clinical populations where MEPs are not reliable. We recently published a study testing a population of chronic stroke patients in which we were not able to record reliable MEPs from the affected hemisphere (Koch et al., 2018). In this view, the combination of EEG recordings during TMS represents an innovative and promising approach to assess interhemispheric dynamics.

Competing interest

The authors declare that they have no conflict of interest.

Author contributions

E. P. C. and G. K. conceived and designed the experiments; E. P. C., M. M., F. P. and A. D. collected the data; E. P. C. analyzed the data; E. P. C. and L. R. wrote the manuscript; E. P. C., M. C. P., L. R. and G. K. revised the manuscript. All authors approved the final version of the manuscript. All the authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship. Experiments were carried out at the Santa Lucia Foundation (Rome).

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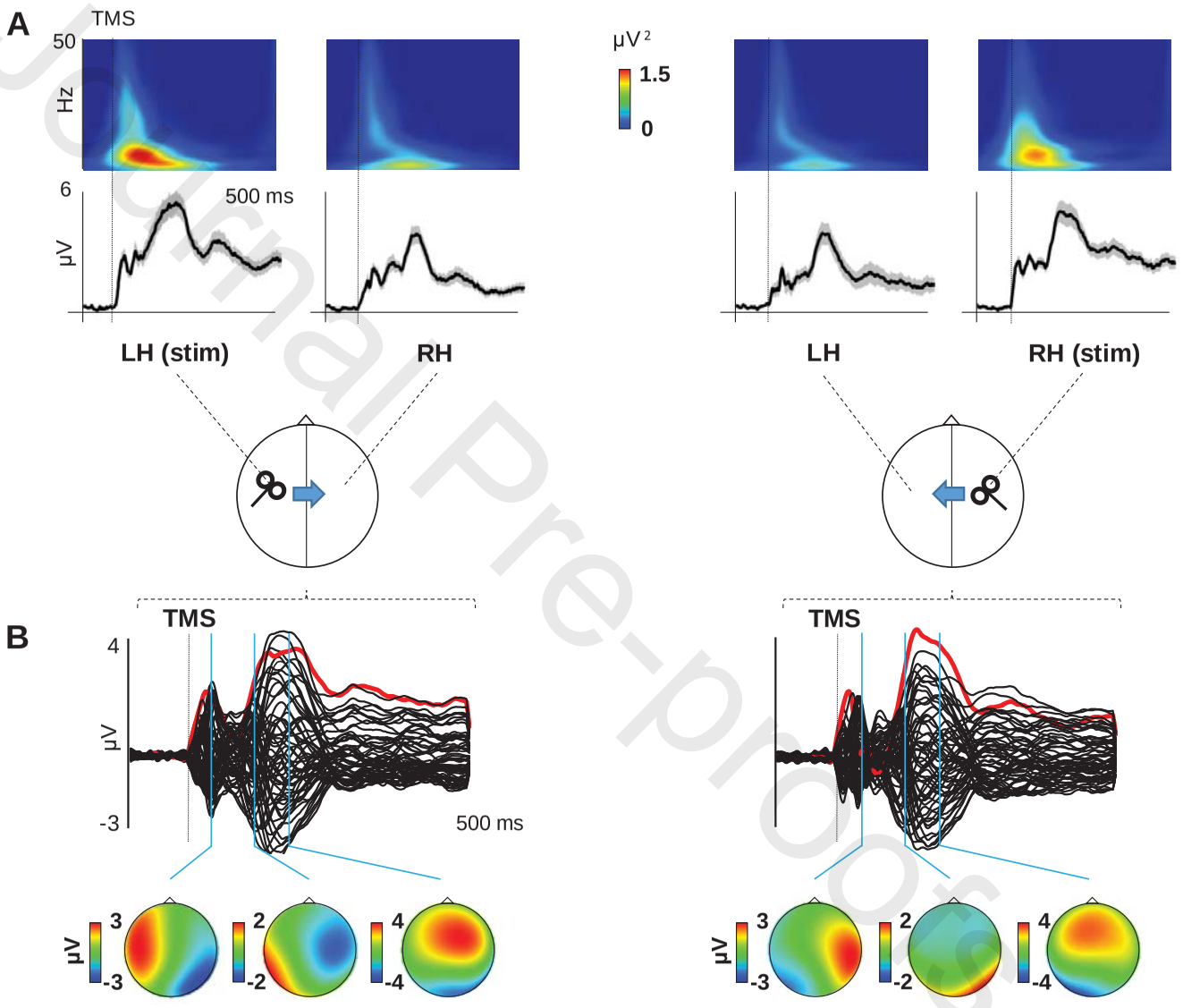
Figure captions

Figure 1. Local and global TMS-evoked cortical response after stimulation of the left (LH) and right hemisphere (RH). Local cortical response (panel A) are displayed in terms of TMS-evoked activity and cortical oscillations evoked over M1. Global cortical response (panel B) are displayed in terms of TMS-evoked potentials (TEPs) recorded over all the scalp with the scalp voltage distribution at the three main peaks of activity (20-40 ms; 40-70 ms; 70-150 ms).

