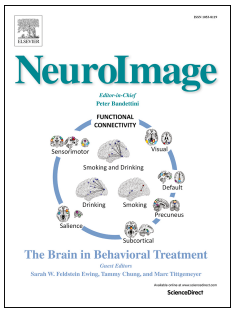
Accepted Manuscript



Dynamic reorganization of TMS-evoked activity in subcortical stroke patients

Maria Concetta Pellicciari, Sonia Bonnì, Viviana Ponzo, Alex Martino Cinnera, Matteo Mancini, Elias Paolo Casula, Fabrizio Sallustio, Stefano Paolucci, Carlo Caltagirone, Giacomo Koch

PII: S1053-8119(18)30299-4

DOI: 10.1016/j.neuroimage.2018.04.011

Reference: YNIMG 14858

To appear in: *NeuroImage*

Received Date: 14 September 2017

Revised Date: 4 April 2018

Accepted Date: 6 April 2018

Please cite this article as: Pellicciari, M.C., Bonnì, S., Ponzo, V., Cinnera, A.M., Mancini, M., Casula, E.P., Sallustio, F., Paolucci, S., Caltagirone, C., Koch, G., Dynamic reorganization of TMS-evoked activity in subcortical stroke patients, *NeuroImage* (2018), doi: 10.1016/j.neuroimage.2018.04.011.

Dynamic reorganization of TMS-evoked activity in subcortical stroke patients

Maria Concetta Pellicciari[1], Sonia Bonnì[1], Viviana Ponzo[1], Alex Martino Cinnera[1], Matteo Mancini[2], Elias Paolo Casula[1], Fabrizio Sallustio[3], Stefano Paolucci[4], Carlo Caltagirone[1,5], Giacomo Koch[1,3*].

[1]*Non-invasive Brain Stimulation Unit, Department of Behavioral and Clinical Neurology, Santa Lucia Foundation IRCCS, Rome, Italy*

[2]*Centre for Medical Image Computing, University College London, London, UK*

[3] *Stroke Unit, Tor Vergata Hospital, Rome, Italy*

[4]*Clinical Laboratory of Experimental Neurorehabilitation, Santa Lucia Foundation IRCCS, Rome, Italy*

[5]*Department of System Medicine, Tor Vergata University, Rome 00133, Italy*

***Corresponding author:**

Giacomo Koch, MD, PhD

Non Invasive Brain Stimulation Unit, Department of Behavioural and Clinical Neurology
IRCCS Santa Lucia Foundation
Via Ardeatina 306, 00179 Rome, Italy
Tel: +39 0651501181
E-mail: g.koch@hsantalucia.it

Conflicts of Interest: None

Keywords: stroke, recovery, TMS-EEG, cortical excitability, oscillatory activity, motor cortex, parietal cortex.

Abbreviations: TMS, transcranial magnetic stimulation; EEG, electroencephalography; MEP, motor-evoked potential; TEP, TMS-evoked potential; M1, primary motor cortex; PPC, posterior parietal cortex; ICA, independent component analysis; RMT, resting motor threshold; GMFP, global mean field power; EOR, evoked oscillatory response; MRI, magnetic resonance imaging; MCA, middle cerebral artery; AH, affected hemisphere; UH, unaffected hemisphere; FMA, Fugl-

Meyer Assessment; BBS, Berg Balance Scale; BI, Barthel Index; SSQoL, Quality of Life scale; NIHSS, National Institutes of Health Stroke Scale; GABA, gamma-aminobutyric acid.

Funding: This study was funded by grants of the Italian Ministry of Health (GR-2013-02358852 to SB and MCP and RF-2011-02349953 to GK).

Abstract

Since early days after stroke, the brain undergoes a complex reorganization to allow compensatory mechanisms that promote functional recovery. However, these mechanisms are still poorly understood and there is urgent need to identify neurophysiological markers of functional recovery after stroke. Here we aimed to track longitudinally the time-course of cortical reorganization by measuring for the first time EEG cortical activity evoked by TMS pulses in patients with subcortical stroke.

Thirteen patients in the sub-acute phase of ischemic subcortical stroke with motor symptoms completed the longitudinal study, being evaluated within 20 days and after 40, 60 and 180 days after stroke onset. For each time-point, EEG cortical activity evoked by single TMS pulses was assessed over the motor and parietal cortex of the affected and unaffected hemisphere. We evaluated global TMS-evoked activity and TMS-evoked oscillations in different frequency bands. These measurements were paralleled with clinical and behavioral assessment.

We found that motor cortical activity measured by TMS-EEG varied across time in the affected hemisphere. An increase of TMS-evoked activity was evident at 40 days after stroke onset. Moreover, stroke patients showed a significant increase in TMS-evoked alpha oscillations, as highlighted performing analysis in the time-frequency domain. Notably, these changes indicated that crucial mechanisms of cortical reorganization occur in this short-time window. These changes coincided with the clinical improvement. TMS-evoked alpha oscillatory activity recorded at baseline was associated to better functional recovery at 40 and 60 days' follow-up evaluations, suggesting that the power of the alpha rhythm can be considered a good predictor of motor recovery. This study demonstrates that cortical activity increases dynamically in the early phases of recovery after stroke in the affected hemisphere. These findings point to TMS-evoked alpha oscillatory activity as a potential neurophysiological markers of stroke recovery and could be helpful to determine the temporal window in which neuromodulation should be potentially able to drive neuroplasticity in an effective functional direction.

1. Introduction

Stroke is a prominent source of permanent adult disability, resulting in huge social costs. Among the clinical damages, post-stroke motor impairment represents one of the most relevant, influencing strongly the rehabilitation process (Bonita and Beaglehole 1988; Lai et al., 2002). Despite its devastating impact, the neural mechanism at the basis of functional recovery is still poorly understood. This is especially relevant to favor the development of tailored therapeutic strategies (for a review see, Di Pino et al., 2014).

Following stroke, there is a time-limited window of neuroplasticity during which the greatest gains in recovery occur (Murphy and Corbett, 2009). The choice of any neuro-rehabilitative intervention must necessarily take into account that, after the stroke event, the brain undergoes several stages of recovery reorganizing spontaneously neural circuitry and giving rise to neuroplasticity phenomena (for a review see, Carmichael, 2003; Rossini et al., 2003; Nowak et al., 2009).

At this regard, surrogate biomarkers of spontaneous recovery from stroke and restored neuronal networks, as predictive tools of functional outcome, are increasingly needed (Milot and Cramer et al., 2008; Burke and Cramer, 2013). Understanding the evolution of neurophysiological recovery and its cortical markers, might be useful in guiding ad-hoc neuro-rehabilitative interventions, such as non-invasive brain treatments (Hummel and Cohen, 2006; for a review see, Sale et al., 2015; Kubis, 2016).

Neurophysiological changes occurring in spontaneous manner during the weeks to months after cerebral damage have been widely characterized by various techniques (e.g. functional magnetic resonance imaging, MRI; positron emission tomography; electroencephalography, EEG; and transcranial magnetic stimulation, TMS) capable to map local blood flow and metabolic changes linked with neuronal firing or able to detect cortical excitability (for a review, see Auriat et al., 2015). Nowadays, several studies using different neuroimaging approaches have investigated longitudinally and in vivo the specific role of brain areas for functional recovery in order to understand the underlying mechanisms of the clinical course of post-stroke spontaneous recovery

(Marshall et al., 2000; Ward et al., 2003; Bashir et al., 2010) and to detect cortical targets and critical periods for therapeutic intervention (Calautti et al. 2001; Feydy et al., 2002; Freundlieb et al., 2015).

Residual motor functioning and post-stroke recovery processes related to specific patterns of cortical activity and neuronal excitability, have been highlighted (Grefkes and Ward, 2013; Stinear and Ward, 2013; Rehme and Grefkes, 2013; Grefkes and Fink, 2014), as well as abnormal brain activity in the affected and unaffected hemisphere (AH and UH) have been associated to incomplete motor recovery (Ward et al., 2003; Grefkes et al., 2008).

Nevertheless, until now, motor cortical excitability changes following stroke over ipsilesional and contralesional hemispheres and their underlying mechanisms have been indirectly inferred by assessing the modifications in corticospinal excitability using motor-evoked potentials (MEPs) (Di Lazzaro and Ziemann, 2013). However, the functional evaluation of motor physiology through TMS applied over the primary motor cortex (M1) must take in account the difficulty to investigate the neurophysiological correlates of a paretic hand unable to produce a measurable MEP, which can be absent in most patients, especially in the early phases after stroke (Byblow et al., 2015). Although TMS can be used to identify patients with 'notable' potential for recovery, especially when combined with structural MRI and with standard clinical examination (i.e. Stinear et al., 2012), the lack of MEP recordings can limit the potential of TMS in predicting functional recovery.

Considering that in the first weeks/months after stroke, changes in motor cortex excitability are dynamic as a function of time and recovery (Byrnes et al. 1999; Thickbroom et al. 2002), the application of novel multimodal approaches paves the way for non-invasive insights into the neural mechanisms underlying recovery of function and reorganization of motor cortical networks.

In the present study, we sought to determine whether a novel multimodal neuroimaging approach that combines TMS with EEG (Ilmoniemi and Kicic, 2010) could be effective in tracking cortical reorganization in patients with stroke.

Albeit EEG is potentially a sensitive method to detect functional changes due to regional brain pathology after an ischemic stroke (Moyanova and Dijkhuizen, 2014), the limited spatial resolution reduces its usefulness. At this regard, the integration of EEG with TMS might add synergetic effects for the assessment of neural processing through objective measurements of cortical reactivity and oscillatory dynamics (Borich et al., 2016). Specifically, the combined use of TMS and EEG can offer measures of brain responses and functionality both in healthy and pathophysiological conditions (Julkunen et al., 2011; Ragazzoni et al., 2013; Rosanova et al., 2013; Pellicciari et al., 2017; Casula et al., 2017; Koch et al., 2018).

TMS-evoked potentials (TEPs), the electrophysiological responses induced by TMS, represent a cortical measure in nature, not influenced by non-cortical confounds such as spinal cord excitability, which can limit MEP-based measures of cortical excitability (Chung et al., 2015). Moreover, TEPs, reflecting the direct activation of the cortical neurons at the site of the stimulation can be used to estimate regional excitability of the motor and extra-motor cortices (Lioumis et al., 2009; Kahkonen et al., 2005) as well as in clinical condition characterized by disrupted sensorimotor input/output pathways (Sato et al., 2015).

The general aim was to promote this approach as method to characterize not only the hemispheric changes but also the critical cortical excitation-inhibition balance in the stroke. Starting from this scenario, we report the first results of an ongoing longitudinal study, in which we investigated both spontaneous cortical activity and evoked cortical reactivity, using a multimodal experimental approach. We employed TEPs as a novel probe of cortical excitability re-organization of motor and parietal cortices of affected and unaffected hemispheres. TMS-evoked oscillatory response (i.e., EOR) was also analyzed to investigate directly their cortical oscillatory activity and functional connectivity (for a review see Rogasch and Fitzgerald, 2012). To track the time-course of cortical reorganization following stroke, we collected the data from sub-acute stroke patients until six months after the ischemic event. Additionally, with the aim to characterize the cortical patterns after

stroke event, we compared spontaneous cortical activity, TEPs and EOR of our patient samples with that obtained from a population of control healthy subjects.

2. Materials and Methods

2.1. Stroke patients

Seventy consecutive patients with a history of first ever unilateral, subcortical ischemic stroke, admitted at the Santa Lucia Foundation for a standard rehabilitation, were screened for inclusion in this study. Lesions were considered as subcortical if involved the deep white matter inferior to the corpus callosum, including the internal capsule, thalamus, and basal ganglia. Inclusion criteria were as follows: 1) ischemic stroke in the middle cerebral artery (MCA) territory; 2) neuroradiological diagnosis of ischemic brain damage 3) < 20 days' post stroke; 4) moderate to moderately severe upper extremity hemiparesis (UEFMA score: 28-50). Exclusion criteria were as follows: 1) seizure history; 2) hemorrhagic stroke; 3) concomitant neurological disorders or other medical serious complications; 4) cardiac pacemaker; 5) inability to give informed consent; 6) any contraindications for TMS (Rossi et al., 2015).

Twenty-four patients resulted eligible according to the inclusion and exclusion criteria but only twenty of them agreed to participate in the study. Two patients were excluded for the occurrence of a second ischemic event during the 6-months of follow-up and five patients were not able to take part in every follow-up evaluations and were discarded from the study. Finally, a total of 13 stroke patients (mean age: 60.3 ± 12.1 years; 9 males) with ischemic lesions (8 right-sided and 5 left-sided) have completed the study. Demographic and clinical characteristics of stroke patients are shown in Table 1.

A group of 10 age-matched healthy volunteers (mean age 59.2 ±12.3 years; 3 males) were used as a control group. All control subjects had no history of neurological or psychiatric disease, were under no pharmacological treatment and did not report contraindications for TMS. The control group was used to evaluate the cortical activity in healthy condition.

