



Human Motor Cortex Functional Changes in Acute Stroke: Gender Effects

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The acute phase of stroke is accompanied by functional changes in the activity and interplay of both hemispheres. In healthy subjects, gender is known to impact the functional brain organization. We investigated whether gender influences also acute stroke functional changes. In thirty-five ischemic stroke patients, we evaluated the excitability of the affected (AH) and unaffected hemisphere (UH) by measuring resting and active motor threshold (AMT) and motor-evoked potential amplitude under baseline conditions and after intermittent theta burst stimulation (iTBS) of AH. We also computed an index of the excitability balance between the hemispheres, laterality indexes (LI), to evidence hemispheric asymmetry. AMT differed significantly between AH and UH only in the male group ($p = 0.004$), not in females ($p > 0.200$), and both LI_{AMT} and LI_{RMT} were significantly higher in males than in females (respectively $p = 0.033$ and $p = 0.042$). LTP-like activity induced by iTBS in AH was more frequent in females. Gender influences the functional excitability changes that take place after human stroke and the level of LTP that can be induced by repetitive stimulation. This knowledge is of high value in the attempt of individualizing to different genders any non-invasive stimulation strategy designed to foster stroke recovery.

Keywords: acute cerebral infarction, gender, neurophysiology, stroke, transcranial magnetic stimulation

INTRODUCTION

Gender related functional asymmetries between the two cerebral hemispheres have been documented in healthy human brain (Tomasi and Volkow, 2012). It has been suggested that they might be responsible for gender differences in cognitive styles (Proust-Lima et al., 2008), in the incidence of neuropsychiatric disorders (Narr et al., 2001; Baron-Cohen et al., 2005), and for a gender-specific influence on the functional outcome after unilateral cerebral lesion (Draca, 2010). Sex-related differences have been also reported after stroke with a worse functional outcome in women (Lisabeth et al., 2015), however the causes of this sex disparity in stroke outcome are still largely unknown because demographics, prestroke and clinical factors cannot explain it. One possibility is that gender has a significant influence on the functional changes underlying recovery that take place in the brain after a stroke. Non-invasive brain stimulation techniques provide the opportunity for the functional evaluation of the human brain. Thanks to these techniques it has been shown that pronounced asymmetrical functional changes take place in cortex in the acute phase of stroke (Di Pino et al., 2014a,b).

These changes involve both the affected (AH) and unaffected (UH) hemispheres and might be correlated with long term recovery (for review see Di Pino et al., 2014a). Along this line, it is still unknown whether gender has an effect in stroke-related acute functional changes in the excitability of AH and UH.

The present study aims at investigating whether gender influences the cortical functional changes observed in the acute phase of stroke. To this end, in patients with acute stroke we evaluated motor cortex excitability by using single pulse transcranial magnetic stimulation (TMS) and the propensity of the cortex to undergo LTP- and LTD-like plasticity by means of a repetitive TMS (rTMS) paradigm, known as intermittent theta burst stimulation (iTBS). iTBS produces LTP-like changes in the stimulated hemisphere and LTD-like changes in the contralateral hemisphere (Di Lazzaro et al., 2008; Suppa et al., 2008). Similar interhemispheric effects have been observed also using a different TBS protocol known as continuous TBS, a rTMS paradigm that produces opposite effects on cortical excitability with LTD-like changes in the stimulated hemisphere and LTP-like changes in the contralateral hemisphere (Stefan et al., 2008).

Electrophysiological findings after single pulse TMS and after iTBS were compared between genders.

MATERIALS AND METHODS

Patients

Thirty-five patients with first-ever stroke were recruited (mean age = 71.4 years, SER = 1.96, 15F). Inclusion criteria were: (1) single ischemic stroke (both cortical and subcortical) involving the middle cerebral artery territory; (2) less than 10 days post-stroke; (3) hand weakness; (4) recordable muscle evoked potential (MEP) after TMS of the AH. Exclusion criteria were: (1) history of seizure; (2) hemorrhagic stroke; (3) concomitant neurological or other severe medical problems; (4) complete paralysis of the hand; (5) inability to give informed consent; (6) concomitant treatment with drugs acting on the central nervous system; (7) contraindications for TMS studies. In order to identify at risk patients for post-stroke epilepsy, all patients underwent an EEG before entering the study and none of them showed any epileptic abnormality (Rossini and Johnston, 2005). The evaluation of neurological impairment was based on the National Institutes of Health Stroke Scale (NIHSS).

