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Intracortical and interhemispheric excitability changes in arm amputees: A TMS study

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HIGHLIGHTS

- Amputation causes the reorganization of sensory-motor areas and reshapes intra- and inter-hemispheric connectivity.
- The "orphan" cortical areas maintain hushed connections with the corresponding peripheral areas.
- Amputees show changes in corticospinal excitability depending on amputation level.

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ABSTRACT

Objective: To evaluate cortical circuits and excitability of the motor cortex in the hemisphere contralateral to the affected (AH) and to the unaffected arm (UH), in upper limb amputees.
Methods: Motor evoked potentials (MEP) were recorded in 17 subjects who had upper limb amputation: 11 trans-radial (TR) and 6 trans-humeral (TH). Motor thresholds (MT), short interval intracortical inhibition (SICI), and interhemispheric inhibition (IHI) in the available arm muscles of the stump were evaluated.
Results: There was no significant difference in MT between hemispheres. SICI was preserved in TR but not in TH group. Additionally, in the TR group, the MEP amplitudes in AH were higher than in UH. A significant IHI was observed in the whole sample but not in each hemisphere or patient group.
Conclusions: In our population of TR amputees, we found increased corticospinal excitability in the AH with preserved intracortical inhibition. This finding was not observed in the TH population.
Significance: Understanding the changes in intracortical excitability in amputees may enhance knowledge of the functional reorganization of the brain in the post-amputation phase, bringing useful information for prosthetic rehabilitation.

1. Introduction

Cerebral plasticity and reorganization of afferent and efferent pathways underpin the functional recovery (Chen et al., 2013; Pilato et al., 2009) after brain injuries but these physiological processes are essential also in amputees (Candido Santos et al., 2020). The cerebral cortex may adapt to injuries by changing its activity at different levels. Neuronal activity changes may occur at the cellular and network levels by reshaping the functional connectivity of intact cortical areas and activating new neural pathways (Barth and Stanfield, 1990; Hicks and D'Amato, 1970; Waxman, 1988). In physiological conditions, the sensory-motor cortices are constantly modulated by the sensory feedback from skin and muscle afferents, generated by activities of daily living or by specific and repeated movements (Bütefisch et al., 2000; Vahdat et al., 2011). Previous studies demonstrated that cortical excitability and cortical maps may be affected by amputation resulting in a reorganization (Gunduz et al., 2020; Schwenkreis et al., 2000). The amputation, by interrupting the unceasing information exchange between the limb and the brain along peripheral and

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central sensory-motor pathways, causes plastic changes in the brain, whose underlying mechanisms remain mostly unknown.

In orphaned cortical areas, the remaining sensorimotor connections have a "memory" of the amputated limbs, and this phenomenon provides the ground for the so-called phantom limb pain (PLP). Overall, although several explanations have been proposed for PLP, such as it is either a maladaptive plasticity phenomenon (Flor et al., 2006) or a persistent functional representation as a complementary process, it is known that neuroplastic changes occur in the involved brain areas (DI Pino et al., 2021; Weiss et al., 2022). After upper limb amputation, sensorimotor re-adaptation phenomena occur in the brain and in the hemisphere corresponding to the amputated limb, causing the expansion of the motor cortex and adjacent cortical areas (Cohen et al., 1991; Pascual-Leone et al., 1996; Röricht et al., 1999). PLP is often a painful condition involving patients with limb amputation and it is thought to be due to a maladaptive plasticity (Flor et al., 2006). Some studies revealed that plastic phenomena in the motor cortex are more evident in subjects with PLP (Karl et al., 2001; Kew et al., 1997; Pascual-Leone et al., 1996) whereas other studies demonstrated that subjects with amelia, who generally do not report PLP, do not show substantial cortical asymmetry (Reilly and Sirigu, 2011). PLP is associated with better cortical reorganization phenomena (Flor et al., 1998, 1995; Montoya et al., 1998) and, in turn, these phenomena may improve phantom pain, as it has been shown after training sessions with bionic hand prosthesis to restore sensory feedback (Rossini et al., 2010).

In humans, transcranial magnetic stimulation (TMS) and functional neuroimaging have provided in-vivo pieces of information on the functional reorganization of the motor cortex (Di Lazzaro et al., 1998; Pilato et al., 2020, 2009; Wilson et al., 1993). TMS activates non-invasively corticospinal motor neurons and cortical interneurons and this tool has been used for studying intracortical phenomena (Di Lazzaro et al., 2004; Di Pino et al., 2014; Pilato et al., 2012). Moreover, it allows studying spatial reorganization of motor cortical representation maps (Wilson et al., 1993).