All participants, right-handed according to the Edinburgh Handedness Inventory (Oldfield, 1971). provided informed written consent approved from the local Ethical Committee. The study was conducted in accordance with the Code of Ethics expressed in the Declaration of Helsinki.

Table 1 about here

2.2 General procedure

To investigate the time-course of cortical and clinical re-organization following stroke, neurophysiological assessments and motor/clinical evaluations were achieved at baseline, i.e. within 20 days (t0), and at several time-points during the follow-up period, specifically at: 40 (t1), 60 (t2) and 180 days (t3) after stroke onset. Moreover, a neurophysiological assessment was performed also in an age-matched healthy volunteers group, to compare the normal and dysfunctional cortical patterns at baseline. At this regard and according to the study design, both the hemispheres were evaluated in healthy control subjects. The assignment of AH and UH in the healthy subjects was based matching their age with that of stroke patients.

For each time-point and each participant, neurophysiological assessment began with an EEG session followed by TMS-EEG sessions. Each EEG session consisted of a 3 min recording during an open-eyes resting state. In each TMS-EEG session, 80 single TMS pulses were applied at a random inter-stimulus interval of 0.25-0.5 Hz with an intensity of 90% of the resting motor threshold (rMT) over motor (M1) and parietal cortices (PPC), both ipsilateral (affected hemisphere, AH) and contralateral (unaffected hemisphere, UH) to the infarct, in counterbalanced manner. During the neurophysiological recordings, the participants were seated on a dedicated, comfortable armchair in a Faraday-cage, sound-proofed room and were instructed to keep their hands completely relaxed, passively sitting and fixing their eyes on a visual target directly in front of them.

Neurophysiological assessments were paralleled with standardized motor and clinical evaluations, both at baseline and at each time-points. For the statistical analysis, the sphericity of data was tested

with Mauchly's test; when sphericity was violated (i.e. Mauchly's test < 0.05), the Greenhouse-Geisser correction was used. Bonferroni-adjusted pairwise comparisons were then performed. The p-values less than 0.05 were considered significant. All statistical analyses were performed using SPSS 23.0 (SPSS Inc., Chicago, IL, USA). ANOVAs were performed for statistical analysis ($p<0.05$) and Bonferroni adjusted multiple comparisons were performed as post-hoc tests.

2.3. EEG and TMS/EEG recordings

A TMS-compatible EEG equipment (BrainAmp 32MRplus, BrainProducts GmbH, Munich, Germany) was used to record the EEG activity from the scalp. The EEG was continuously acquired from 29 scalp sites positioned according to the 10-20 International System, using TMS-compatible Ag/AgCl sintered ring electrodes mounted on an elastic cap. Additional electrodes were used as ground and reference. The ground electrode was positioned in AFz, while an active reference was positioned on the tip of the nose. The EEG and EOG signals were band-pass filtered at 0.1-1000 Hz and digitized at a sampling rate of 5 kHz. Skin/electrode impedance was maintained below 5 kΩ. Horizontal and vertical eye movements were detected by recording the electro-oculogram (EOG), to monitor participant behavior on line and to reject off-line the trials with ocular artifacts.

2.4 TMS

Single-pulse TMS was carried out by a Magstim Super Rapid magnetic stimulator connected to one booster module and a standard figure-of-eight shaped coil with an outer winding diameter of 70 mm (Magstim Company, Whitland, UK) that generates 2.2 T as a maximum output. To define the rMT, the coil was placed tangentially to the scalp, both over the left and right M1, with the handle pointing backwards and laterally, at about 45° angle from the mid-sagittal axis of the participant's head, so that the direction of current flow in the second phase was anteromedial-posterolateral. The stimulation started at a supra-threshold intensity. The optimal stimulus sites to elicit motor evoked potentials (MEPs), both in the right and left first dorsal interosseous (FDI), termed the "motor

hotspots", were identified by positioning the coil approximately over the central sulcus and moving it on the scalp by 0.5 cm steps on left and right M1. On this site, rMTs was assessed as the lowest stimulus intensity needed to produce a response of at least 50 μV in amplitude in the relaxed muscle for at least 5 out of 10 consecutive trials, at a resolution of 1% of the maximal stimulator output (Rossini et al., 2015). If no MEPs were evoked in AH at maximum stimulator output, then the intensity was set to 90% of rMT of UH. In order to target left and right PPC, the coil was positioned over the caudal part of the intraparietal sulcus approximately in correspondence with P3 and P4 and orientated 15° from the midline, so that current was induced in a posterior-anterior direction (Koch et al. 2007; Koch et al., 2013). For PPC, both hemispheres were symmetrically stimulated. To ensure a high degree of reliability during and across each recording session, the coil positioning and orientation on the hotspot were constantly online monitored by means of the SofTaxic neuronavigation system (EMS, Bologna, Italy) coupled with a Polaris Vicra infrared camera (NDI, Waterloo, Canada) (Carducci and Brusco, 2012).

2.5 Motor and clinical evaluations

Fugl-Meyer Assessment scale (FMA; Fugl-Meyer et al., 1975) and Berg Balance Scale (BBS; Berg et al., 1991) were used to measure the patient's sensory-motor and balance functions, whereas to evaluate the ability in daily living, quality of life and more global aspects of clinical recovery, the Barthel Index (BI; Colin et al., 1988), Specific Stroke Quality of Life scale (SSQoL; Williams et al., 1999) and National Institutes of Health Stroke Scale (NIHSS; Brott et al., 1989) were administered.

3. Data analysis

3.1 Behavioral and clinical data analysis

In stroke patients, for each behavioral and clinical outcome assessed in the different times of evaluation, a repeated-measures ANOVA or, when appropriate, a non-parametric Friedman test was

used with Time (t0, t1, t2 and t3) as within-subject factor. A non-parametric Wilcoxon rank sum test was adopted for post-hoc comparison.

3.2 EEG analysis

EEG data acquired during the resting state were analyzed off-line with dedicated software (Brain Vision Analyzer, Brain Products GmbH, Munich, Germany), separately for the baseline in both groups (healthy subjects and stroke patients) and for each time point following stroke (t0, t1, t2, t3). Continuous EEG recordings were segmented in 2-second epochs and those with excessive drift, eye movements, blinks or muscle artifacts were excluded from the analysis. Power density was estimated by means of the fast Fourier transform (10% Hanning-window; frequency resolution 1 Hz) for all the frequencies ranging from 2 to 45 Hz, and divided into four bands as follows: delta (2-4 Hz), theta (5-7 Hz), alpha (8-12 Hz) and beta (13-30 Hz). In line with previous EEG studies (Dubovik et al., 2012; Gerloff et al., 2006; Sheorajpanday et al., 2011; Nicolo et al., 2015; Thibaut et al., 2017), gamma band oscillations were not investigated due to difficulty to separate this frequency from muscular artifact and background noise. The mean band power was then obtained by averaging the power values of all the single-trial epochs for each participant, normalizing such values using the total spectral power. We calculated the total spectral power as the sum of all the channel powers for each frequency band. For the baseline comparison between the healthy subjects and stroke patients, a repeated-measures mixed ANOVA was performed with the spectral power as dependent variable, Group (healthy control subjects and stroke patients) as between-subjects factor and frequency bands as within-subject factor (delta, theta, alpha and beta). In order to investigate if there were significant changes across the four time-points of evaluation for the stroke patients, we performed repeated measure ANOVAs using total spectral powers, separately for each frequency band.

3.3 TMS-EEG analysis

TMS-EEG data were analyzed off-line (Brain Vision Analyzer, Brain Products GmbH, Munich, Germany), with different approaches both in time and frequency domains. Independent component analysis (ICA) was then used to identify and remove components reflecting residual muscle activity, eye movements, blink-related activity, and residual TMS-related artifacts. After these steps, the artifact induced by pulse delivery was removed using an interpolation for a conservative interval from 1 ms before to 10 mafter the TMS pulse. Consequently, the first 10 ms following the pulse were excluded from the analysis. Bad channels were interpolated using spherical interpolation function when needed. The signal was re-referenced offline to the mean signal across all electrodes, downscaling (1000 Hz), band-pass filtered (1 and 80 Hz, Butterworth zero phase filters, with a 50 Hz notch filter). Epochs with excessively noisy EEG, eye-movement artifacts or muscle artifacts were excluded from the analysis after a visual inspection.

3.4 Time-domain analysis

To evaluate the cortical response to TMS in the time-domain, the TMS-evoked response was averaged in the whole epoch from 100 before to 500 ms after single TMS pulse, for each time-point of evaluation and stimulated area. All epochs were baseline corrected to a time period of 100 ms recorded before TMS pulse. The time course of the total EEG response to TMS was determined by calculating the global mean field power (GMFP) as follows:

$$GMFP(t) = \sqrt{\frac{[\sum_i^k (V_i(t) - V_{mean}(t))^2]}{K}}$$

where *t* is time, *K* the number of channels, V_i the voltage in channel *i* averaged across participants, and V_{mean} is the mean of the voltages in in all the channels (Lehmann and Skrandies, 1980). In order to obtain temporal indices of cortical excitability evoked by the delivering of TMS pulse, the GMFP was cumulated within three temporal windows following TMS-pulse: 10-50 (Peak 1), 50-100 (Peak

2) and 100-150 ms (Peak 3) (Romero Lauro et al., 2014). In the first instance, the cortical excitability at baseline evaluation (t0) was compared by means of a repeated-measures mixed ANOVA, with GMFP values as dependent variable, Group (healthy control subjects and stroke patients) as between-subjects factor, and Hemisphere (AH and UH) and Peak (P1, P2 and P3) as within-subject factors. To longitudinally evaluate the cortical excitability changes following stroke, a repeated-measure ANOVA with factors Hemisphere (AH and UH), Time (t0, t1, t2 and t3) and Peak (P1, P2 and P3) was performed on GMFP values of each time point of evaluation.

3.5 Time/Frequency-domain analysis

To evaluate the cortical response to TMS in the time/frequency domain from the different cortical sites of AH and UH, the evoked oscillatory response was detected in epochs starting 1 s before to 1 s after the TMS pulse. A time/frequency decomposition based on a complex Morlet wavelet transform (2-40 Hz, 38 frequency steps, c = 3.5) was applied to averaged epochs in each participant normalizing the data to a window of 500 ms preceding TMS onset. The global EOR (Pellicciari et al., 2017) was computed by averaging the oscillatory activity of all channels in each time point of evaluation. To minimize the effect of possible artifacts occurring at the time of stimulation, the frequency values were calculated by averaging the EOR values over a 20-200 ms time window, corresponding to the main activity evoked by single TMS pulse (see results section). Subsequently, the spectral power in the frequency ranges between 2-4 Hz (delta), 4-7 Hz (theta), 8-12 Hz (alpha) and 13-30 Hz (beta) was extracted from the wavelet dataset. The EOR values were compared between groups (healthy control subjects and stroke patients) at baseline evaluation by means of mixed repeated-measures ANOVA with Hemisphere (AH and UH) and Frequency (delta, theta, alpha and beta) as within-subject factor. To longitudinally assess the changes of cortical oscillations evoked by TMS, the EOR was compared among each time point of evaluation, by means of repeated-measure ANOVA with factors Time (t0, t1, t2 and t3) and Hemisphere, separately for each frequency band.

Correlations were performed to investigate possible predictive value of TMS/EEG based oscillatory patterns and functional outcomes. Pearson's correlations were carried out between the EOR at baseline and each measure of clinical outcomes (i.e. FMA, BBS and NIHSS), in both the hemispheres and in each time point of evaluation.

4. Results

4.1 Behavioral and Clinical outcomes

A significant effect of the factor Time was observed both in the clinical and behavioral scales. Specifically, the FMA scores showed a significant difference between t0 and all time-points after stroke ($\chi^2_{13,3}$ =19.77, p<0.001). Post-hoc analysis revealed a significant improvement from t0 to t1 (Z= 3.17), t2 (Z= 3.11) and t3 (Z=3.04) (all ps< 0.01). BBS showed significant changes between t0 and all times of evaluation ($\chi^2_{13,3}$ =24.30; p<0.001), with an improvement of balance functions from t0 to t1 (Z= 3.06), t2 (Z= 3.18) and t3 (Z= 3.07) (all ps< 0.01). Moreover, a balance improvement was observed from t1 to t2 (Z= 2.29, p<0.05). A significant effect was observed also in the ability of daily living evaluated through BI ($\chi^2_{13,3}$=24.29, p<0.001), Specifically, post-hoc analysis revealed a significant improvement from t0 to t1 (Z= 2.93), t2 (Z= 3.05) and t3 (Z= 2.98) (all ps<0.01), from t1 to t2 (Z= 2.54, p=0.01) and to t3 (Z=2.22, p<0.05). Moreover, an enhancement of quality of life, as assessed by means of SSQoL, was detected ($F_{3,36}$=14.85; p<0.001) between t0 and t1, t2 and t3 (all ps<0.001). Finally, a regression of signs and symptoms in the NIHSS ($F_{3,36}$=8.83; p<0.001) was reported by stroke patients from t0 to t1, t2 and t3 (all ps<0.05). For all scales, no further difference was highlighted in the comparisons between the other time points (Figure 1).