All patients underwent brain MRI with a 1.5-T scanner (GE Signa; General Electric, Milwaukee, WI), and lesion size was estimated by using the Alberta Stroke Program Early CT Score (ASPECTS) (Barber et al., 2000).

All the patients signed a written informed consent form. This study was conducted in accordance with the Helsinki Declaration of 1975 and was approved by the Ethics Committee of Campus Bio-Medico University of Rome.

Magnetic Stimulation

Motor Cortex Excitability to Single Pulse TMS

Magnetic stimulation was performed with a high-power Magstim 200 (MagstimCo., Whitland, Dyfed). 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 contralateral first dorsal interosseous muscle (FDI). The induced current flowed in a postero-anterior direction. We evaluated the threshold and amplitude of MEPs. The resting motor threshold (RMT) was defined as the minimum stimulus intensity, expressed as the percentage of the maximal output intensity deliverable by the stimulator, which produced a liminal MEP (about 50 μ V in 50% of 10 trials) at rest (Rossini, 2014). The active motor threshold (AMT) was defined as the minimum stimulus intensity that produced a liminal MEP (about 200 μ V in 50% of 10 trials) during isometric contraction of the tested muscle (Rossini, 2014). We evaluated the RMT, AMT, and MEP amplitude elicited stimulating both the AH and UH. The MEP amplitude was evaluated using a stimulus intensity of 120% RMT with the muscle at rest. Audio-visual feedback of the electromyographic (EMG) signal at high gain was given to subjects in order to assist them in maintaining complete relaxation; trials contaminated by EMG activity were discarded. Ten data sweeps were collected, and the mean peak-to-peak amplitude of the MEPs was calculated.

Intermittent Theta Burst Stimulation

iTBS was delivered over the affected motor cortex “hot spot” for MEPs in the contralateral FDI muscle using a MagPro stimulator (Medtronic A/S Denmark) connected to a figure-of-eight coil (MCF B65). The magnetic stimulus had a biphasic waveform with a pulse width of about 280 μ s and a maximum magnetic field strength of 1.5 T. The initial direction of the current induced in the brain was anterior to posterior. The stimulation intensity was defined in relation to the AMT evaluated using the MagPro stimulator. An intensity of 80% AMT was used. We applied the iTBS protocol in which 10 bursts of high frequency stimulation (3 pulses at 50 Hz) are delivered at 5 Hz every 10 s, for a total of 600 pulses (Huang et al., 2005). iTBS effects on both hemispheres were assessed by evaluating the changes of the RMT, AMT, and MEP amplitude stimulating the AH and UH, before and immediately after iTBS. MEP amplitude was evaluated as detailed above.

Statistical Analysis

Main aim of the statistical analysis is to assess the effect of gender on excitability and plasticity measures. Baseline and iTBS-dependent excitability changes were tested on RMT, AMT, MEP amplitude and on the Laterality Index (LI) (Cramer et al., 1997; Di Lazzaro et al., 2015). The latter is a derived compound estimate of inter-hemispheric excitability imbalance. In the case of MEP amplitude, LI is expressed by the following equation: $LI_{MEP} = (MEP_{UH} - MEP_{AH}) / (MEP_{UH} + MEP_{AH})$. On the contrary, in the case of AMT and RMT the correlation with excitability is opposite (the lower are the thresholds the higher is the excitability). Thus, LI is calculated as follow: $LI_{RMT} = (MEP_{AH} - MEP_{UH}) / (MEP_{UH} + MEP_{AH})$ and $LI_{AMT} = (MEP_{AH} - MEP_{UH}) / (MEP_{UH} + MEP_{AH})$. LI ranges between -1 and $+1$; positive values always indicate higher excitability of the UH. The bigger the difference from 0, the higher is the inter-hemispheric imbalance. Gender effect on baseline RMT, AMT, and MEP is evaluated applying a mixed model ANOVA with *Hemisphere*

(two levels: *Affected -AH and Unaffected -UH*) as within-subjects factor and *Gender* (two levels: *Female and Male*) as between-subjects factor. A two-tailed independent sample t-test is used to assess the LI between groups difference. iTBS effect is tested on RMT, AMT, and MEP amplitude by using a mixed model ANOVA with *Hemisphere (Affected -AH and Unaffected -UH)* and *iTBS (Pre and Post)* as within-subjects factor and *Gender (Female and Male)* as between-subjects factor. The same model without the factor hemisphere is applied to study iTBS-related LI changes. Differences between females and males for non-normal distributed data were checked applying Mann-Whitney tests. In order to better address the variability of iTBS effects on MEP amplitude, the proportion of iTBS-induced AH excitability increase and UH excitability decrease are compared between groups, by means of Chi-Square test. The correlation between the clinical status and the neurophysiological data was performed using Pearson's correlation coefficients and partial correlations. The statistical distribution of all the variables is tested by means of Kolmogorov and Smirnov test. The significance level is set to 0.05. Descriptive statistic is reported as Mean \pm Standard Error of the Mean (SEM).