In both animal and human subjects, there is evidence of muscular representation expansion within the deafferented region, encompassing muscles adjacent to the amputated limb, including facial and stump muscles. This expansion has been observed to occur even after a span of 20 years following the amputation (Röricht et al., 1999). Remarkably, this phenomenon has been documented in individuals during childhood (Hall et al., 1990) as well as in adulthood (Cohen et al., 1991; Pascual-Leone et al., 1996).

In addition to the motor cortex, the process of brain remodeling extends to other brain areas. Electrophysiological and neuroimaging investigations have provided evidence of neuroplastic changes occurring in the deafferented somatosensory cortex (Elbert et al., 1994; Yang et al., 1994). These changes involve functional expansion towards intact areas. However, the mechanisms underlying this phenomenon, which hold significant implications for functional recovery, remain poorly understood.

We used single- and paired-pulse TMS paradigms on both hemispheres, in order to study neuroplastic changes in the motor cortex circuits in trans-radial (TR) and trans-humeral (TH) amputees,.

2. Methods

2.1. Experimental design

To evaluate cortical circuits of the primary motor cortex (M1) of the hemisphere contralateral to the affected (AH) and to the unaffected limb (UH), we recorded motor evoked potentials (MEP) elicited by TMS of M1 of both hemispheres. We measured active motor threshold (AMT), resting motor threshold (RMT), short interval intracortical inhibition (SICI), and interhemispheric inhibition (IHI) in upper limb muscles proximal to the amputation site.

2.2. Subjects

We included patients with stable amputation of the upper limb. We enrolled 17 subjects (1 female; mean age: 48.6 ± 14.6 (SD) years; age at amputation: 33.3 ± 16.8 years). Among them, 11 had TR and 6 TH amputations, right side (n = 10) and left side (n = 7). Manual preference before amputation was right side in all patients and most patients had a long-standing amputation (15.3 ± 15.7 years) (Table 1).

Exclusion criteria were contraindications to magnetic fields exposure (e.g., cardiac pacemakers, other stimulators, or internal non-paramagnetic metallic objects); cognitive impairment (i.e., MMSE score < 24) and inability to express consent for study participation; neuroactive drugs, epilepsy and comorbidities that could affect the study results.

The study was approved by the Ethics Committee of Campus Bio-Medico University and was conducted in accordance with the Declaration of Helsinki and the following amendments, protocol code: AMP-PLAST15.

2.3. Electrophysiological evaluation

TMS was performed with a high-power Magstim 200^2 stimulator (Magstim Co Ltd, Whitland, UK). A figure-of-eight coil with external loop diameters of 9 cm was held over the motor cortex at the optimum scalp position to elicit MEPs in the distal muscles available in the stump and in the same muscles of the contralateral limb. Specifically, we used as target muscles the Biceps brachii (BB) in TR amputees and the Trapezius upper head (TPZ) in TH amputees (Fig. 1).

The induced current flowed in a posterior-anterior direction. Stimulation intensity was expressed as a percentage of the maximum stimulator output (MSO). MEPs were recorded via two 9-mm diameter Ag-AgCl surface electrodes. The electromyogram (EMG) was amplified and filtered (bandwidth 3 Hz-3 kHz) by a Digitimer D360 amplifier (Digitimer, Welwyn Garden City, UK). Data were collected on a computer with a sampling rate of 10 kHz per channel and stored for later analysis using a CED 1401 analog-to-digital converter (Cambridge Electronic Design, Cambridge, UK). Subjects were given audio-visual feedback of the EMG signal to assist in maintaining complete muscular relaxation. EMG continuous activity was sampled to 3.10 s-epochs, and in protocols performed at rest, trials displaying EMG activity > 0.1 mV preceding TMS were discarded. MEP amplitudes were measured from peak to peak.

The excitability of the M1 area of the target muscle was assessed by measuring RMT and AMT, which were expressed as a percentage of MSO.

Because TMS protocols were performed on different days and not all participants were available for both protocols only 10 subjects were included in the IHI study.

Short interval intracortical inhibition (SICI) was measured using the technique described by the original paradigm developed by Kujirai et al. (Kujirai et al., 1993). Two magnetic stimuli were given through the same stimulating coil over the M1 at an interstimulus interval (ISI)s of 2 and 3 ms, and the effect of the first (conditioning) stimulus on the second (test) stimulus was measured. Ten tests and 10 conditioned stimuli for each ISI – i.e., 2 and 3 ms – were delivered in a pseudorandomized order. The conditioning stimulus was set at an intensity of 5 % MSO below AMT. The intensity of the test stimulus was adjusted to elicit an unconditioned test MEP in the relaxed target muscle of ~0.5 mV

Table 1
Participants' clinical and demographic characteristics.