Figure 1 about here

4.2 Spontaneous EEG activity

No significant differences in all frequency bands was found both between the two groups (i.e., healthy control subjects and stroke patients) at baseline comparison and longitudinally in stroke patients (all ps>0.05).

4.3 TMS-evoked cortical activity

The TMS-EEG procedure was well-tolerated in each participant. In the healthy subjects group, the TMS intensity was: 61.1±10.2% and 62.4±9.3% of maximum stimulator output (MSO), respectively in AH and UH. In the stroke patients, the TMS intensity was: 62.1±13.8% (AH) and 61.6± 12.9 (UH) at t0; 64.8±13.1 (AH) and 62.7±12.1 (UH) at t1; 66.1±13.9 (AH) and 62.5±12.3 (UH) at t2; 68.5±15.3 (AH) and 62.6±9.9 (UH) at t3.

Stimulation of M1 and PPC resulted in a sequence of positive and negative polarity deflections starting a few milliseconds post-stimulation, as reported in a previous study (Casula et al., 2016). Figures 2 and 3 illustrate the grand-averaged TEPs for the stroke patients and healthy subjects over each recording site, i.e. M1 and PPC, of the stimulated AH and UH. TEPs displayed similar morphology across healthy subjects and patients, although the amplitude of the all components was clearly reduced in stroke patients (Figure 2 and 3).

Figure 2 and 3 about here

Baseline cortical excitability (GMFP) was significantly higher in the control group than in the stroke group ($F_{1,21}$=10.744, p=0.005). Moreover, a significant interaction of the factors Group and Peak ($F_{2,42}$=7.432, p=0.002) was observed. Post-hoc comparisons revealed that GMFP in the stroke group showed a decrease in amplitude relative to control group (Peak 3: p<0.001), regardless the

stimulated hemisphere. No other significant differences were highlighted between two groups ($p>0.05$).

The longitudinal analysis of cortical excitability showed a significant Hemisphere, Time and Peak interaction ($F_{6,72}=3.491$, $p<0.05$). Specifically, a significant increase of AH excitability compared to UH was observed after 40 (t1: AH-Peak 2=2.63±0.28 *vs.* UH-Peak 2=1.95±0.16 μV; p=0.043) and 60 days (t2: AH-Peak 2=2.95±0.39 *vs.* UH-Peak 2=2.01±0.14 μV; p=0.042) following stroke. No hemispheric difference was highlighted at baseline (t0), nor at the last time point of evaluation (t3) (Figure 4).

The evaluation of the TMS-evoked activity of PPC did not reveal any significant difference between the two groups at baseline level, nor within the stroke group among the time points of evaluation (all ps>0.05) (Figure 5).

Figure 4 and 5 about here

4.4 TMS-evoked oscillatory activity

TMS pulse over M1 produced an initial broadband response in each stimulated hemisphere, followed by widespread fast EEG oscillations, specifically in the alpha frequency band (Brignani et al., 2008; Fuggetta et al., 2005).

To establish whether the TMS-evoked oscillatory activity over M1 changed following the stroke event, the EOR values were compared between the two groups, in all frequency bands. ANOVA revealed a significant Hemisphere, Frequency and Group interaction ($F_{3,63}= 3.943$, $p=0.012$). Post-hoc analysis showed a decrease in both hemispheres of alpha (AH: p=0.017; UH: p=0.001) and beta activity (AH: p=0.006; UH: p=0.029) and in the contralesional hemisphere only for the delta activity (UH: p=0.029), in the stroke group respect to control group.

The statistical analysis performed on the longitudinal changes of EOR in the alpha frequency band revealed a significant main effect of Hemisphere ($F_{1,12}=15.620$, $p=0.002$) and a significant Time and Hemisphere interaction ($F_{3,36}=3.490$, $p=0.025$). No hemispheric difference in alpha EOR at t0

(p=0.417) was observed, although a hemispheric difference was revealed among the subsequent three time points, with higher values over AH compared to UH (t1: p=0.007; t2: p=0.007 and t3: p=0.015). No significant difference was found for the other frequency bands (Figure 6).

ANOVA on EOR evoked from PPC did not reveal any difference between the baselines of the two groups ($p > 0.05$) nor between the time points of evaluation in of stroke patients ($p > 0.05$) (Figure 7).

Figure 6 and 7 about here

4.5 Relation between TMS-evoked oscillatory activity and clinical outcomes

Starting from the prediction that the magnitude of oscillatory activity at baseline would correlate positively with the longitudinal recovery, Pearson's correlation coefficients were computed between the baseline value of alpha-EOR evoked by TMS, the key measure related to cortical reorganization, and the BBS, NIHSS and FMA scales assessed at each time-points during follow-up. A significant linear correlation was observed between alpha EOR evaluated over M1 of AH at baseline and BBS score both at baseline (t0: r=0.67, p=0.013) and at the following two time-points of evaluation (t1: r=0.61, p=0.028; t2: r=0.67, p=0.011). A similar trend, although marginally significant, was observed between the alpha EOR recorded at baseline and the FMA and the NIHSS scores, at each time-points after stroke onset (Figure 8).

Figure 8 about here

5. Discussion

Our study illustrates that TMS-EEG can be used in an innovative manner to longitudinally track the neurophysiological correlates of spontaneous recovery following stroke. For the first time, we

combined TMS with EEG to detect directly the cortical changes in stroke patients affected by a subcortical stroke lesion, with a consequent motor impairment resulting in a paretic hand function.

Our findings show that spontaneous clinical recovery is paralleled by changes in cortical excitability and oscillatory activity occurring at specific time-points after the injury within the affected hemisphere, as previously reported (Ward et al., 2003; for a review see, Murphy and Corbett, 2009). Although preliminary, these results highlight how combining TMS and EEG can characterize cortical changes induced by a stroke event and to track them longitudinally in stroke patients with damaged peripheral pathways by circumventing subcortical structures and directly assessing cortical excitability (Sato et al., 2015).

Until now, several TMS studies have used the corticospinal excitability measures (i.e., MEPs), in terms of resting motor threshold, stimulus-response curves and ipsilateral silent periods as outcomes to detect the motor function after injury (Bashir et al., 2010; Stinear et al., 2015). However, MEPs are an indirect measure of pyramidal tract excitability, since they are affected by a combination of cortical, subcortical and spinal mechanisms. Thus, although MEPs can provide valuable information about the state of the corticomotor projection, it could be not sufficiently informative of the cortical state (Cortes et al., 2012). Additionally, it is important to consider that in the early stage after stroke, MEPs are often not detectable in the AH, due to several factors such as the loss of cortical-motoneurons, altered membrane excitability in the remaining cells, dispersion of the excitatory volleys onto motoneurons, and compromised conduction and increased cortical inhibition (Stinear et al., 2012; Byblow et al., 2015). Moreover, a high MEP heterogeneity is detectable not only immediately after stroke but it can persist also for several months, as observed in our sample of stroke patients. The different MEP rates longitudinally reported in our study could be ascribable to the involvement of different neuroplasticity mechanisms, level of hand motor impairment, as well as to residual spared corticospinal functions (Auriat et al., 2015; Pellicciari et al., 2015). Thus, for the above reasons, it could be conceivable that MEPs are not sufficiently informative of the longitudinal functional recovery after stroke.

Differently, TEPs are quantifiable markers of the cerebral neurophysiological state, representing the direct result of activating excitatory and inhibitory postsynaptic potentials (Ilmoniemi et al., 1997). Moreover, in contrast to the high variability of MEPs, the TEPs are generally highly reproducible, provided that the delivery and targeting of TMS is well controlled and stable from pulse to pulse and between experimental sessions (for a review see, Ferreri and Rossini, 2013). In this case, TEPs could be potential direct markers of the state of the motor cortex in patients in whom TMS fails to produce peripheral markers of central excitability, i.e. MEPs in the affected hand. This was the specific case of stroke patients evaluated in our study, which presented a motor impairment of contralesional hand as demonstrated by the limited upper limb capacity evaluated by means of the Fugl-Meyer Upper Extremity Scale (Hoonhorst et al., 2015).

5.1 Changes in TMS-evoked cortical response

Global cortical activity of stroke patients resulted reduced immediately after the event respect to healthy control subjects, in both the hemispheres. After 40 days following stroke, we observed a shift of cortical activity, as measured by TEPs, within the AH paralleled to a clinical and behavioral amelioration. When a single TMS pulse is applied over a cortical area, a network of neuronal connections is engaged with a consequent cortical activation that extends from the stimulation site to other parts of the brain. In the acute stage of recovery after stroke, we observed that the cortical responses evoked from both the hemispheres was damaged probably due to an altered excitability of stimulated cortical neurons and connected cortico-cortical circuits.

More interestingly, in the longitudinal evaluation of cortical changes after the stroke we observed a significant increase of TMS-evoked cortical response evident as early as one month after stroke, which persists until at least six months. Several evidences reveal that the cortical response to the TMS depends on the neuronal activation state (Amassian et al., 1989; Esser et al., 2006; Huber et al., 2008; Romei et al., 2008; Silvanto et al., 2008) and that the amount of activity displayed on the scalp in terms of the strength of the evoked field reflects the synchronous activation of a neural

population (Lehmann and Skrandies, 1980). Therefore, considering that the amplitude of TMS-evoked response appears to relay information on the excitability of the underlying cortical networks, and is sensitive to its functional changes (Massimini et al., 2005; Morishima et al., 2009), the increase of cortical response evoked by TMS observed in the affected motor cortex represents a direct measure of the spontaneous neuronal modulation of ipsilesional hemisphere after the stroke. In this perspective, we hypothesize a post-stroke re-organization at cortical level, in terms of decrease in synaptic activation threshold, increasing the probability that the single TMS pulse recruits a larger neuronal population and facilitating the induction of action potentials. It is well-established that following stroke, synaptic changes occur both in the AH and UH. From a functional point of view, the recovery after stroke is sustained by brain plasticity involving synaptogenesis, dendritic arborisation, and synaptic and axonal recruitment (Rossini et al., 2007) and is likely to be influenced by the degree of ischemia induced synaptic transmission failure (Bolay et al., 2002). Considering the positive changes in the clinical and behavioral functions observed in our stroke patients, the significant changes in motor cortical response may be a neurophysiological indicator of adaptive plasticity triggered by spontaneous recovery. The increase of motor cortical excitability over AH is in agreement with previous studies in which motor cortical plasticity changes resulted directly related with positive prognosis in acute stroke (Di Lazzaro et al., 2010, for a review see Hummel and Cohen, 2006).

The increased cortical reactivity observed longitudinally in the AH of stroke patients could be explained also considering the specific time course of the change in TMS-evoked cortical activity. Different temporal components of TEPs may reflect independent mechanisms conveying specific information on excitatory and inhibitory neural activity of local cortical populations and wider cortico-cortical and cortico-thalamic networks (Ilmoniemi and Kicic 2010; Rogasch and Fitzgerald 2012). If the initial components appear to result from the activation of the stimulated target area, the later peaks are partially due to activity triggered by axonally propagated signals (Ilmoniemi and Kicic, 2010). We speculate that the increase of cortical response in the affected hemisphere respect

to the unaffected one could be related to increased axonal signal propagation, that happens about one months after the injury, when the natural recovery usually occurs.

Moreover, the later peaks of cortical response to TMS pulse, starting around 50 ms and lasting up to a few hundred milliseconds, have been closely associated with γ-aminobutyric acid (GABA)-ergic transmission, the principal inhibitory neurotransmitter in the cortex (McDonnell et al., 2006; Premoli et al., 2014). We hypothesize that the mechanism underlying the cortical excitability increase in the later GMFP component of the affected hemisphere could be identified in GABAergic mediated excitation/inhibition balance (Corti et al., 2012).

In a more specific manner, the spontaneous cortical excitability increase observed in the affected hemisphere could have been determined by the spontaneous decreasing of GABA-mediated inhibitory processes (Carmichael, 2012). Support evidence to this hypothesis comes from a study in which a similar time course of cortical changes, as evaluated by combining TMS with EEG, was observed after the application over the motor cortex of a paired pulse TMS protocol able to induce cortical inhibition and in which a cortical activity suppression was related to GABA-receptor inhibitory neurotransmission (Fitzgerald et al., 2009). Our findings are strongly in agreement with several evidence that suggest how modulations in inhibitory processing are necessary for occurring of synaptic plasticity (Castro-Alamancos et al., 1995; Clarkson et al., 2010), supporting also the recovery of motor function after stroke (Bachtiar and Stagg, 2014; Huynh et al., 2015).