RESULTS

The average NIHSS at onset was 5.21 ± 0.413 . Gender groups were matched regarding age (F age = 69.87 ± 3.16 ; M age = 72.55 ± 2.50 , $p = 0.505$) and clinical status (NIHSS) at stroke onset (F NIHSS = 4.73 ± 0.66 ; M NIHSS = 5.58 ± 0.53 , $p = 0.317$). Groups were also matched regarding the percentage of patients with different lesion site (subcortical or cortical-subcortical), a pure cortical lesion was present in 3 out of 15 female patients (20%) and in 4 out of 20 male patients (20%). This is relevant because functional changes in cortical excitability may be influenced by stroke location and distribution (Ameli et al., 2009). Lesion size, as evaluated with the ASPECT score, was comparable in the two groups ($p > 0.200$) and resulted 7.47 ± 0.47 for females and 7.40 ± 0.36 for males. In a subgroup of 7 females and 8 males we measured the stroke volume using the procedure described in Di Lazzaro et al. (2010). The median stroke volume was 1463 mmc (range 653–26,514) for females and 2614 mmc (range 576–30,102) for males and it was not significantly different between males and females (Mann-Whitney U -test = 27.000, $p = 0.955$).

Baseline Brain Excitability Measures

Table 1 summarizes the gender-related difference in basal and iTBS-induced changes. Considering all patients together, UH excitability is higher than AH excitability, as probed by RMT, AMT and MEP amplitude [Factor *Hemisphere*: $p = 0.001$, $p = 0.001$, $p < 0.001$, respectively. **Figure 1** Upper Panel]. The effect of *Gender* on hemispheric excitability asymmetry, revealed by the *Hemisphere by Gender* interaction, is significant for AMT [$F_{(1, 32)} = 4.449$, $p = 0.043$], with a trend toward significance for RMT [$F_{(1, 32)} = 3.412$, $p = 0.074$], not significant for MEP amplitude [$F_{(1, 32)} = 0.511$, $p = 0.480$]. The *post-hoc* analysis reveals that AMT is significantly lower

TABLE 1 | Summary of the gender-related difference in basal and iTBS-induced changes.

DIFFERENT POST-STROKE FUNCTIONAL CHANGES	
On MTs	Males have higher AMT in the AH than in the UH ($p = 0.004$) Females have higher AMT in the UH than in the AH ($p = 0.056$)
On inter hemispheric balance	Males have higher inter-hemispheric asymmetry than females ($LI_{AMT} p = 0.033$ and $LI_{RMT} p = 0.042$) Males and Females have opposite inter-hemispheric balance (positive LI in males and negative in females)
DIFFERENT PROPENSITY TO UNDERGO PLASTIC CHANGES	
Pooling both hemispheres together	Females undergo a cumulative (pooling AH and UH together) increase of brain excitability, while males a decrease of it
Rate of iTBS effect	In the female group there is a higher rate of increase of AH excitability than in the male group ($p = 0.022$)

over the UH only in the *Male* group ($p = 0.004$), not in the *Female* group ($p > 0.200$) [**Figure 1** Lower Panel and **Tables 1, 2**].

Laterality Index

Both LI_{AMT} and LI_{RMT} show significant higher hemispheric asymmetry for the *Male* group ($p = 0.042$), while no significant difference has been found for LI_{MEP} (**Figure 2** and **Table 2**).