Id	Amputation type	Side	Age	Sex	Hot-spot muscle	Amputation time (years before study)	Handedness (before amputation)	PLP
1	TR	R	61	M	BB	45	R	N
2	TR	L	52	M	BB	5	R	N
3	TR	R	47	M	BB	27	R	N
4	TH	L	33	M	TPZ	13	R	N
5	TR	L	29	M	BB	5	R	Y
6	TH	L	59	M	TPZ	25	R	N
7	TR	R	46	M	BB	3	R	Y
8	TH	R	38	M	TPZ	8	R	Y
9	TR	R	25	M	BB	0	R	N
10	TR	L	59	M	BB	4	R	Y
11	TH	L	48	M	TPZ	12	R	N
12	TH	R	60	M	TPZ	29	R	Y
13	TR	R	57	M	BB	37	R	N
14	TR	L	49	F	BB	2	R	N
15	TR	R	56	M	BB	43	R	N
16	TR	R	80	M	BB	0.5	R	N
17	TH	R	27	M	TPZ	1	R	N

TH: trans-humeral amputation; TR: trans-radial amputation; TPZ: trapezius muscle; BB: biceps brachii muscle; R: right; L: left; PLP: Phantom limb pain.

peak-to-peak amplitude. The protocol was applied in both hemispheres.

Interhemispheric inhibition (IHI) was assessed with two high-power Magstim 200^2 stimulators, each one connected to a figure-of-eight coil. The coils were held over the right and left M1 targets with the induced current flowing in a posterior-anterior direction. IHI was evaluated using the technique described by Ferbert et al. (Ferbert et al., 1992). The effect of the first (conditioning) stimulus over one hemisphere M1 on the second (test) stimulus over the other hemisphere M1 was measured. Both conditioning and test shock intensities were adjusted to obtain in the relaxed target muscle MEPs of $\sim$ 0.5 mV peak-to-peak amplitude. ISIs of 8, 10 and 15 ms were investigated, with ten repeats per ISI in a pseudo-randomized order. The protocol was applied in both hemispheres.

3. Statistical analysis

RMT and AMT of the two hemispheres were compared using paired t-tests (the normality of the data was checked using the Shapiro-Wilks test). MEP values were split on the basis of the tested electrophysiological evaluation (SICI or IHI). Data (amplitudes of MEPs) were analyzed with generalized linear mixed models (GLMM). We chose the most appropriate family (gamma, gaussian or inverse gaussian) and link function (identity, log or inverse) as the model with the lowest Akaike information criterion (AIC). The participants were modeled as the random effects factor, and the amputation level (TR and TH), stimulation site (AH and UH), and stimulation condition (test and conditioned stimulus) were modeled as the fixed effects factors. Therefore, the data were processed in an ANOVA-like analysis, resulting in a 2 $\times$ 2 $\times$ 2 model design. This has several advantages over the classic, repeated measures ANOVA approach allowing to effectively fit large and unbalanced data sets (e.g., missing data) and requires less restrictive assumptions to run the analysis properly (Baayen et al., 2008). If any significant main factor or interaction was identified, the data were split based on the significant factor and different groups were analyzed with separated GLMM (e.g., if amputation level interaction was significant, two 2 $\times$ 2 model design ANOVA-like analyses were performed, one analysis for each group of amputees).

In order to evaluate the amount of inhibition (i.e., inhibition ratio) between amputation levels and sides of stimulation, the conditioned MEP values were normalized by dividing them by the mean test values. Also in this case, the data were analyzed with GLMM. The amputation level (TR and TH level), and stimulation site (AH and UH) were modeled as the fixed effects factors.

In order to identify a possibly different cortical inhibition pattern in subjects showing PLP compared to the other ones, an additional analysis was performed on the Inhibition ratio data for both protocols: a GLMM analysis with fixed factors amputation level (TR and TH level), stimulation site (AH and UH) and presence of PLP (PLP and No-PLP).

The statistical analysis was performed with JASP software.

4. Results

There was no significant difference in RMT and AMT values between the AH and the UH (RMT: 67.4 ± 3.3 % vs 63.3 ± 3.1 %, p = 0.36; AMT: 52.3 ± 3.4 % vs 50.6 ± 2.5 %, p = 0.69).

SICI was studied in 17 subjects (n:11 TR, n:6 TH). The GLMM analysis with gamma family and log link function showed a significant effect of stimulation condition (test MEP amplitude was higher than the conditioned one: $\chi2$ (1) = 10.83, p < 0.001) and a significant interaction between the stimulation site and amputation level factors ($\chi2$ (1) = 4.64, p < 0.031) (for the other factors and interaction: $\chi2$ (1) > 2.84, p > 0.092). Considering such significant interaction, a GLMM analysis for each group of amputation level (TR and TH group) was performed. The GLMM analysis with gamma family and log link function performed on the TR group showed a significant main effect of stimulation condition (test MEP amplitude was higher than the conditioned one: $\chi2$ (1) = 11.20, p < 0.001) and of stimulation site (MEP amplitudes relative to AH were higher than those of UH: $\chi2$ (1) > 5.95, p < 0.015) whereas the GLMM analysis with gamma family and log link function (fixed factors: stimulation site and condition) performed on the TH group did not show any significant main effect or interaction between factors ($\chi2$ (1) > 2.39, p > 0.122) (Fig. 2).