Figure 2. Analysis of local TMS-evoked cortical activity evoked from LH and RH in young and adult participants. The plots depict the amplitude of the TMS-evoked cortical activity evoked in the stimulated hemisphere and in the contralateral one for each single participant.

Figure 3. Analysis of interhemispheric signal propagation (ISP, panel A), interhemispheric balance (IHB, panel B), interhemispheric inhibition (IHI, panel C) and correlations between ISP and IHI after stimulation of LH (panel D) and RH (panel E). Light red areas in panel C, D and E indicate inhibition, whereas light green areas indicate facilitation.

Figure 1



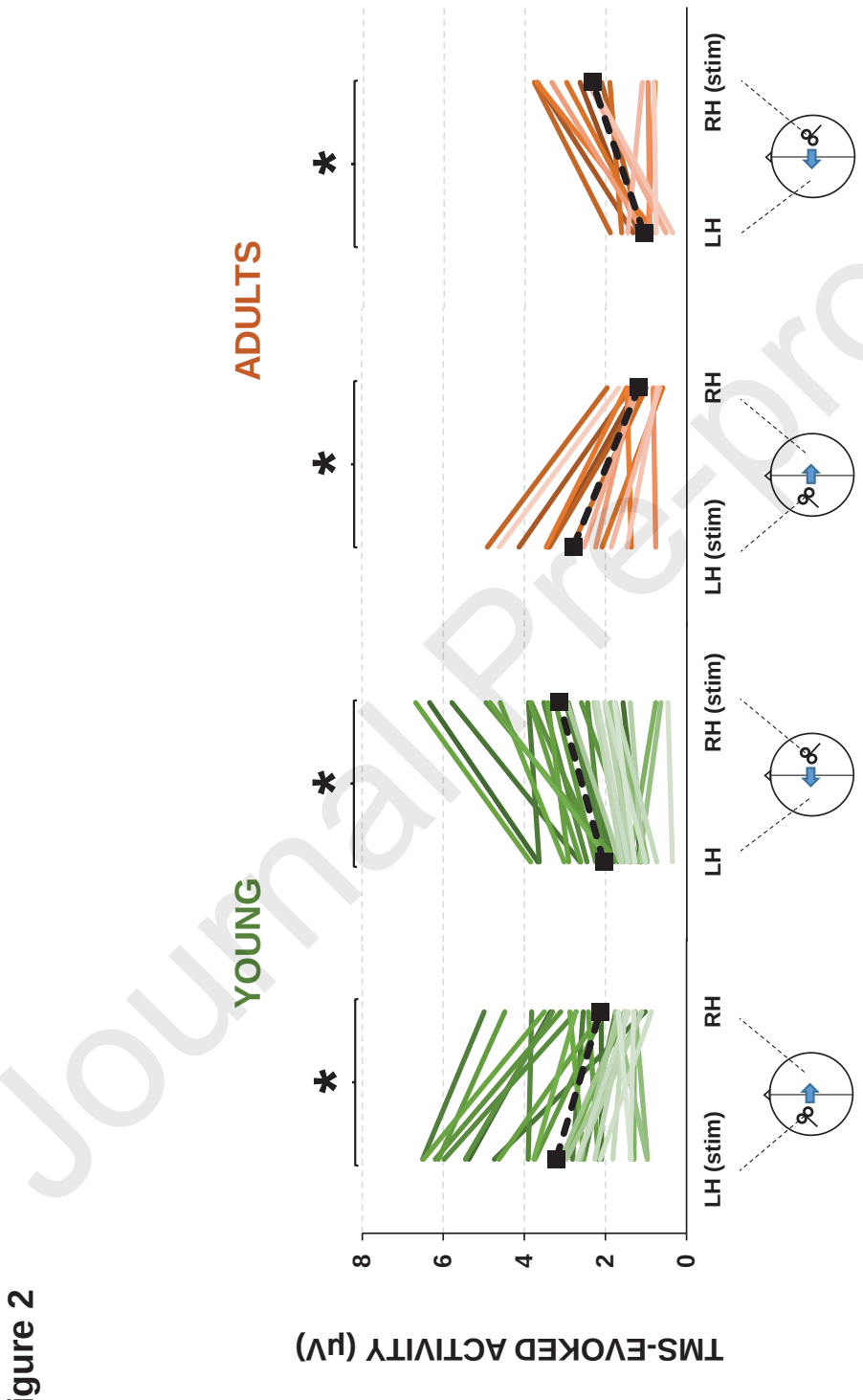


Figure 2

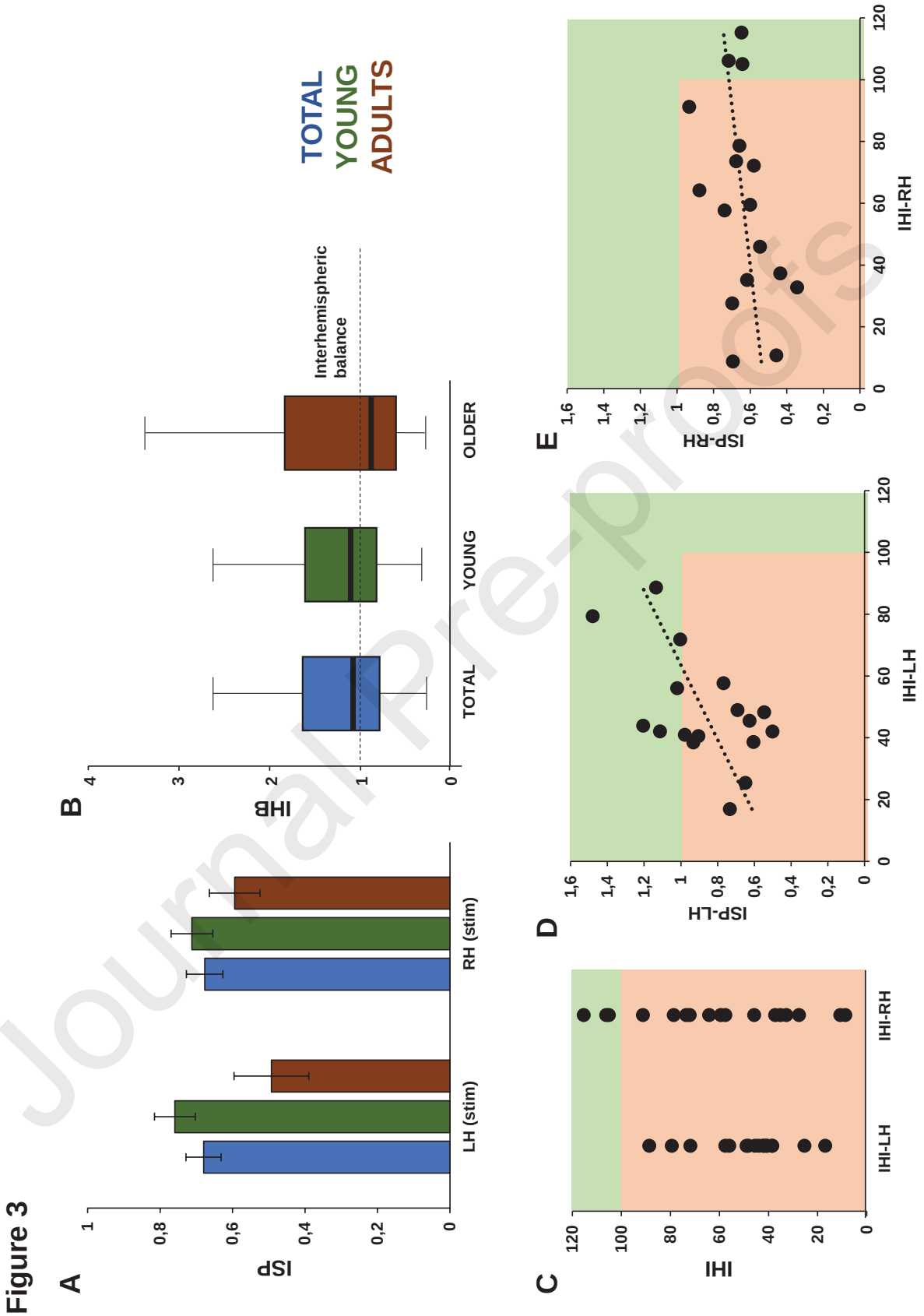


Figure 3