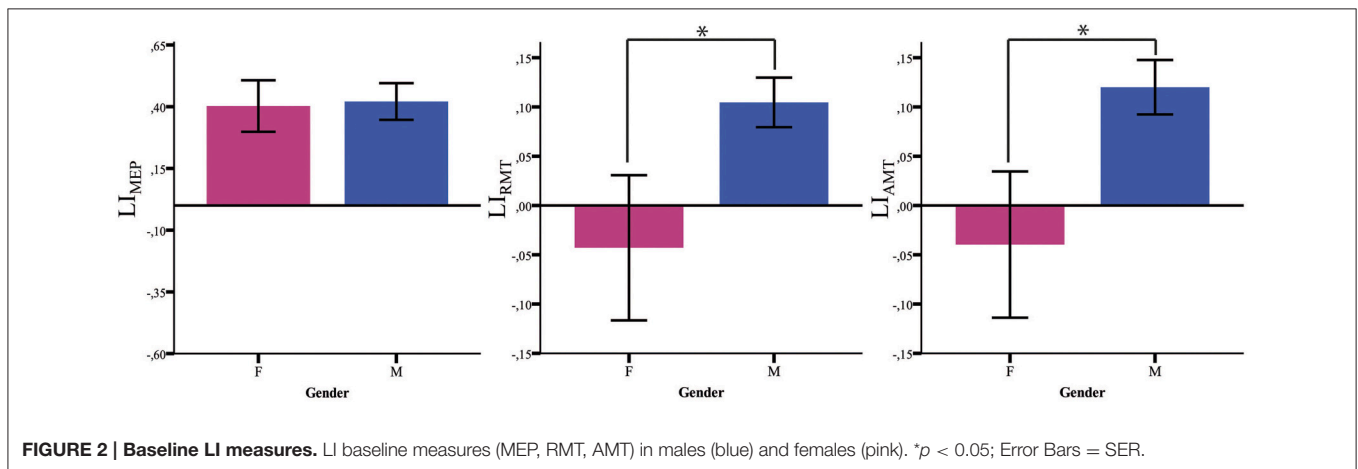
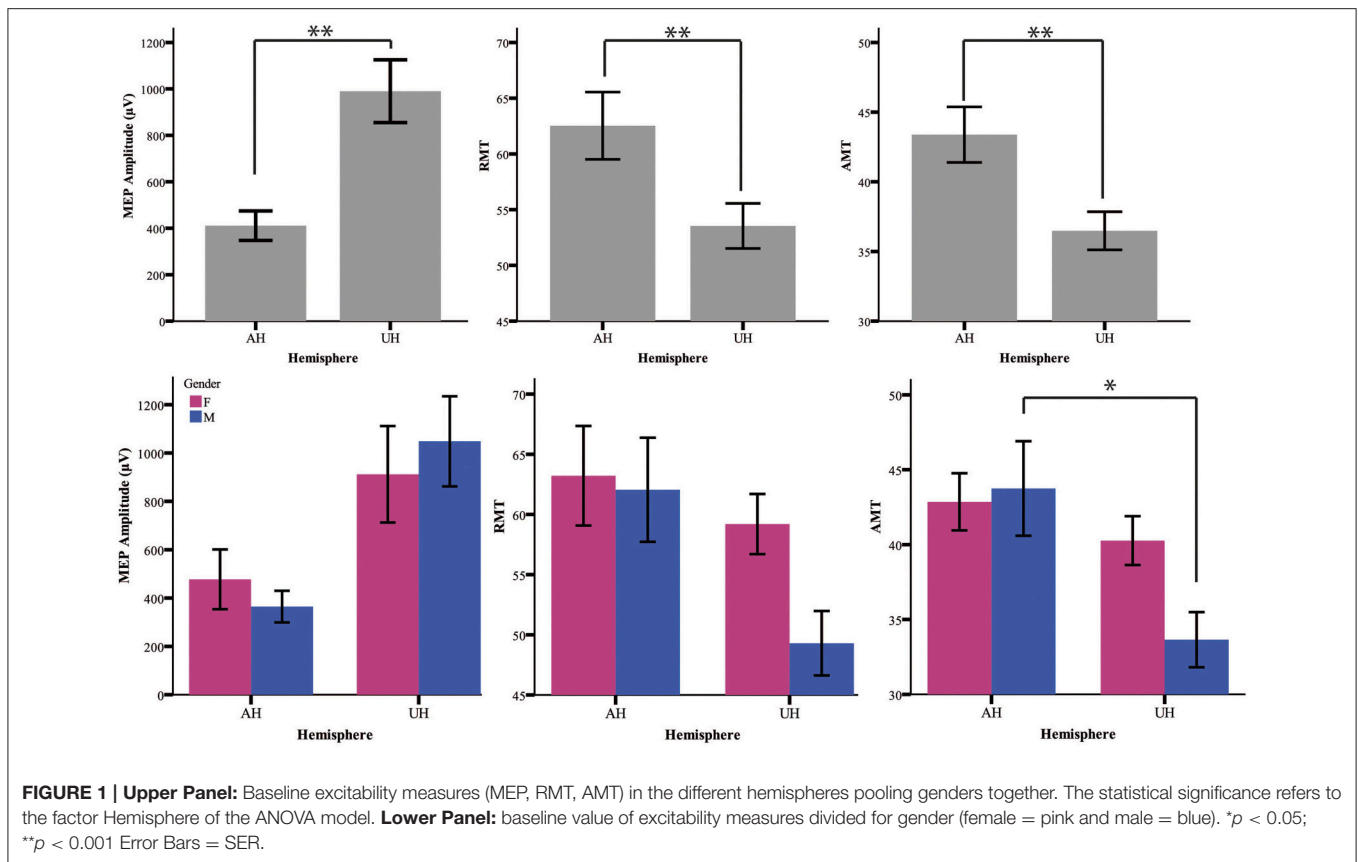
iTBS Effects