GLMM analysis on inhibition ratio data with gamma family and log link function did not show any significant main factor or interaction ($\chi2$ (1) > 3.16, p > 0.076) (Fig. 3).

In case of SICI protocol, the GLMM analysis with gamma family and identity link function showed a significant interaction between the amputation level and the presence of PLP factors ($\chi2$(1) = 7.28, p = 0.007) (for the other factors and interaction: $\chi2$(1) > 3.38, p > 0.068). Considering such significant interaction, a GLMM analysis for each group of amputation level (TR and TH group) was performed. The GLMM analysis with gamma family and log link function performed on the TR group did not show any significant

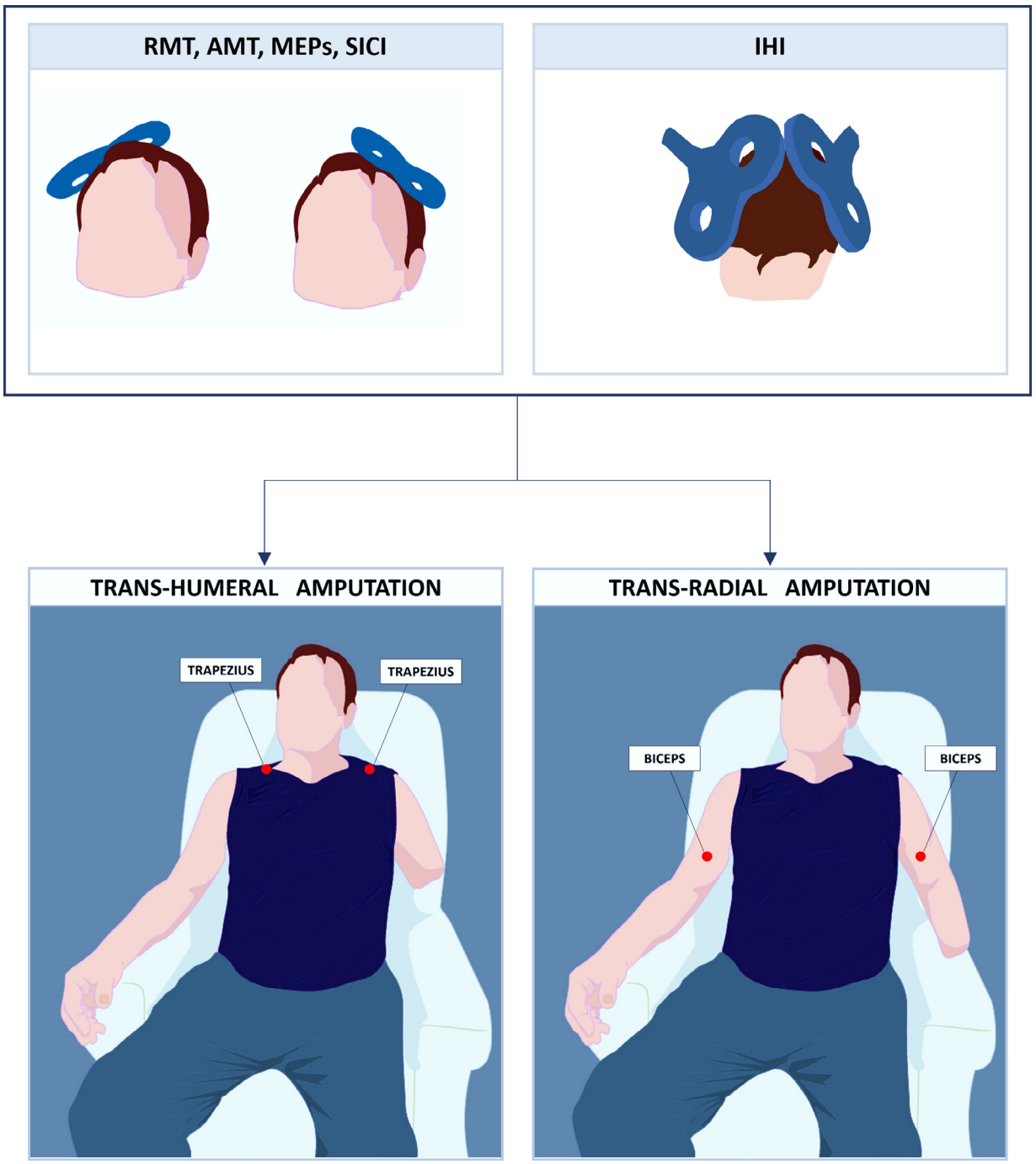

Fig. 1. Representation of study setting. RMT, AMT, MEPs, SICI and IHI were recorded bilaterally. The coil was positioned over the scalp area optimal for eliciting a MEP in the contralateral Trapezius muscle (upper head) for trans-humeral amputation, and in the contralateral Biceps brachii for trans-radial amputation. The coil's handle pointed posteriorly at an angle of approximately 45 degrees relative to the medial-sagittal plane of the subjects' head, inducing a posterior-to-anterior directed electric field perpendicular to the central sulcus. RMT = Resting Motor Threshold; AMT = Active Motor Threshold; MEPs = Motor Evoked Potentials; IHI = interhemispheric inhibition.