5.2 Changes in TMS-evoked oscillatory activity

Another main finding of our study regards the frequency-dependent changes observed in the evoked cortical oscillations. In the first instance, the widespread oscillatory activity observed in our stroke group at baseline fully correspond to the patterns reported in previous studies performed in healthy subjects, in which the delivering of a single TMS pulse over M1 mainly evoked oscillations in the entire frequency range, with a prominent alpha band activation reflecting the typical phase resetting of ongoing motor cortical oscillations (Brignani et al., 2008; Veniero et al., 2011). Nevertheless, our

findings indicate a specific reduction of fast frequencies evoked by TMS over both the hemispheres and a more local change of slow frequency in the contralesional hemisphere in stroke patients respect to healthy subjects.

Currently, the prominent neurophysiological indices of tissue dysfunction following stroke are considered the spontaneous oscillatory activity changes measured by means of different neurophysiological methods of investigation, such as quantitative electroencephalographic (Sheorajpanday et al., 2011; Finnigan et al., 2016) and functional connectivity analysis (Dubovik et al., 2012). Studies on resting state EEG activity show that stroke affects the synchrony of electrical oscillations in neural networks, with attenuation of faster activity, particularly in the alpha and beta frequency range (Foreman and Claassen, 2012; Rabiller et al., 2015), whereas the preservation of fast frequencies is indicative of neuronal survival and a good prognosis (Niedermeyer, 2005). Therefore, in this framework, considering that the neuronal oscillations reflect the synchronized activity of large populations of neurons and have been implicated in brain function and behavior, and that TMS when combined with EEG is able to trigger sustained long-range and complex oscillatory patterns of activation, we used the TMS-EEG approach because it guarantees a more precise measurement of cortical network properties with a major spatiotemporal resolution respect to EEG alone.

In this context, in our study the largest changes were observed on the stimulated AH respect to UH in specific time-points of spontaneous recovery post-stroke, in the alpha frequency band. We found a highly specific time-dependent modulation of TMS-evoked alpha oscillatory activity over the affected hemisphere after 40 days, implying a dynamic process of ongoing cortical reorganization. This finding revealed a restoration process of cortical activity, as indirectly compared with the baseline patterns of healthy control group.

At general level, the neural oscillations in cortical networks are the result of the synchronous firing of neuronal populations and are important in the functional coordination of brain activity (Uhlhaas et al., 2009). After stroke, abnormalities in neural synchronization mechanisms have been widely

related to dysfunctional outcomes (de Vos et al., 2008), but at the same time improved functional outcomes are associated with an increase in perilesional alpha-band functional connectivity (Westlake et al., 2012; for a review see, Assenza et al., 2017). Starting from the assumption that alpha band is widely associated with aspects of motor functioning (Neuper et al., 2006) and the evidence that TMS-evoked oscillations over the motor cortex result from the resetting of ongoing alpha oscillatory activity, we speculate that the longitudinal TMS-evoked alpha oscillatory activity increase observed in our patients would be the marker of the spontaneous recovery of motor cortical organization in the AH, as triggered and magnified by means of TMS-EEG. Moreover, considering that alpha wave is driven by thalamic pacemaker cells (Sauseng et al., 2008), and the thalamus appears to play an important role in the generation and amplification of the TMS-evoked cortical oscillations, we hypothesize also an active involvement of the thalamo-cortical network in the reorganization of functional electrical activity in the brain.

Our findings could appear in contrast with the inverse relationship between oscillatory brain alpha activity around and cortical activation level that show as high EEG alpha power is associated with neural deactivation or inhibition (for a review see, Sauseng and Klimesch, 2008). However, recently an active role of alpha oscillatory activity (Palva and Palva, 2007) has been not only related but also considered to predict behavioral performance in distinct functions (Jensen et al., 2007; Hanslmayr et al., 2005), and its disruption associated to neurological deficits. Moreover, we chose to focus our study on resting-state oscillations excluding the evaluation of cortical oscillatory activity during a task, because our patients presented a moderate to moderately severe functional deficits, with a strong motor impairment that would have prevented an accurate assessment of motor function if involved in a task-based study.

Furthermore, we found exclusively a significant correlation between TMS-evoked alpha oscillations evaluated over M1 and the clinical functional score assessed by the BBS. Bearing in mind that BBS is used to measure static and dynamic balance abilities, we hypothesize that the alpha oscillatory

activity could be considered as a potential predictor of motor recovery, especially in terms of balance. However, these correlations need to be confirmed in larger samples of stroke patients.

6. Limitations

Despite its novel findings, our study has some limitations. The first one is the relative small sample size of evaluated stroke patients. However, the longitudinal assessment of spontaneous course of neurophysiological, behavioral and clinical outcomes up six months after stroke onset represents the starting point for wider studies and it is important to notice that TMS/EEG measurements were reliably measured in every session across time. Finally, our sample included only patients with sub-cortical stroke. It would be important in future studies to investigate whether the current findings can be generalized also to stroke patients with large ischemic lesions involving the cerebral cortex; patients' cohorts with lesions in different vascular territories, as well as with a broader spectrum of clinical impairment, or with recurrent stroke.

7. Conclusions

Understanding the mechanisms underlying the motor dysfunction after stroke and the brain reorganization during the motor recovery process could have important clinical implications leading to more effective rehabilitation strategies for patients with hemiparesis. The combined approach of TMS with EEG would be highly useful in clinical practice to assess and monitor longitudinally the state of neural circuits at single patient level, also before and after a rehabilitative program to detect the longitudinal changes in the state of cortical circuits and their relationship with a low or high functional recovery, also in silent brain areas affected by injury. Last but not least, our results could pay the way to a more focused definition both in terms of cortical target that modality of application for the potential therapeutic applications of rhythmic brain stimulation with rehabilitative aims (Koch et al., 2012; Avenanti et al., 2012).

References

Amassian VE, Cracco RQ, Maccabee PJ. Focal stimulation of human cerebral cortex with the magnetic coil: a comparison with electrical stimulation. Electroencephalogr Clin Neurophysiol. 1989, 74: 401-416, 1989.

Assenza G, Capone F, di Biase L, Ferreri F, Florio L, Guerra A, Marano M, Paolucci M, Ranieri F, Salomone G, Tombini M, Thut G, Di Lazzaro V. Oscillatory Activities in Neurological Disorders of Elderly: Biomarkers to Target for Neuromodulation. Front Aging Neurosci. 2017 Jun 13; 9:189.

Auriat AM, Neva JL, Peters S, Ferris JK, Boyd LA. A Review of Transcranial Magnetic Stimulation and Multimodal Neuroimaging to Characterize Post-Stroke Neuroplasticity. Front Neurol. 2015 Oct 29; 6:226.

Avenanti A, Coccia M, Ladavas E, Provinciali L, Ceravolo MG. Low-frequency rTMS promotes use-dependent motor plasticity in chronic stroke: a randomized trial. Neurology. 2012 Jan 24;78(4):256-64.

Bachtiar V, Stagg CJ. The role of inhibition in human motor cortical plasticity. Neuroscience. 2014 Oct 10; 278:93-104

Bashir S, Mizrahi I, Weaver K, Fregni F, Pascual-Leone A. Assessment and modulation of neural plasticity in rehabilitation with transcranial magnetic stimulation. PM&R. 2010 Dec; 2 (12 Suppl 2): S253-68.

Berg K, Wood-Dauphinee S, Williams JI. The Balance Scale: reliability assessment with elderly residents and patients with an acute stroke. Scand J Rehabil Med. 1995, 27:27- 36.

Bolay H, Gürsoy-Ozdemir Y, Sara Y, Onur R, Can A, Dalkara T. Persistent defect in transmitter release and synapsin phosphorylation in cerebral cortex after transient moderate ischemic injury. Stroke. 2002 May;33(5):1369-75.

Bonita R, Beaglehole R. Recovery of motor function after stroke. Stroke. 1988 Dec;19(12):1497-500

Borich MR, Wheaton LA, Brodie SM, Lakhani B, Boyd LA. Evaluating interhemispheric cortical responses to transcranial magnetic stimulation in chronic stroke: A TMS-EEG investigation Neurosci Lett. 2016 Apr 8; 618:25-30.

Brignani D, Manganotti P, Rossini PM, Miniussi C. Modulation of cortical oscillatory activity during transcranial magnetic stimulation. Hum Brain Mapp. 2008 May;29(5):603-12.

Brott T, Adams HP Jr, Olinger CP, Marler JR, Barsan WG, Biller J, Spilker J, Holleran R, Eberle R, Hertzberg V, et al. Measurements of acute cerebral infarction: a clinical examination scale. Stroke 1989; 20: 864–870.

Burke E, Cramer SC. Biomarkers and predictors of restorative therapy effects after stroke. Curr Neurol Neurosci Rep. 2013 Feb;13(2):329.

Byblow WD, Stinear CM, Barber PA, Petoe MA, Ackerley SJ. Proportional recovery after stroke depends on corticomotor integrity. Ann Neurol. 2015;78(6):848-59.

Byrnes ML, Thickbroom GW, Phillips BA, Wilson SA, Mastaglia FL. Physiological studies of the corticomotor projection to the hand after subcortical stroke. Clin Neurophysiol. 1999 Mar;110(3):487-98.

Calautti C, Leroy F, Guincestre JY, Baron JC. Dynamics of motor network over-activation after striatocapsual stroke. A longitudinal PET study using a fixed performance paradigm. Stroke, 2001, 32:2534–42.

Carducci F and Brusco R. Accuracy of an individualized MR-based head model for navigated brain stimulation. Psychiatry Res. 2012 Jul 30;203(1):105-8.

Carmichael ST. Plasticity of cortical projections after stroke. Neuroscientist. 2003 Feb;9(1):64-75.

Carmichael ST. Brain excitability in stroke: the yin and yang of stroke progression. Arch Neurol. 2012; 69:161-167.

Castro-Alamancos MA, Donoghue JP, Connors BW. Different forms of synaptic plasticity in somatosensory and motor areas of the neocortex. J Neurosci. 1995 Jul;15(7 Pt 2):5324-33.

Casula EP, Pellicciari MC, Ponzo V, Stampanoni Bassi M, Veniero D, Caltagirone C, Koch G. Cerebellar theta burst stimulation modulates the neural activity of interconnected parietal and motor areas. Sci Rep. 2016 Oct 31; 6:36191.

Casula EP, Bassi MS, Pellicciari MC, Ponzo V, Veniero D, Peppe A, Brusa L, Stanzione P, Caltagirone C, Stefani A, Koch G. Subthalamic stimulation and levodopa modulate cortical reactivity in Parkinson's patients. Parkinsonism Relat Disord. 2017 Jan 1;34:31-7.

Chung SW, Rogasch NC, Hoy KE, Fitzgerald PB. Measuring brain stimulation induced changes in cortical properties using TMS-EEG. Brain Stimul 2015; 8:1010–20.

Clarkson AN, Huang BS, Macisaac SE, Mody I, Carmichael ST. Reducing excessive GABA-mediated tonic inhibition promotes functional recovery after stroke. Nature. 2010 Nov 11;468(7321):305-9.

Collin C, Wade DT, Davies S, Horne V. The Barthel ADL Index: a reliability study. Int Disabil Stud. 1988;10(2):61-3.

Cortes M, Black-Schaffer RM, Edwards DJ. Transcranial magnetic stimulation as an investigative tool for motor dysfunction and recovery in stroke: an overview for neurorehabilitation clinicians. Neuromodulation 2012 Jul;15(4):316-25.

Corti M1, Patten C, Triggs W. Repetitive transcranial magnetic stimulation of motor cortex after stroke: a focused review. Am J Phys Med Rehabil. 2012 Mar;91(3):254-70.

de Vos CC, van Maarseveen SM, Brouwers PJAM, and van Putten MJAM. Continuous EEG monitoring during thrombolysis in acute hemispheric stroke patients using the brain symmetry index. J. Clin. Neurophysiol. 2008, 25, 77–82.

Di Lazzaro V and Ziemann U. The contribution of transcranial magnetic stimulation in the functional evaluation of microcircuits in human motor cortex. Front. Neural Circuits, 2013, 7:18.

Di Lazzaro V, Profice P, Pilato F, Capone F, Ranieri F, Pasqualetti P, Colosimo C, Pravatà E, Cianfoni A, Dileone M. Motor cortex plasticity predicts recovery in acute stroke. Cereb Cortex. 2010 Jul;20(7):1523-8.

Di Pino G, Pellegrino G, Assenza G, Capone F, Ferreri F, Formica D, et al. Modulation of brain plasticity in stroke: a novel model for neurorehabilitation. Nat. Rev. Neurol. 2014, 10, 597–608.

Dijkhuizen RM, Zaharchuk G, Otte WM. Assessment and modulation of resting-state neural networks after stroke. Curr Opin Neurol. 2014 Dec;27(6):637-43.

Dubovik, S, Pignat, JM, Ptak R, Aboulafia T, Allet L, Gillabert N, et al. The behavioral significance of coherent resting-state oscillations after stroke. Neuroimage, 2012, 61, 249–257.

Esser SK, Huber R, Massimini M, Peterson MJ, Ferrarelli F, Tononi G. A direct demonstration of cortical LTP in humans: a combined TMS/EEG study. Brain Res Bull, 2006, 69:86 –94.