Our analysis shows an *iTBS by Hemisphere* interaction [RMT $p = 0.025$; AMT $p = 0.001$; MEP $p < 0.001$], suggesting that there is an effect of iTBS characterized by an excitability increase over the AH and an excitability decrease over the UH (**Figure 3**, **Tables 2, 3**). RMT shows that *Gender* influences *iTBS* effects on brain excitability [*iTBS by Gender* interaction $F_{(1, 32)} = 7.860$, $p = 0.009$]. This effect does not differ depending on the *Hemisphere* [*iTBS by Gender by Hemisphere* interaction $F_{(1, 32)} = 0.081$, $p = 0.777$]. The significant *iTBS by Gender* interaction is motivated by a cumulative (both hemispheres together) mild increase of brain excitability for *Females* and decrease of brain excitability for *Males* (**Figure 3**, **Tables 2, 3**). *Gender* does not significantly impact on the effect of *iTBS* on AMT and MEP.

Laterality Index

iTBS reduces the LI (LI_{RMT} , LI_{AMT} , LI_{MEP}) regardless of the *Gender* [*iTBS by Gender* interaction: $LI_{RMT} F_{(1, 33)} = 0.030$, $p = 0.864$; $LI_{AMT} F_{(1, 33)} = 0.223$, $p = 0.640$; $LI_{MEP} F_{(1, 33)} = 0.001$, $p = 0.979$]. More in details:

- LI_{RMT} : *Pre-iTBS* 0.04 ± 0.04 ; *Post-iTBS* 0.03 ± 0.04 , $p = 0.022$;
 - LI_{AMT} : *Pre-iTBS* 0.05 ± 0.04 ; *Post-iTBS* 0.03 ± 0.04 , $p = 0.001$;
- after *iTBS* LI_{AMT} remains negative for *Female* and positive for



Male [factor Gender: $F_{(1, 33)} = 4.842, p = 0.035$; Post-*i*TBS LI_{AMT} Male = 0.092 ± 0.028 ; Female = -0.060 ± 0.04];

- LI_{MEP} : Pre-*i*TBS 0.41 ± 0.06 ; Post-*i*TBS 0.30 ± 0.08 ; $p = 0.014$.

To better characterize *i*TBS effects, we also looked at the rate of subjects reporting *i*TBS-related effects in the two groups. The rate of *i*TBS-induced AH excitability increase is higher in Females (14 out of 15, 93%) than in Males (11 out of 20, 55%; Chi-square $p = 0.022$). Even if the comparison does not reach a significant level (Chi-square $p = 0.266$), the rate of *i*TBS-induced UH excitability decrease is higher in Males (16 out of 20, 80%) than in Females (9 out of 15 female, 60%).

The individual level of brain excitability and *i*TBS effects are reported in **Supplementary Figure 1**.