main effect or interaction between factors ($\chi2(1) > 0.73$, $p > 0.392$), whereas the GLMM analysis with gamma family and identity link function performed on the TH group showed a significant main effect of the presence of PLP (inhibition ratio was lower for group of participants with PLP: $\chi2(1) = 11.77$, $p < 0.001$) (for the other factor and interaction: $\chi2(1) > 3.99$, $p > 0.050$) (Fig. S1).

IHI was studied in 10 subjects (6 TR, 4 TH). The GLMM analysis with gamma family and log link function highlighted a significant effect of stimulation condition (test MEP amplitudes were significantly higher than the conditioned ones: $\chi2\ (1) = 8.98$, $p = 0.003$), but not of stimulation site, amputation level and interactions (Fig. 4).

GLMM analysis on inhibition ratio data with Gamma family and log link function did not show any significant main factor or interaction ($\chi2\ (1) > 3.16$, $p > 0.076$) (Fig. 5).

Similar results were obtained from GLMM analysis (gamma family and identity link function) performed to investigate the effect of the presence of PLP ($\chi2\ (1) > 3.32$, $p > 0.068$) (Fig. S2).

An additional analysis was performed for both protocols to ensure no significant differences in test MEP values between the

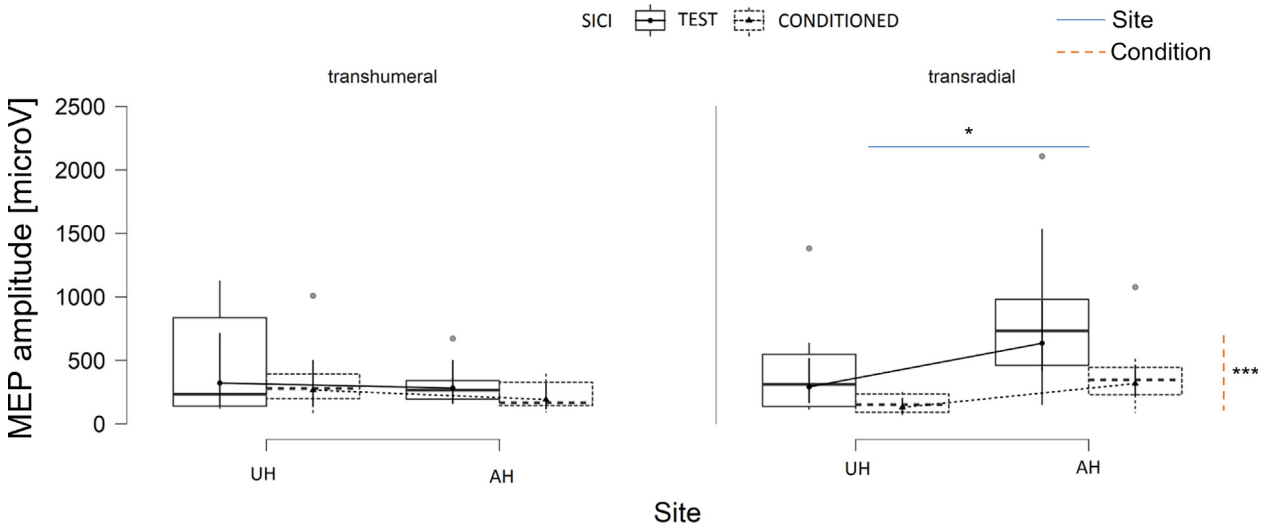

Fig. 2. MEP obtained using the paired-pulse protocol of short-interval intracortical inhibition (SICI) in 6 subjects with trans-humeral amputation (TH) (left panel) and 11 subjects with trans-radial amputation (TR) (right panel). Asterisk indicates a significant difference (*: $p < 0.05$; ***: $p < 0.001$).