Ferreri F and Rossini PM. TMS and TMS-EEG techniques in the study of the excitability, connectivity and plasticity of the human motor cortex. Rev. Neurosci. 2013, 24, 431–442.

Feydy A, Carlier R, Roby-Brami A, Bussel B, Cazalis F, Pierot L, Burnod Y, Maier MA. Longitudinal study of motor recovery after stroke: recruitment and focusing of brain activation. Stroke. 2002 Jun;33(6):1610-7.

Finnigan S, Wong A, Read S. Defining abnormal slow EEG activity in acute ischaemic stroke: Delta/alpha ratio as an optimal QEEG index. Clin Neurophysiol. 2016 Feb;127(2):1452-9.

Fitzgerald PB, Maller JJ, Hoy K, Farzan F, Daskalakis ZJ. GABA and cortical inhibition in motor and non-motor regions using combined TMS-EEG: a time analysis. Clin Neurophysiol. 2009 Sep;120(9):1706-10.

Foreman B and Claassen J. Quantitative EEG for the detection of brain ischemia. Crit Care. 2012 Dec 12;16(2):216.

Freundlieb N, Philipp S, Drabik A, Gerloff C, Forkert ND, Hummel FC. Ipsilesional motor area size correlates with functional recovery after stroke: a 6-month follow-up longitudinal TMS motor mapping study. Restor Neurol Neurosci. 2015;33(2):221-31.

Fuggetta G, Fiaschi A, Manganotti P. Modulation of cortical oscillatory activities induced by varying single-pulse transcranial magnetic stimulation intensity over the left primary motor area: a combined EEG and TMS study. Neuroimage. 2005 Oct 1;27(4):896-908.

Fugl-Meyer AR, Jääskö L, Leyman I, Olsson S, Steglind S The post-stroke hemiplegic patient. 1. a method for evaluation of physical performance. Scand J Rehabil Med. 1975;7(1):13-31.

Gerloff C, Bushara K, Sailer A, Wassermann EM, Chen R, Matsuoka T, Waldvogel D, Wittenberg GF, Ishii K, Cohen LG, Hallett M. Multimodal imaging of brain reorganization in motor areas of the contralesional hemisphere of well recovered patients after capsular stroke. Brain. 2006 Mar;129(Pt 3):791-808.

Gloor P, Ball G, Schaul N. Brain lesions that produce delta waves in the EEG. Neurology. 1977 Apr;27(4):326-33.

Grefkes C, Nowak DA, Eickhoff SB, Dafotakis M, Kust J, Karbe H, et al. Cortical connectivity after subcortical stroke assessed with functional magnetic resonance imaging. Ann Neurol. 2008, 63:236–46.

Grefkes C, Nowak DA, Wang LE, Dafotakis M, Eickhoff SB, and Fink GR. Modulating cortical connectivity in stroke patients by rTMS assessed with fMRI and dynamic causal modeling. Neuroimage, 2010, 50, 233–242.

Hachinski V, Donnan GA, Gorelick PB, Hacke W, Cramer SC, Kaste M, et al. Stroke: working toward a prioritized world agenda. Int J Stroke. 2010 Aug;5(4):238-56.

Hanslmayr S, Sauseng P, Doppelmayr M, Schabus M, Klimesch W. Increasing individual upper alpha power by neurofeedback improves cognitive performance in human subjects. Appl Psychophysiol Biofeedback. 2005 Mar;30(1):1-10.

Hari R and Salmelin R. Human cortical oscillation: a neuromagnetic view through the skull. Trends Neurosci. 1997. 20, 44–49.

Huynh W, Vucic S, Krishnan AV, Lin CS, Kiernan MC. Exploring the Evolution of Cortical Excitability Following Acute Stroke. Neurorehabil Neural Repair. 2016 Mar;30(3):244-57.

Hummel FC and Cohen LG. Non-invasive brain stimulation: a new strategy to improve neurorehabilitation after stroke? Lancet Neurol. 2006 Aug;5(8):708-12.

Hoonhorst MH, Nijland RH, van den Berg JS, Emmelot CH, Kollen BJ, Kwakkel G. How Do Fugl-Meyer Arm Motor Scores Relate to Dexterity According to the Action Research Arm Test at 6 Months Poststroke? Arch Phys Med Rehabil. 2015 Oct;96(10):1845-9.

Huber R, Määttä S, Esser SK, Sarasso S, Ferrarelli F, Watson A, Ferreri F, Peterson MJ, Tononi G. Measures of cortical plasticity after transcranial paired associative stimulation predict changes in electroencephalogram slow-wave activity during subsequent sleep. J Neurosci. 2008 Jul 30;28(31):7911-8.

Hummel FC, Cohen LG. Non-invasive brain stimulation: a new strategy to improve neurorehabilitation after stroke? Lancet Neurol. 2006, 5:708–12.

Ilmoniemi RJ and Kicic D. Methodology for combined TMS and EEG. Brain Topogr. 2010, 22, 233-248.

Ilmoniemi RJ, Virtanen J, Ruohonen J, Karhu J, Aronen HJ, Naatanen R, Katila T. Neuronal responses to magnetic stimulation reveal cortical reactivity and connectivity. Neuroreport, 1997, 8, 3537–3540.

Jensen O, Colgin LL. Cross-frequency coupling between neuronal oscillations. Trends Cogn Sci. 2007, 11:267–269.

Julkunen P, Jauhiainen AM, Könönen M, Pääkkönen A, Karhu J, Soininen H. Combining transcranial magnetic stimulation and electroencephalography may contribute to assess the severity of Alzheimer's disease. Int J Alzheimers Dis. 2011; 2011:654794.

Kim DJ, Bolbecker AR, Howell J, Rass O, Sporns O, Hetrick WP, Breier A, O'Donnel BF. Disturbed resting state EEG synchronization in bipolar disorder: a graph theoretic analysis. Neuroimage Clinical. 2013, 2, 414–423.

Koch G, Fernandez Del Olmo M, Cheeran B, Ruge D, Schippling S, Caltagirone C, Rothwell JC. Focal stimulation of the posterior parietal cortex increases the excitability of the ipsilateral motor cortex. J Neurosci. 2007 Jun 20;27(25):6815-22.

Koch G, Ponzo V, Di Lorenzo F, Caltagirone C, Veniero D. Hebbian and anti-Hebbian spike-timing-dependent plasticity of human cortico-cortical connections. J Neurosci. 2013 Jun 5;33(23):9725-33.

Koch G, Bonnì S, Giacobbe V, Bucchi G, Basile B, Lupo F, Versace V, Bozzali M, Caltagirone C. θ-burst stimulation of the left hemisphere accelerates recovery of hemispatial neglect. Neurology. 2012 Jan 3;78(1):24-30.

Koch G, Bonnì S, Pellicciari MC, Casula EP, Mancini M, Esposito R, Ponzo V, Picazio S, Di Lorenzo F, Serra L, Motta C, Maiella M, Marra C, Cercignani M, Martorana A, Caltagirone C, Bozzali M.
Transcranial magnetic stimulation of the precuneus enhances memory and neural activity in prodromal Alzheimer's disease. Neuroimage. 2017 Dec 19;169:302-311.

Kubis N. Non-Invasive Brain Stimulation to Enhance Post-Stroke Recovery. Front Neural Circuits. 2016 Jul 27; 10:56.

Lai SM, Studenski S, Duncan PW, Perera S. Persisting consequences of stroke measured by the Stroke Impact Scale. Stroke. 2002;33: 1840-1844.

Lehmann D and Skrandies W. Reference-free identification of components of checkerboard-evoked multichannel potential fields. Electroencephalogr. Clin. Neurophysiol. 1980, 48, 609–621.

Lioumis P, Kicic D, Savolainen P, Mäkelä JP, Kähkönen S, Lioumis P, et al. Reproducibility of TMS-evoked EEG responses. Hum. Brain Mapp.2009, 30, 1387–1396.

Marshall RS, Perera GM, Lazar RM, Krakauer JW, Constantine RC, De La Paz RL. Evolution of cortical activation during recovery from corticospinal tract infarction. Stroke, 2000, 31:656–61.

Massimini M, Ferrarelli F, Huber R, Esser SK, Singh H, Tononi G. Breakdown of cortical effective connectivity during sleep. Science. 2005 Sep 30;309(5744):2228-32.

McDonnell MN, Orekhov Y, Ziemann U. The role of GABA(B) receptors in intracortical inhibition in the human motor cortex. Exp. Brain Res. 2006, 173 (1), 86–93.

Milot MH and Cramer SC. Biomarkers of recovery after stroke. Curr Opin Neurol. 2008 Dec;21(6):654-9.

Miniussi C and Thut G. Combining TMS and EEG offers new prospects in cognitive neuroscience. Brain Topogr. 2009, 22, 249–256.

Morishima Y, Akaishi R, Yamada Y, Okuda J, Toma K, Sakai K. Task-specific signal transmission from prefrontal cortex in visual selective attention. Nat Neurosci. 2009 Jan;12(1):85-91.

Moyanova SG, Dijkhuizen RM. Present status and future challenges of electroencephalography- and magnetic resonance imaging-based monitoring in preclinical models of focal cerebral ischemia. Brain Res Bull. 2014 Mar; 102:22-36.

Murphy TH, Corbett D. Plasticity during stroke recovery: from synapse to behaviour. Nat Rev Neurosci. 2009 Dec;10(12):861-72.

Nagata K. Topographic EEG mapping in cerebrovascular disease. Brain Topogr. 1989 Fall-Winter;2(1-2):119-28.

Neuper C, Wörtz M, Pfurtscheller G. ERD/ERS patterns reflecting sensorimotor activation and deactivation. Prog Brain Res. 2006; 159:211-22.

Nicolo P, Rizk S, Magnin C, Pietro MD, Schnider A, Guggisberg AG. Coherent neural oscillations predict future motor and language improvement after stroke. Brain. 2015 Oct;138(Pt 10):3048-60.

Niedermeyer E, Lopes da Silva F, editors. Electroencephalography: basic principles, clinical applications and related fields. 3rd ed. Philadelphia, Lippincott: Williams & Wilkins; 2005. p. 339-62.

Nowak DA, Grefkes C, Ameli M, Fink GR. Interhemispheric competition after stroke: brain stimulation to enhance recovery of function of the affected hand. Neurorehabil Neural Repair. 2009 Sep;23(7):641-56.

Oldfield RC. The assessment and analysis of handedness: The Edinburgh inventory. Neuropsychologia 1971; 9:97–113.

Palva S, Palva JM. New vistas for alpha-frequency band oscillations. Trends Neurosci. 2007 Apr;30(4):150-8

Paus P, Sipila PK, Strafella AP. Synchronization of neuronal activity in the human primary motor cortex by transcranial magnetic stimulation: an EEG study. J Neurophysiol 2001; 86:1983–90.

Pellicciari MC, Veniero D, Miniussi C. Characterizing the Cortical Oscillatory Response to TMS Pulse. Front Cell Neurosci. 2017 Feb 27; 11:38.

Pellicciari MC, Ponzo V, Caltagirone C, Koch G. Restored Asymmetry of Prefrontal Cortical Oscillatory Activity after Bilateral Theta Burst Stimulation Treatment in a Patient with Major Depressive Disorder: A TMS-EEG Study. Brain Stimul. 2017 Jan - Feb;10(1):147-149.

Premoli I, Rivolta D, Espenhahn S, Castellanos N, Belardinelli P, Ziemann U, Müller-Dahlhaus F. Characterization of GABAB-receptor mediated neurotransmission in the human cortex by paired-pulse TMS-EEG. Neuroimage. 2014 Dec; 103:152-62.

Rabiller G, He JW, Nishijima Y, Wong A, Liu J. Perturbation of Brain Oscillations after Ischemic Stroke: A Potential Biomarker for Post-Stroke Function and Therapy. Int J Mol Sci. 2015 Oct 26;16(10):25605-40.

Ragazzoni A, Pirulli C, Veniero D, Feurra M, Cincotta M, Giovannelli F, Chiaramonti R, Lino M, Rossi S, Miniussi C. Vegetative versus minimally conscious states: a study using TMS-EEG, sensory and event-related potentials. PLoS One. 2013;8(2): e57069.

Rogasch NC, Fitzgerald PB. Assessing cortical network properties using TMS-EEG. Hum Brain Mapp. 2013 Jul;34(7):1652-69

Romei V, Brodbeck V, Michel C, Amedi A, Pascual-Leone A, Thut G. Spontaneous fluctuations in posterior alpha-band EEG activity reflect variability in excitability of human visual areas. Cereb. Cortex, 2008, 18, 2010–2018.

Romero Lauro LJ, Rosanova M, Mattavelli G, Convento S, Pisoni A, Opitz A, Bolognini N, Vallar G. TDCS increases cortical excitability: direct evidence from TMS-EEG. Cortex. 2014 Sep; 58:99-111.

Rosanova M, Casali A, Bellina V, Resta F, Mariotti M, Massimini M. Natural frequencies of human corticothalamic circuits. J Neurosci. 2009, 29:7679–7685.