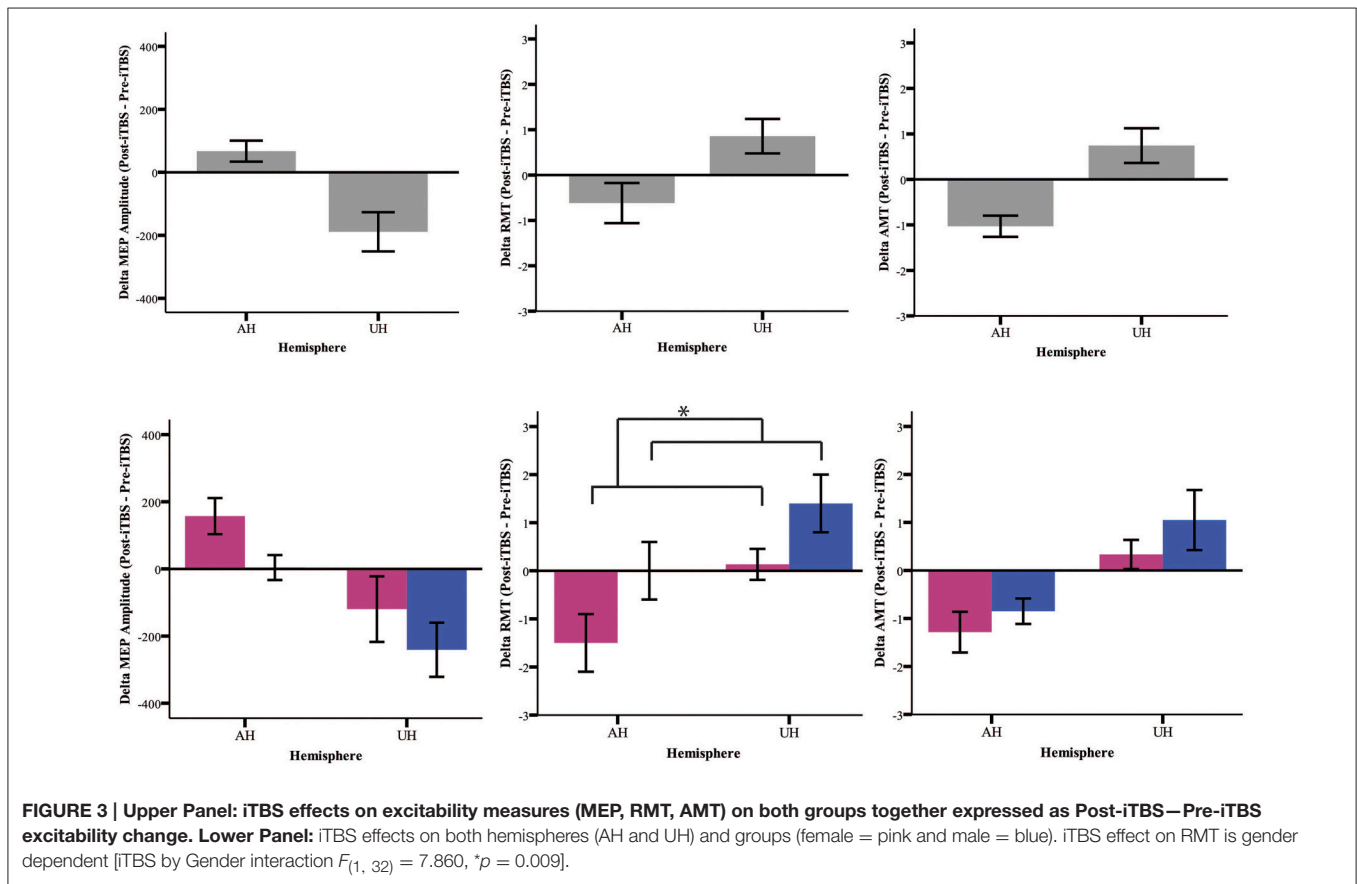
Relationship between Clinical Condition and Neurophysiological Measures

Pooling together all patients, NIHSS showed a significant correlation with the AMT LI both before *i*TBS (AMT LI pre-*i*TBS Pearson's $R = 0.328, p = 0.029$) and after *i*TBS (AMT LI post-*i*TBS Person's $R = 0.327, p = 0.030$). This correlation pattern did not survive the correction by sex, suggesting that sex might in fact play also a role in the relationship between measure of

TABLE 2 | Excitability measures for both AH and UH under baseline conditions and after iTBS.

		RMT						AMT						MEP					
		PRE			POST			PRE			POST			PRE			POST		
		AH	UH	LI	AH	UH	LI	AH	UH	LI	AH	UH	LI	AH	UH	LI	AH	UH	LI
F	Mean	63.21	59.20	-0.04	61.71	59.33	-0.06	42.86	40.27	-0.04	41.57	40.60	-0.06	477.42	911.95	0.40	634.75	792.27	0.29
	SER	4.14	2.50	0.07	4.35	2.49	0.07	1.91	1.63	0.07	1.94	1.63	0.07	123.64	199.45	0.10	170.69	160.87	0.12
M	Mean	62.05	49.30	0.10	62.05	50.70	0.09	43.75	33.65	0.12	42.90	34.70	0.09	364.51	1048.34	0.42	368.69	807.60	0.31
	SER	4.32	2.68	0.03	4.17	2.63	0.03	3.16	1.84	0.03	3.14	1.68	0.03	65.43	186.41	0.07	66.99	130.89	0.11
M+F	Mean	62.53	53.54	0.04	61.91	54.40	0.03	43.38	36.49	0.05	42.35	37.23	0.03	411.00	989.89	0.41	478.25	801.03	0.30
	SER	3.02	2.02	0.04	2.99	1.96	0.04	1.99	1.37	0.04	1.99	1.27	0.04	63.43	135.09	0.06	82.25	100.20	0.08