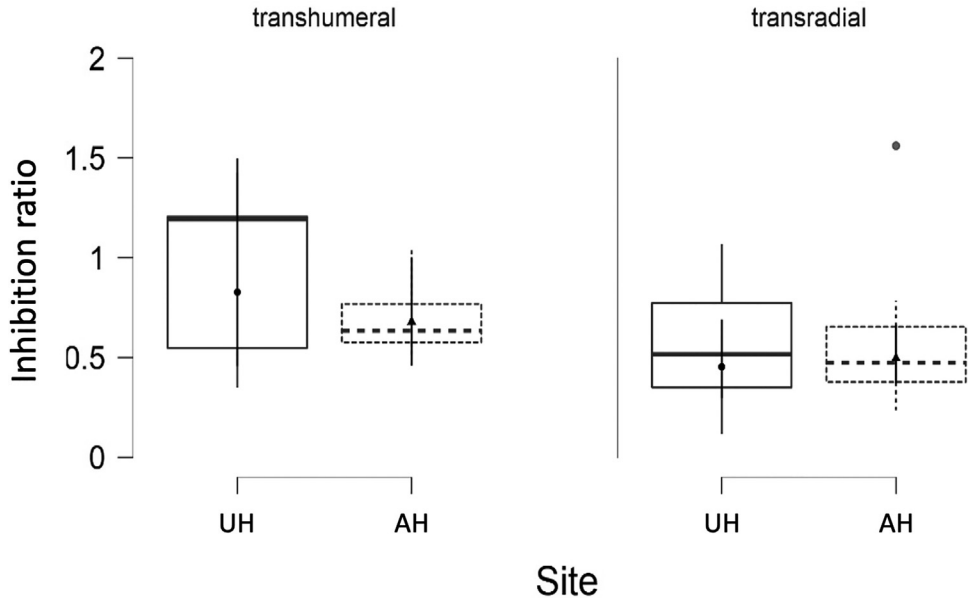

Fig. 3. Inhibition ratio obtained using the paired-pulse protocol of short-interval intracortical inhibition (SICI) in 6 subjects with trans-humeral amputation (TH) (left panel) and 11 subjects with trans-radial amputation (TR) (right panel).

hemispheres. The mean MEP values for each subject, condition of TMS stimulation and site of stimulation were calculated and two Friedman tests (no normal distribution of the data) for each group of amputees were performed to compare the test MEP values in the tested hemisphere (UH and AH). No significant difference between the tested hemispheres in test MEPs was found both for SICI ($\chi 2$ (1) < 3.60, $p > 0.058$) and IHI protocol ($\chi 2$ (1) < 0.51, $p > 0.414$), confirming the results of GLMM analysis.

5. Discussion

Previous neurophysiological studies have evaluated the changes in cortical excitability in amputees and their influence on clinical effects such as PLP (Schwenkreis et al., 2000; Teixeira et al., 2021). Here, we evaluated corticospinal excitability and intracortical neuronal activity in unilateral upper-limb amputated patients at TH and TR levels by using single and paired-pulse TMS protocols. We assessed motor thresholds as a measure of cortical excitability. Moreover, we used paired stimulation protocols of SICI and IHI to assess intracortical and interhemispheric inhibition. We did not find any difference in AMT and RMT between hemispheres in both groups. SICI is a largely used protocol to assess GABAergic activity in the motor cortex (Di Lazzaro et al., 2002, 2007a). We studied SICI in both hemispheres (AH vs UH) and in both groups of patients (TH vs TR). We found that conditioned MEPs were suppressed compared to test MEPs only in TR patients; in this group, the suppression of conditioned MEPs was comparable and not statistically different between AH and UH.

Most previous studies found SICI reduction in AH, but other studies found no difference between hemispheres (reviewed in Candido Santos et al., 2020). This discrepancy may have several explanations mainly related to the heterogeneity of study designs and patients' selection. Time from amputation can be crucial in