Rossi S, Hallett M, Rossini PM, Pascual-Leone A, Safety of TMS Consensus Goup. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol. 2009, 120:2008 –2039.

Rossini PM, Calautti C, Pauri F, Baron JC. Post-stroke plastic reorganisation in the adult brain. Lancet Neurol. 2003 Aug;2(8):493-502.

Rossini PM, Altamura C, Ferreri F, Melgari JM, Tecchio F, Tombini M, Pasqualetti P, Vernieri F. Neuroimaging experimental studies on brain plasticity in recovery from stroke. Eura Medicophys. 2007 Jun;43(2):241-54.

Rossini PM, Burke D, Chen R, Cohen LG, Daskalakis Z, Di Iorio R, Di Lazzaro V, Ferreri F, Fitzgerald PB, George MS, Hallett M, Lefaucheur JP, Langguth B, Matsumoto H, Miniussi C, Nitsche MA, Pascual-Leone A, Paulus W, Rossi S, Rothwell JC, Siebner HR, Ugawa Y, Walsh V, Ziemann U. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. Clin Neurophysiol. 2015 Jun;126(6):1071-107.

Sale MV, Mattingley JB, Zalesky A, Cocchi L. Imaging human brain networks to improve the clinical efficacy of non-invasive brain stimulation. Neurosci Biobehav Rev. 2015 Oct;57:187-98.

Sato S, Bergmann TO, Borich MR. Opportunities for concurrent transcranial magnetic stimulation and electroencephalography to characterize cortical activity in stroke. Front Hum Neurosci. 2015 May 5; 9:250.

Sauseng P, Klimesch W. What does phase information of oscillatory brain activity tell us about cognitive processes? Neuroscience and Biobehavioral Reviews 32 (2008) 1001–1013

Sheorajpanday RV, Nagels G, Weeren AJ, van Putten MJ, De Deyn PP. Quantitative EEG in ischemic stroke: correlation with functional status after 6 months. Clin Neurophysiol. 2011 May;122(5):874-83.

Silvanto J, Muggleton N, Walsh V. State-dependency in brain stimulation studies of perception and cognition. Trends Cogn. Sci. 2008, 12, 447–454.

Stinear CM, Barber PA, Petoe M, Anwar S, Byblow WD. The PREP algorithm predicts potential for upper limb recovery after stroke. Brain. 2012 Aug;135(Pt 8):2527-35.

Stinear CM, Ward NS. How useful is imaging in predicting outcomes in stroke rehabilitation? Int J Stroke. 2013 Jan;8(1):33-7.

Stinear CM, Petoe MA, Byblow WD. Primary Motor Cortex Excitability During Recovery After Stroke: Implications for Neuromodulation. Brain Stimul. 2015 Nov-Dec;8(6):1183-90.

Thickbroom GW, Byrnes ML, Archer SA, Mastaglia FL. Motor outcome after subcortical stroke: MEPs correlate with hand strength but not dexterity. Clin Neurophysiol. 2002 Dec;113(12):2025-9.

Thibaut A, Simis M, Battistella LR, Fanciullacci C, Bertolucci F, Huerta-Gutierrez R, Chisari C, Fregni F. Using Brain Oscillations and Corticospinal Excitability to Understand and Predict Post-Stroke Motor Function. Front Neurol. 2017 May 10; 8:187.

Uhlhaas PJ, Pipa G, Lima B, Melloni L, Neuenschwander S, Nikolic D, Singer W. Neural synchrony in cortical networks: History, concept and current status. Front Integr Neurosci. 2009, 3:17.

van Meer MP, Otte WM, van der Marel K, Nijboer CH, Kavelaars A, van der Sprenkel JW, Viergever MA, Dijkhuizen RM. Extent of bilateral neuronal network reorganization and functional recovery in relation to stroke severity. J Neurosci. 2012 Mar 28;32(13):4495-507.

Veniero D, Brignani D, Thut G, Miniussi C. Alpha-generation as basic response-signature to transcranial magnetic stimulation (TMS) targeting the human resting motor cortex: a TMS/EEG coregistration study. Psychophysiology, 2011, 48:1381–1389.

Ward NS, Brown MM, Thompson AJ, Frackowiak RS. Neural correlates of motor recovery after stroke: a longitudinal fMRI study. Brain. 2003 Nov;126(Pt 11):2476-96

Westlake KP, Hinkley LB, Bucci M, Guggisberg AG, Byl N, Findlay AM, Henry RG, Nagarajan SS. Resting state α-band functional connectivity and recovery after stroke. Exp Neurol. 2012 Sep;237(1):160-9.

Williams LS, Weinberger M, Harris LE, Clark DO, Biller J. Development of a stroke-specific quality of life scale. Stroke 1999 Jul;30(7):1362-9.

Figure Legend

Figure 1. Effectiveness of behavioral (Fugl-Meyer Assessment scale [FMA], Berg Balance Scale: [BBS]) and clinical (Barthel Index [BI], Specific Stroke Quality of Life scale [SSQoL] and National Institutes of Health Stroke Scale [NIHSS]) outcome measures, assessed in the different time-points of evaluation (t0: 20 days; t1: 40 days; t2: 60 days and t3: 180 days). The effectiveness was calculated comparing the value in the different time-points of evaluation with the baseline for each stroke patients, as follows:

$$\text{Effectiveness} = \left(\frac{\Delta t}{t_{max}} * 100\right)$$

where Δt is the differences between the considered time-point and the baseline (t0) and t_{max} is the maximum score for the evaluated outcome measure. Grey symbol indicates a significant difference respect to t0. Black symbol indicates a significant difference compared to the previous time-point of evaluation.

Figure 2. Butterfly plots of grand-averaged response evoked from motor cortex (M1) stimulation of the affected (left panel: AH) and unaffected hemisphere (right panel: UH), in stroke patients (SP, upper plots) and in control healthy subjects (HS, below plots). Red line indicates the global mean field power (GMFP).

Figure 3. Butterfly plots of grand-averaged response evoked from parietal cortex (PPC) stimulation of the affected (left panel: AH) and unaffected hemisphere (right panel: UH), in stroke patients (SP, upper plots) and in control healthy subjects (HS, below plots). Red line indicates the global mean field power (GMFP).

Figure 4. TMS-evoked cortical response (GMFP) evoked from M1 of the affected (AH) and unaffected hemisphere (UH), in control healthy subjects (HS) and in stroke patients (SP), evaluated longitudinally after stroke onset (t0: 20 days; t1: 40 days; t2: 60 days and t3: 180 days) (upper panel). Bar graphs of mean values ± SE of the three Peaks of GMFP (Peak 1:10-50 ms; Peak 2: 50-

100 ms and Peak 3:100-150 ms), evoked from M1 of the AH (grey blank for HS and black blank bars for SP) and UH (grey filled bars for HS and black filled bars for SP) (below panel). Asterisks represent significant differences ($p < 0.05$).

Figure 5. TMS-evoked cortical response (GMFP) evoked from PPC of the affected (AH) and unaffected hemisphere (UH), in control healthy subjects (HS) and in stroke patients (SP), evaluated longitudinally after stroke onset (t0: 20 days; t1: 40 days; t2: 60 days and t3: 180 days) (upper panel). Bar graphs of mean values ± SE of the three Peaks of GMFP (Peak 1:10-50 ms; Peak 2: 50-100 ms and Peak 3:100-150 ms), evoked from M1 of the AH (grey blank bars for HS and black blank bars for SP) and UH (grey filled bars for HS and black filled bars for SP) (below panel). Asterisks represent significant differences ($p < 0.05$).

Figure 6. Time-frequency plots of TMS-evoked cortical oscillatory activity evaluated from M1 of the affected (left panel: AH) and unaffected hemisphere (right panel: UH), in each time-point of evaluation both in control healthy subjects (HS, upper panels) and in stroke patients (SP). The gray scale graph plotted at the right of each time-frequency plot illustrates the power spectrum profile induced by the TMS pulse in the AH (red line) and UH (black line).

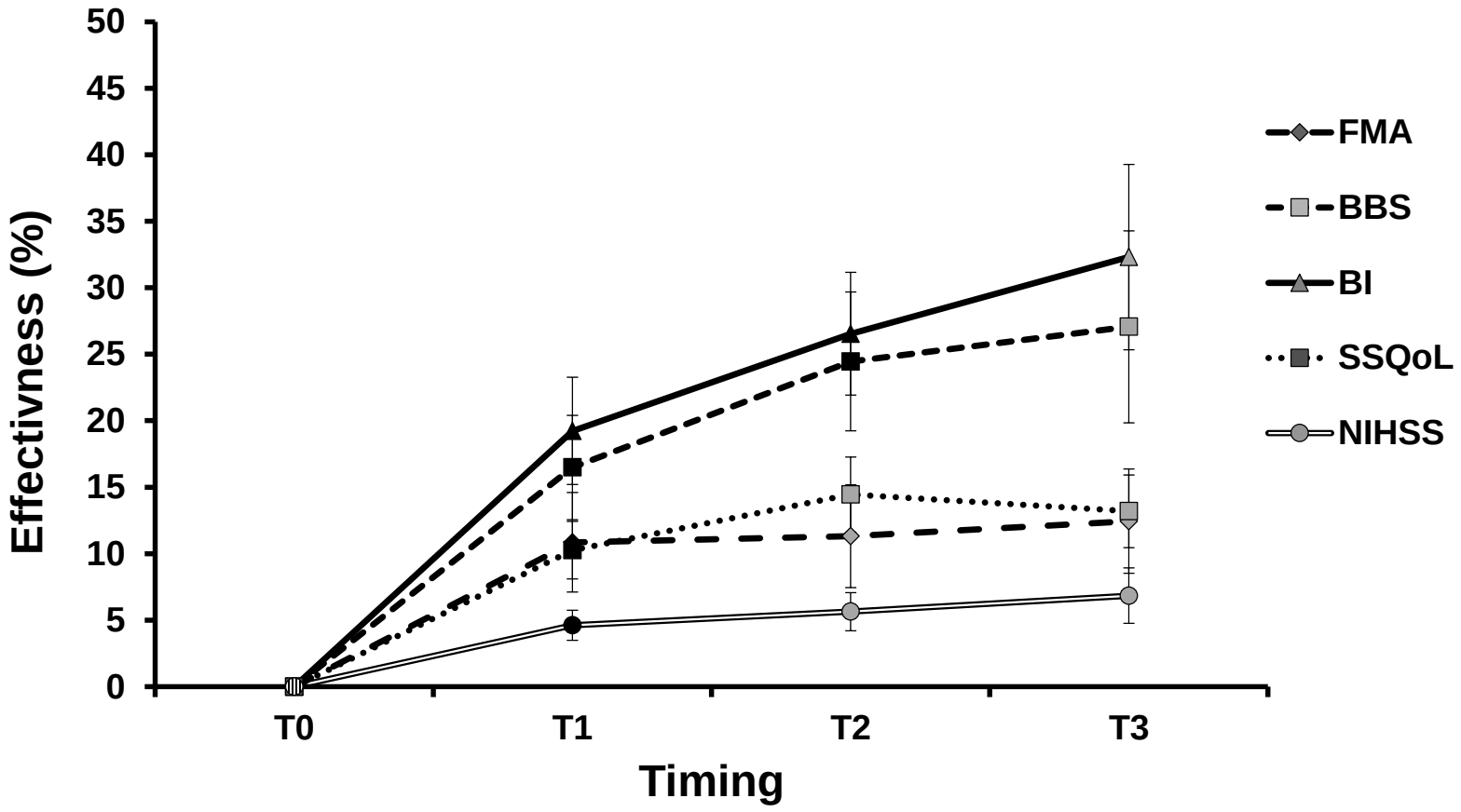
Figure 7. Time-frequency plots of TMS-evoked cortical oscillatory activity evaluated from PPC of the affected (left panel: AH) and unaffected hemisphere (right panel: UH), in each time-point of evaluation both in control healthy subjects (HS, upper panels) and in stroke patients (SP). The gray scale graph plotted at the right of each time-frequency plot illustrates the power spectrum profile induced by the TMS pulse in the AH (red line) and UH (black line).

Figure 8. Correlations between alpha EOR evaluated from M1 of AH at baseline and functional recovery measured by means of Berg Balance Scale (BBS), Fugl-Meyer Assessment (FMA), and National Institutes of Health Stroke Scale (NIHSS), evaluated at t0, t1, t2 and t3 (respectively at 20, 40, 60 and 180 days following stroke).