RMT, resting motor threshold, expressed as percentage intensity of maximal stimulator output; AMT, active motor threshold expressed as percentage intensity of stimulator output; MEP, amplitude of motor evoked potentials at 120% of RMT (μV).



cortical excitability and clinical status. The subsequent analysis performed independently on the two groups showed: (i) absence of correlation in the male group (ii) strong correlation in the female group. More in details, in this subgroup we confirmed the relationship between LI and NIHSS (AMT LI Pre-iTBS Pearson's $R = 0.500, p = 0.029$, AMT LI Post-iTBS Pearson's $R = 0.530, p = 0.021$). In other words, a worst clinical condition is associated to stronger interhemispheric unbalance toward higher excitability of the UH. Additionally, both before and after iTBS higher NIHSS scores were associated to higher AH AMT

(Person's $R = 0.601, p = 0.011$ before iTBS; Person's $R = 0.649, p = 0.006$, after iTBS).

DISCUSSION

Several studies have reported an asymmetry in the excitability of the AH and UH to non-invasive brain stimulation after stroke (Liepert et al., 2000; Manganotti et al., 2002; Shimizu et al., 2002; Cicinelli et al., 2003; Di Lazzaro et al., 2010, 2014). This is the first study evaluating the effects of gender on the changes in human

TABLE 3 | Summary of the ANOVA Mixed Model on iTBS effects on brain excitability measures.

Source	RMT		AMT		MEP	
	F(1, 32)	P	F(1, 32)	P	F(1, 32)	P
Hemisphere	9.462	0.004	8.779	0.006	12.778	0.001
iTBS	0.040	0.843	0.493	0.488	2.284	0.140
Gender	1.192	0.283	0.854	0.362	0.238	0.629
Hemisphere * iTBS	5.558	0.025	13.761	0.001	17.568	0.000
Hemisphere * Gender	3.465	0.072	4.316	0.046	0.834	0.368
iTBS * Gender	7.860	0.009	1.395	0.246	2.575	0.118
Hemisphere * iTBS * Gender	0.081	0.777	0.013	0.909	0.226	0.638

brain excitability observed in the acute phase of stroke. We found sex differences in the functional changes that take place in AH and UH. The AH showed a lower excitability than the UH in both men and women, but males have higher excitability in the UH (lower AMT) and higher inter-hemispheric asymmetry than females. At a group level, the excitability of the AH is always lower than of the UH. However, the study of the LI, which takes into consideration and normalizes subject by subject for the level of excitability of both hemispheres, reveals that males and females have opposite inter-hemispheric balance, with higher excitability of UH in males, *vice versa* in females.

The meaning of these findings is still uncertain; we can speculate that they might be correlated with the existence of gender-related differences in the organizational patterns of functional cortical connectivity between different brain areas. Several studies have demonstrated sex differences in the connectivity of the brain (Gong et al., 2011). The results of the analysis of the structural connectome of the human brain suggest that male brains are structured to facilitate intra-hemispheric cortical connectivity, while female brain displays higher strength of inter-hemispheric connectivity (Ingalhalikar et al., 2014). Thus, we can speculate that to facilitate within-hemisphere connectivity in males there is a higher level of inter-hemispheric inhibition and, in case of a mono-hemispheric brain lesion, a lower level of inter-hemispheric inhibition from AH to UH makes the UH hyper-excitable to transcranial stimulation.

When testing the effects of iTBS of the AH, a rTMS protocol capable of inducing LTP-like changes in the stimulated hemisphere, females undergo a cumulative (pooling AH and UH together) increase of brain excitability, while males a decrease of it. In the female group, there is a higher rate of increase of AH excitability than in the male group and a tendency for a lower rate of decrease of UH excitability. Again men showed a pronounced effect in UH with a partial correction of the hyper-excitability associated with a comparable increase in the excitability of AH. In women, instead, we observed a more pronounced increase in the excitability of the AH that was associated with a slight increase in excitability, and not a suppression, of UH.