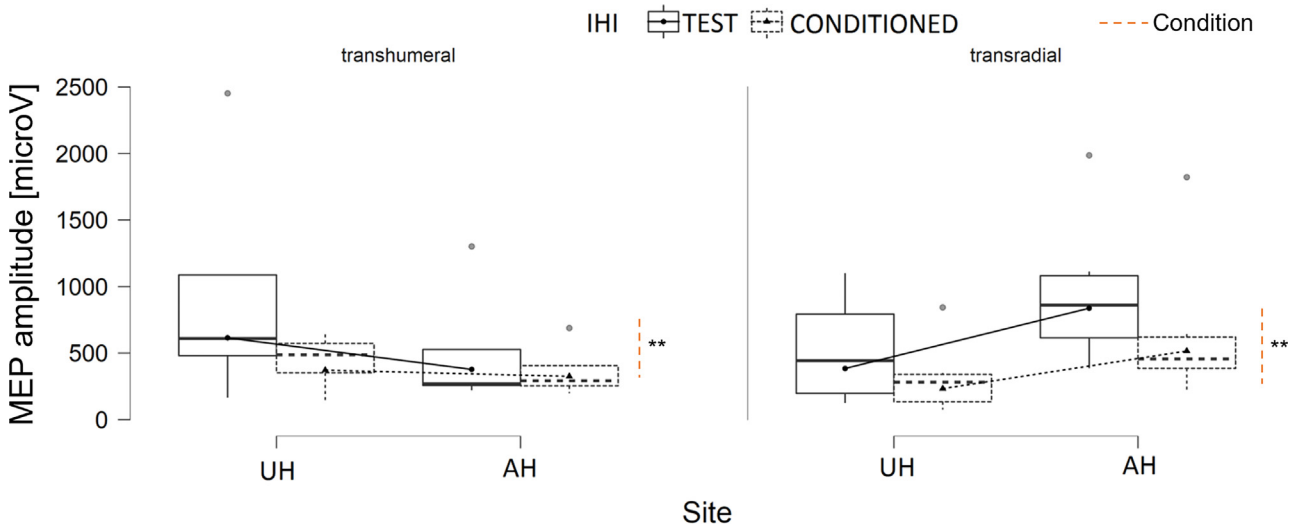

Fig. 4. MEP obtained using the paired-pulse protocol of interhemispheric inhibition (IHI) in 4 subjects with trans-humeral amputation (TH) and 6 subjects with trans-radial amputation (TR). Asterisk indicates a significant difference (**: $p < 0.01$).

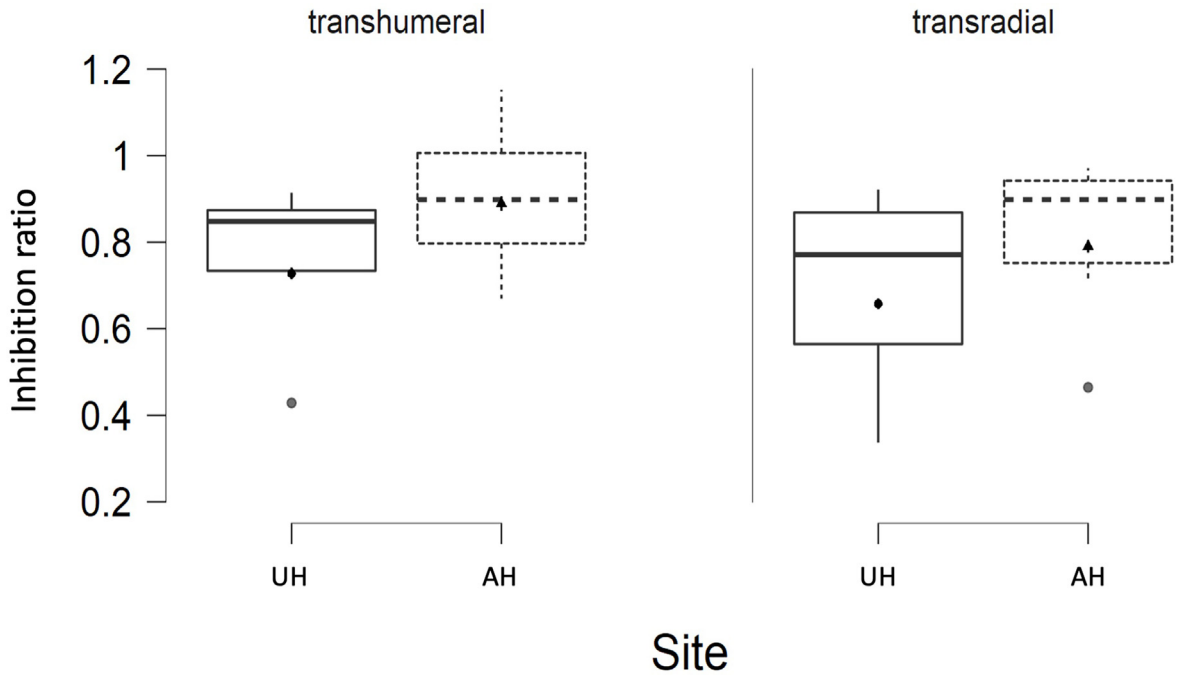

Fig. 5. Inhibition ratio obtained using the paired-pulse protocol of interhemispheric inhibition (IHI) in 4 subjects with trans-humeral amputation (TH) and 6 subjects with trans-radial amputation (TR).

plastic adaptations: although patients' age was comparable among studies, the time from amputation was much longer in our sample as compared to other studies, such as that on PLP patients by Teixeira et al. (33 years vs 22 months) (Teixeira et al., 2021). Moreover, the use of some drugs such as antidepressants and other drugs active on CNS may affect the results: it is observed that most of the previous studies focused on amputees with PLP who required painkiller drugs. Our enrolled subjects did not take any drug active on CNS and only a few of them reported PLP. Moreover, the level of amputation may be another relevant factor.