Patient	Sex	Age	FMA	NIHSS	Lesion location	Lesion hemisphere	Symptoms	MEP t0	MEP t1	MEP t2	MEP t3
1	M	71	195	0	lenticular capsule	L	mild hemiparesis	+	+	+	+
2	M	71	194	2	lenticular capsule	R	mild hemiparesis, facial palsy	+	+	+	+
3	M	69	198	5	internal capsule and thalamus	L	mild hemiparesis, aphasia	+	+	+	+
4	M	52	170	6	corona radiata and semioval centre	R	hemiparesis, facial palsy	-	-	-	-
5	F	78	174	2	lenticular nucleus	R	mild hemiparesis	+	+	+	+
6	M	69	121	9	superior capsule	R	hemiplegia, facial palsy	-	-	-	-
7	M	47	159	4	capsule, periventricular extension	L	severe hemiparesis, dysarthria	-	+	+	+
8	M	43	139	8	internal capsule	L	hemiplegia, facial palsy	-	+	+	+
9	M	55	200	7	corona radiata, internal capsule	R	mild hemiparesis, slight cognitive impairment	+	+	+	+
10	F	50	120	10	internal capsule	R	hemiplegia, facial palsy	-	-	-	-
11	F	48	167	7	internal capsule	R	mild hemiparesis	+	+	+	+
12	M	57	99	18	basal ganglia	L	hemiplegia, facial palsy	-	-	-	-
13	F	74	138	9	corona radiata	R	severe hemiparesis facial palsy	-	-	-	-
Mean		60.3	159.5	6.7							
SD		12.1	33.6	4.6							

Table 1: Patient characteristics

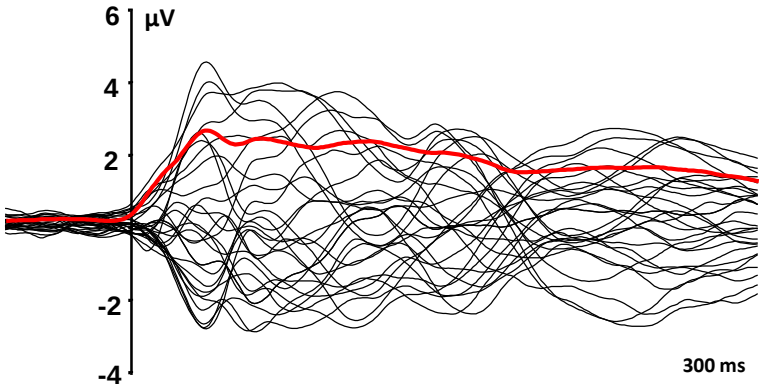
Abbreviations: M: Male, F: Female, R: right, L: left, FMA at baseline: Fugl-Meyer score, NIHSS at baseline: National Institutes of Health Stroke Scale, MEP: motor evoked potential from the affected limb.


50
45
40
35
30
25
20
15
10
5
0
Effectivness (%)
T0
T1
T2
T3
Timing
FMA
BBS
BI
SSQoL
NIHSS