This is a further demonstration that the establishment of inter-hemispheric imbalance after stroke should not be given for

grant, rather it is strictly dependent on patient's individuality. We recently showed that also the haplotype of Brain-Derived Neurotrophic Factor (BDNF) gene has a profound influence on the inter-hemispheric imbalance in cortical excitability (Di Lazzaro et al., 2015). Indeed, the presence of the Val66Met BDNF polymorphism is associated with a nine-fold weaker inter-hemispheric imbalance in cortical excitability as evaluated by comparing the RMT of the AH and the UH.

Is the imbalance in cortical excitability deleterious for recovery? As we suggested for BDNF polymorphism (Di Pino et al., 2014a, 2016), the hyper-excitability of UH might contribute to, or might interfere with, recovery depending on the level of impairment of AH. UH over-activity, observed more commonly in males, might interfere with paretic limb function in patients with less severe damage, while it might have a compensatory role in severely affected patients (Bradnam et al., 2013; Di Pino et al., 2014a). On the other hand, the limited imbalance between the hemispheres in females might represent an advantage in case of limited damage, facilitating the recovery of AH in the absence of a potentially deleterious interference from the UH. However, in more severe lesions the compensatory role of UH seems to be prevalent, and this might be limited in females by their lower UH hyper-excitability. Overall, this would reduce the impact of mild stroke in females and of more severe stroke in males, in line with a lower incidence, but a poorer prognosis of stroke in females and *vice versa* in males (Gibson, 2013).

Moreover, the differential functional changes that take place in the AH and UH in males and females might be adaptive or maladaptive depending on the degree of corticospinal tract damage. Gender influences many aspects of stroke including risk/incidence, diagnosis, symptoms, treatment and outcomes (Reeves et al., 2008; Appelros et al., 2009; Haast et al., 2012; Gibson, 2013); our study strongly contributes to highlight that it also influences the brain response to the damage.

Those considerations warrant further studies aimed at characterizing the interactions that gender and inter-hemispheric imbalance have on recovery.

In conclusion, our study suggests the existence of gender-dependent differences in the functional brain changes that take place after human stroke, in that it seems that male brain has greater asymmetry than the female's. This perfectly fits the recently advanced hypothesis of a higher strength of inter-hemispheric connection owned by the female's healthy brain (Ingalhalikar et al., 2014).

Male and female individuality could conceivably arise from a complex interaction of some sort of gender-specific base with a mosaic of environmental factors. Stroke and its strong plasticity-inducing potential are, in our opinion, optimal examples of events that might unveil and amplify those gender-specific differences, that otherwise might remain unrevealed. Our findings should suggest to be cautious in designing stroke studies, especially since sex differences in stroke that might affect recovery and brain plasticity probably result from a combination of factors, including elements intrinsic to the sex chromosomes, as well as the effects of sex hormone exposure, and not less important cultural and social factors (Cox et al.,

2006; Vagnerova et al., 2008; Cesaroni et al., 2009; Liu et al., 2009; Gibson, 2013). For instance, animal models are often used to provide a better understanding of stroke and of specific brain recovery patterns (Alkayed et al., 1998; Bacigaluppi et al., 2010). However, the majority of experimental stroke studies keeps focusing on using only male animals (Fisher et al., 2009; Gibson, 2013), despite the Stroke Therapy Academic Industry Roundtable (STAIR) recommends that neuro-protective studies should be performed in both male and female rodents (Fisher et al., 2009). Moreover, the impacts of gender on the weight of age and hormone-related risk factors needs to be clarified, since epidemiological studies document an association between the female gender during the premenopausal years and a reduced risk of stroke addressing hormonal factors as potential protective treatments (Gibson et al., 2006, 2009; Suzuki et al., 2009; Liu and Yang, 2013). We envisage that a greater experimental plan and the understanding of the mechanisms underlying gender-related differences in stroke and responsiveness to neuroprotection and brain plasticity will lead

to more appropriate treatment strategies for patients of both sexes.

AUTHOR CONTRIBUTIONS

VD designed the study and wrote the manuscript. GP was in charge of statistic and revised the manuscript, GDP, FR, FL, LF, and FC participated to patients' recruitment, neuromodulation and revised the manuscript. All Authors approved the final version of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fnins.2016.00010>

Supplementary Figure 1 | Scatterplot graph of individual brain excitability levels (Post-iTBS over Pre-iTBS) for RTM, AMT and MEP Amplitude for the Affected (AH) and Unaffected (UH) hemispheres.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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