We also evaluated the interhemispheric connections by assessing IHI: conditioned MEPs were suppressed compared to test MEPs in the whole sample, but no significant IHI could be demonstrated in each hemisphere and patients' group.

Interhemispheric inhibition is essential in bimanual movement control, which involves bilateral cortical and subcortical areas through interhemispheric connections through the corpus callosum (Morishita et al., 2022). Recent data also show that in amputated patients, movement control is based on both higher associative areas (Makin et al., 2015) and interhemispheric communication (Di Pino et al., 2012).

To our knowledge, no TMS data are available on interhemispheric inhibition in subjects with amputation, but recent animal studies suggest that, after amputation, reorganization of the brain connections and functions occur, and callosal pathways mediate interhemispheric plastic changes (Petrus et al., 2020, 2019). Overall, our data show, in TR amputees, an increased test response and significant GABAergic inhibition in the motor cortical areas

contralateral to the amputated side (AH). This finding, combined with a possible rearrangement of the cerebral cortex following amputation, might represent a compensatory phenomenon in the chronic amputation condition. On the other hand, some data show that the time from amputation and the amount of training with the prosthesis can play a role in the plastic changes of the sensorimotor cortex (Di Pino et al., 2009).

Previous studies evaluated the motor cortex reorganization in traumatic lower limb amputees showing an asymmetrical reorganization of the motor cortex depending on the time of limb loss and these effects were not related to the PLP (Pacheco-Barrios et al., 2020).

Our patients' group was mostly composed of long-term amputees. A study by Röricht and coworkers (Röricht et al., 1999) confirms that plastic changes can also occur after 20 years from amputation, although they are distinct in different muscles of the stump. We studied only right-handed subjects, and they were balanced for the amputation side; we studied 2 different muscles of the stump bilaterally. It is conceivable that the level of amputation, causing the loss of a different amount of muscle and sensory fibers and thus their motor-sensory communication with the brain, may cause these distinctive effects. Previous studies reported that in healthy subjects some differences in cortical excitability, facilitatory and inhibitory circuits may depend on the studied muscle (Menon et al., 2018) but also other factors such as polymorphism in neurotrophic factors such as the brain-derived neurotrophic factor (BDNF) gene (Di Lazzaro et al., 2007b; Dubbioso et al., 2022) may have an impact on interhemispheric balance and motor cortex excitability.

Handedness is also a variable factor that may influence neuroplasticity, although Xie et al. (Xie et al., 2013) suggest that manual preference before amputation should not lead to different plasticity phenomena in those who use the prosthesis on the dominant or non-dominant side. An EEG study, carried out by Williams L. and coworkers (Williams et al., 2016), on patients with amputation of the right upper limb, aimed to study the laterality of motor activity and neural reorganization after amputation, showed that there is a remodeling of activations from traditional contralateral motor areas to the posterior parietal areas for motor planning and execution when using the amputated limb. We tried to evaluate also inhibitory pattern differences in TH and TR amputees taking into consideration the presence of PLP. While we did observe a difference exclusively in the TH group among patients experiencing PLP, this finding should be taken with caution due to the restricted sample size.

We are aware of some limitations of the present study. The main limitation is the lack of a control group of healthy age-matched subjects. Indeed, we acknowledge that changes in cortical excitability might occur also in the hemisphere ipsilateral to the amputation, due to interhemispheric interactions, and they may cause compensatory balanced phenomena. A similar condition of excitability imbalance between AH and UH is described in chronic stroke. One study on patients with chronic stroke (Murase et al., 2004), in which the IHI was tested bilaterally, showed increased inhibition of UH on the AH, secondary to disinhibition of the UH and consistent with a model of interhemispheric competition in the motor and sensory systems but also the development of ipsilateral projections may influence this process (Pilato et al., 2009). Moreover, the small sample size may limit the strength of our findings, although subjects with the characteristics of our study population are rare and previous studies evaluated a comparable number of patients. It would have been of interest also evaluating input–output curves and intracortical facilitation in order to characterize excitatory compensatory phenomena and the influence of PLP on intracortical changes.

In conclusion, our data show that in amputees, a rearrangement of intracortical circuitry occurs; the rearrangement depends on the level of amputation, and inhibitory phenomena are involved in this process. Understanding the changes in intracortical excitability in amputees provides information on the reorganization of the brain in the post-amputation phase, bringing useful information for rehabilitation and even prosthetic selection. Understanding neurophysiological markers of cortical reorganization after amputation can help choose the timing and characteristics of prosthetic rehabilitation in an increasingly personalized and patient-friendly medicine. Indeed, the brain's neural activity in prosthetic control can increase the control of currently available myoelectric prostheses, helping develop neurally-interfaced prostheses.

Conflict of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinph.2023.09.017.

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