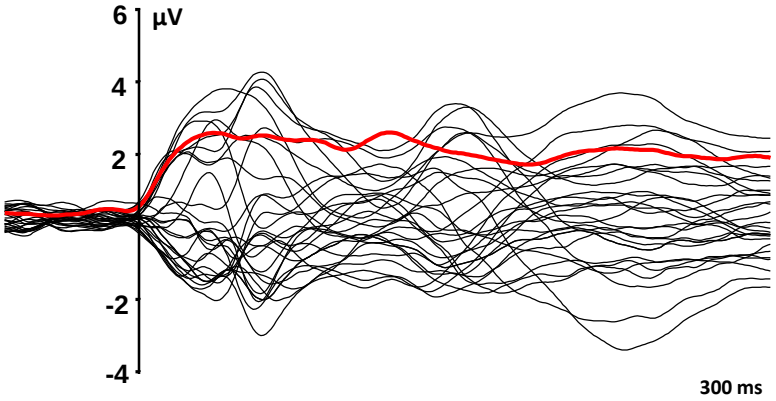
M1

SP

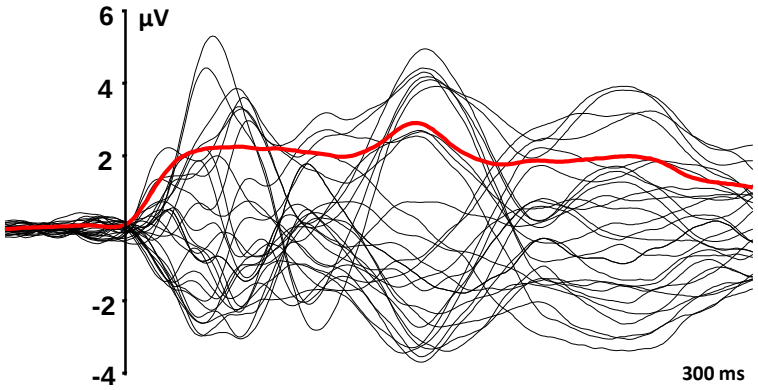
AH

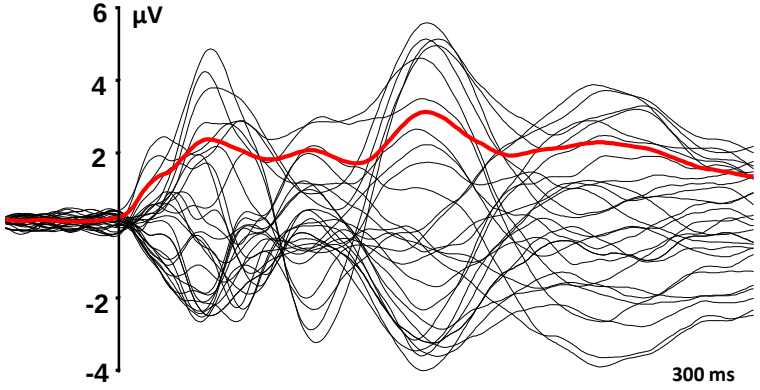


UH



HS



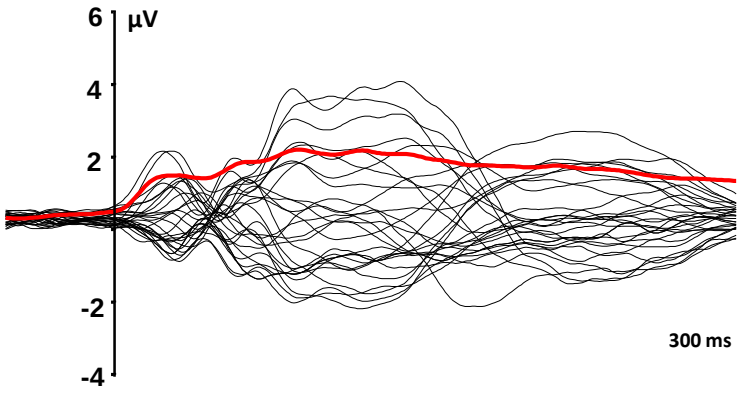


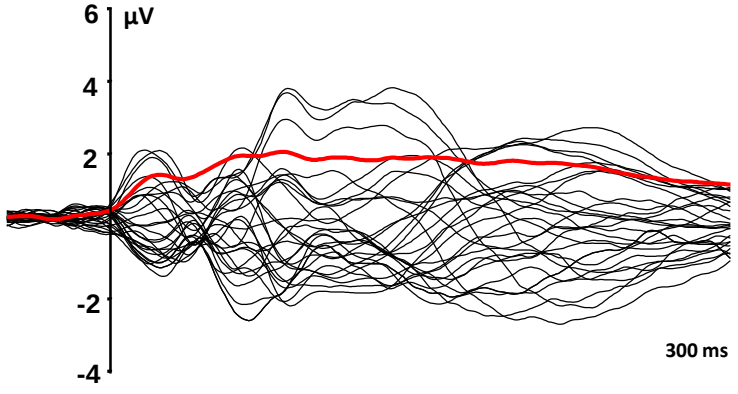
PPC

SP

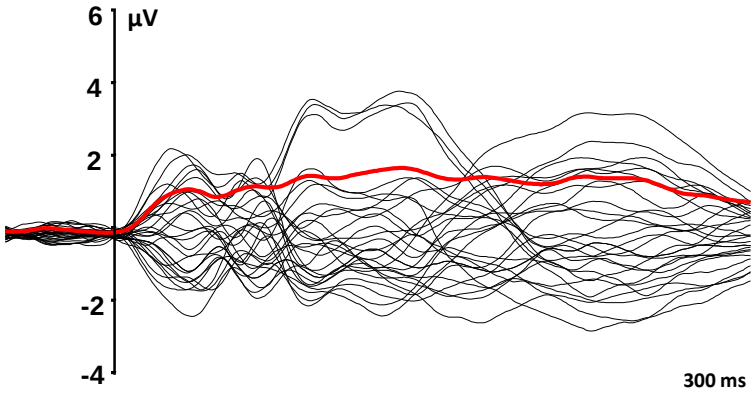
AH

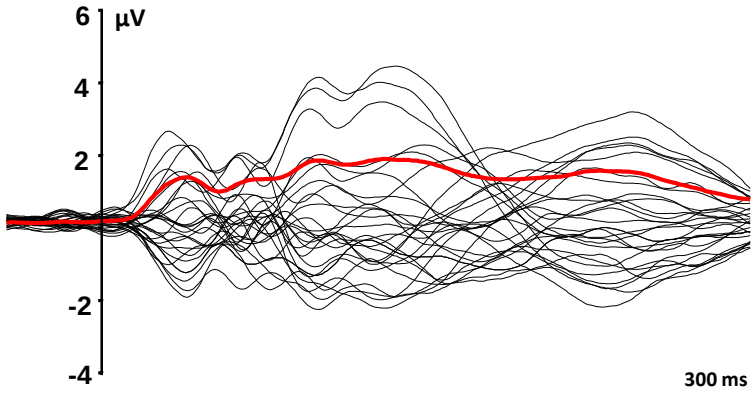
UH



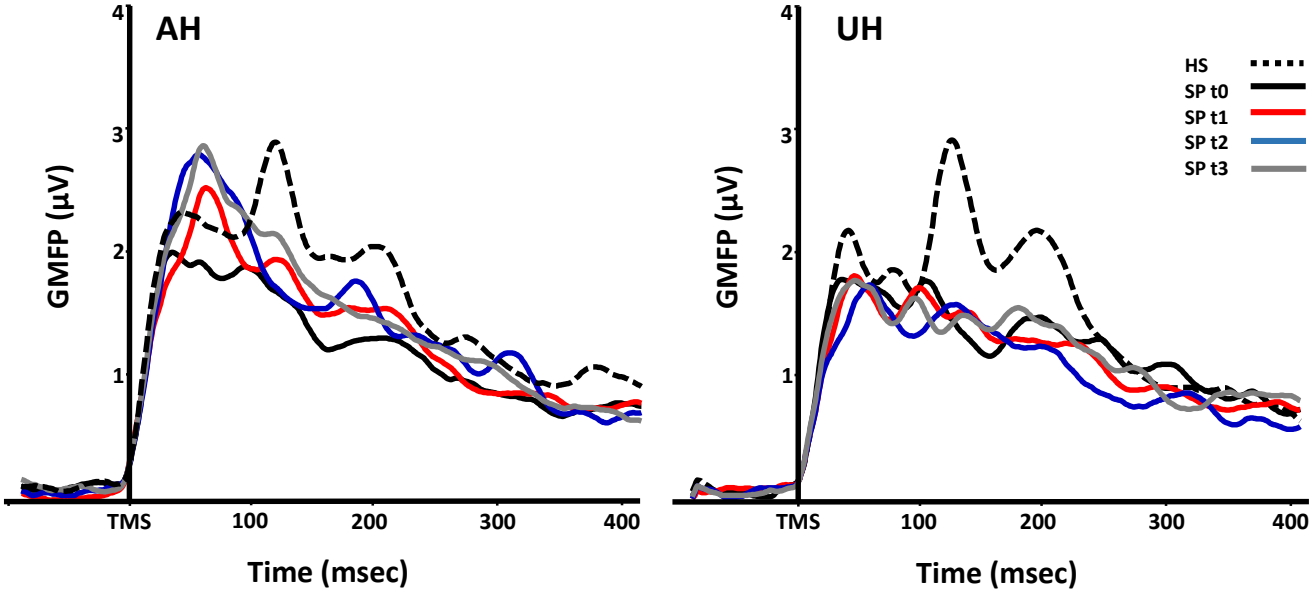


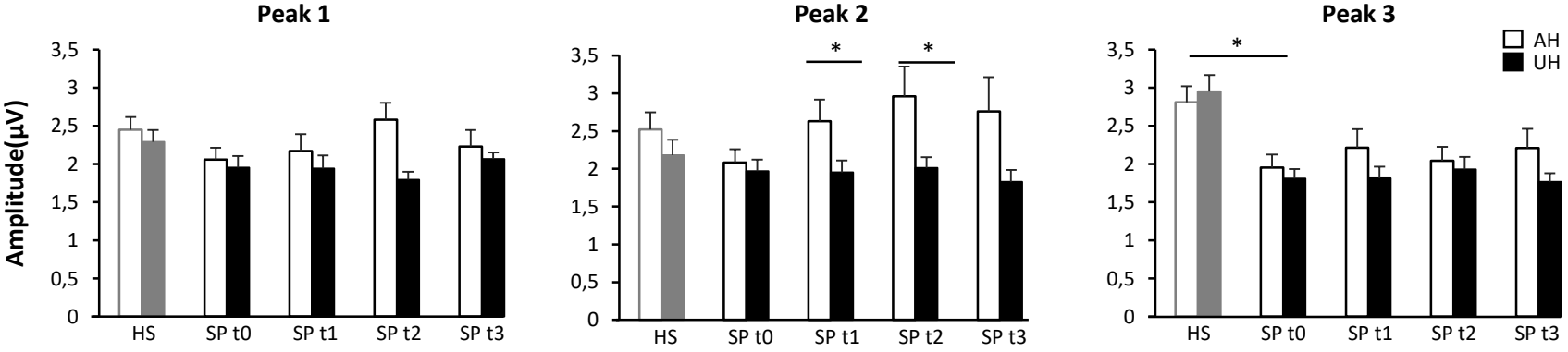
HS



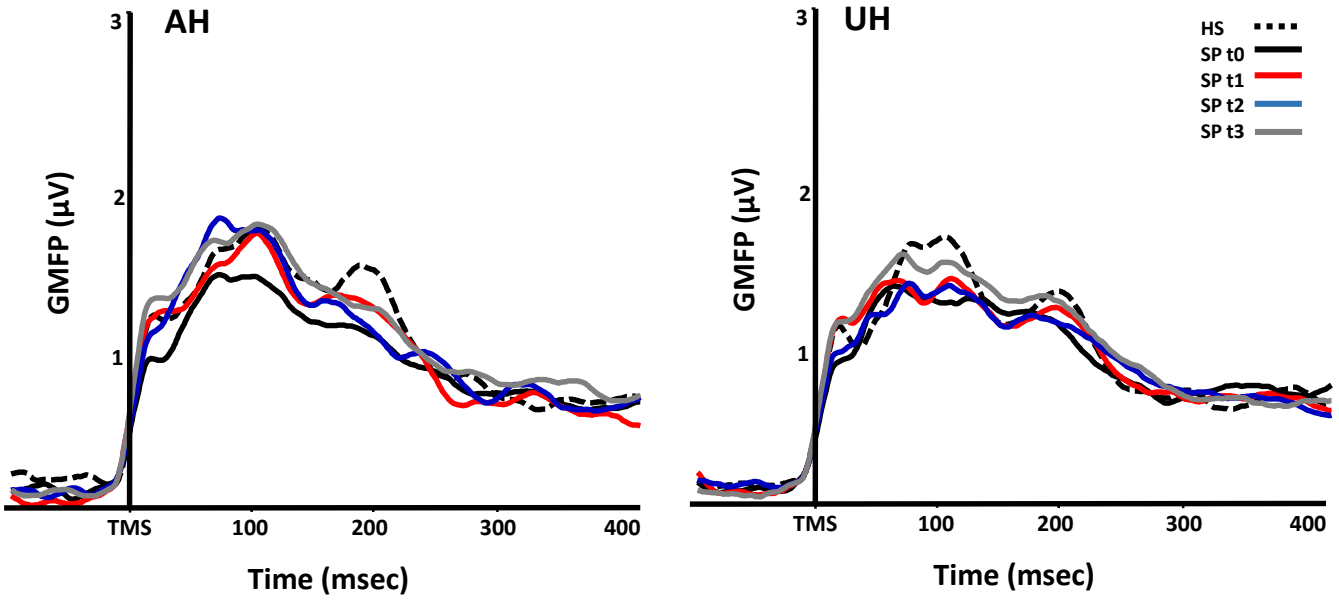


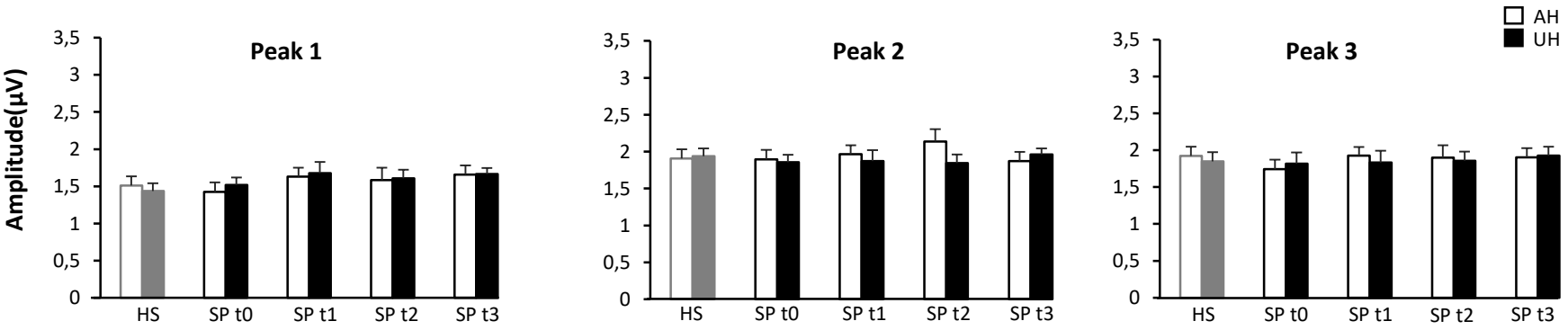
M1


AH
UH
GMFP (µV)
TMS
100
200
300
400
Time (msec)
HS
SP t0
SP t1
SP t2
SP t3

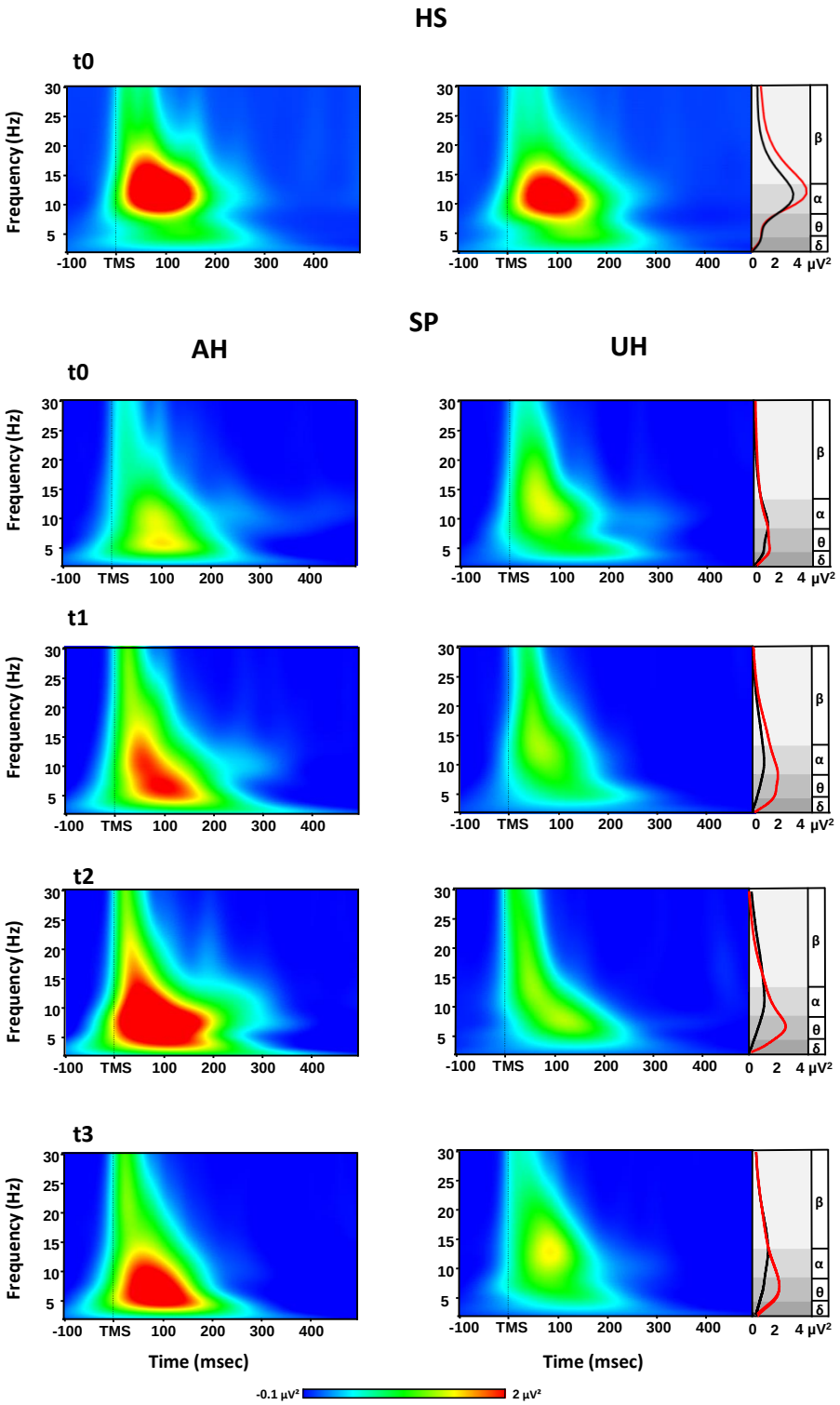

Peak 1
Peak 2
Peak 3
Amplitude(µV)
HS
SP t0
SP t1
SP t2
SP t3
AH
UH
*

PPC

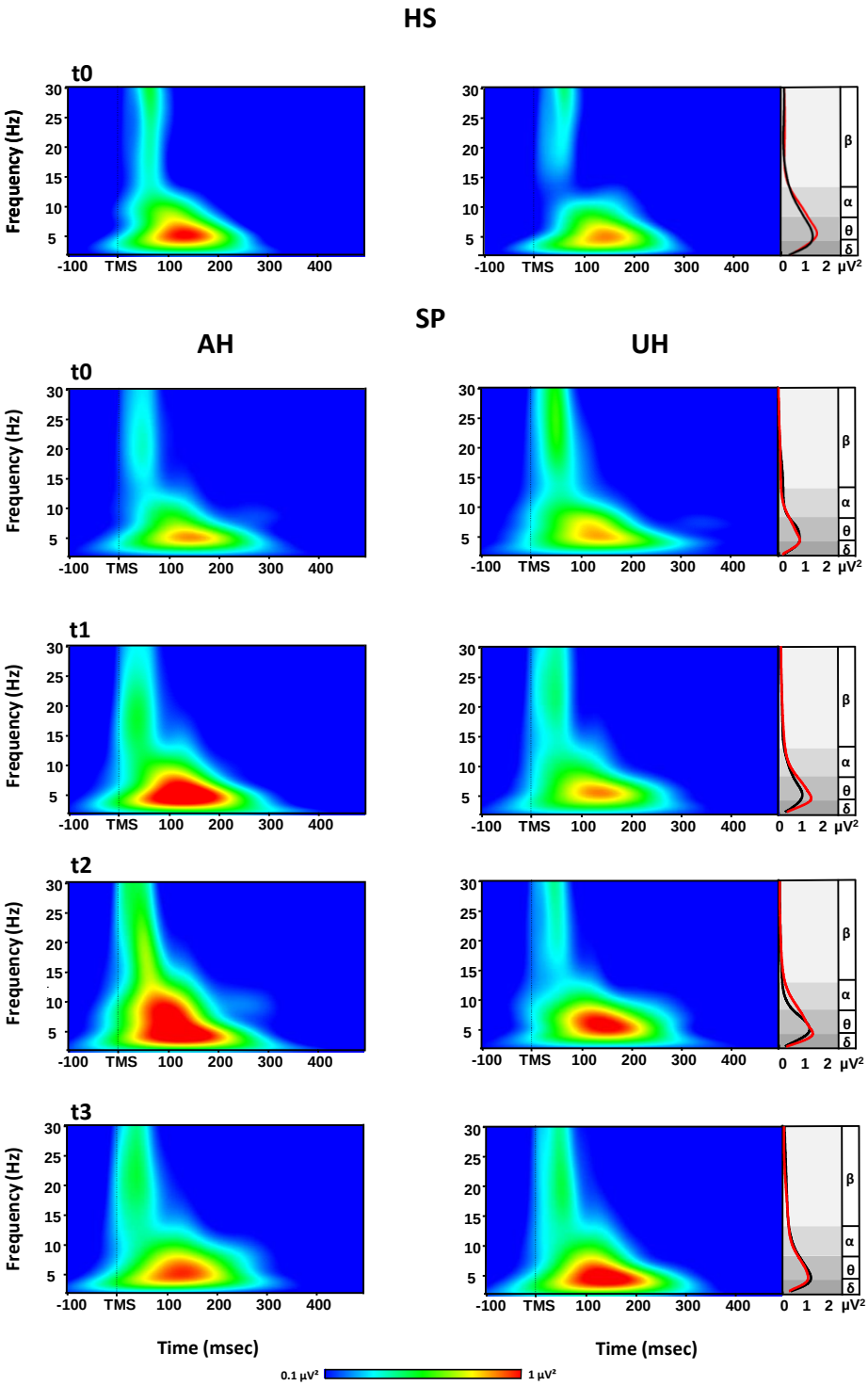

AH
UH
HS
SP t0
SP t1
SP t2
SP t3
GMFP (µV)
3
2
1
TMS
100
200
300
400
Time (msec)

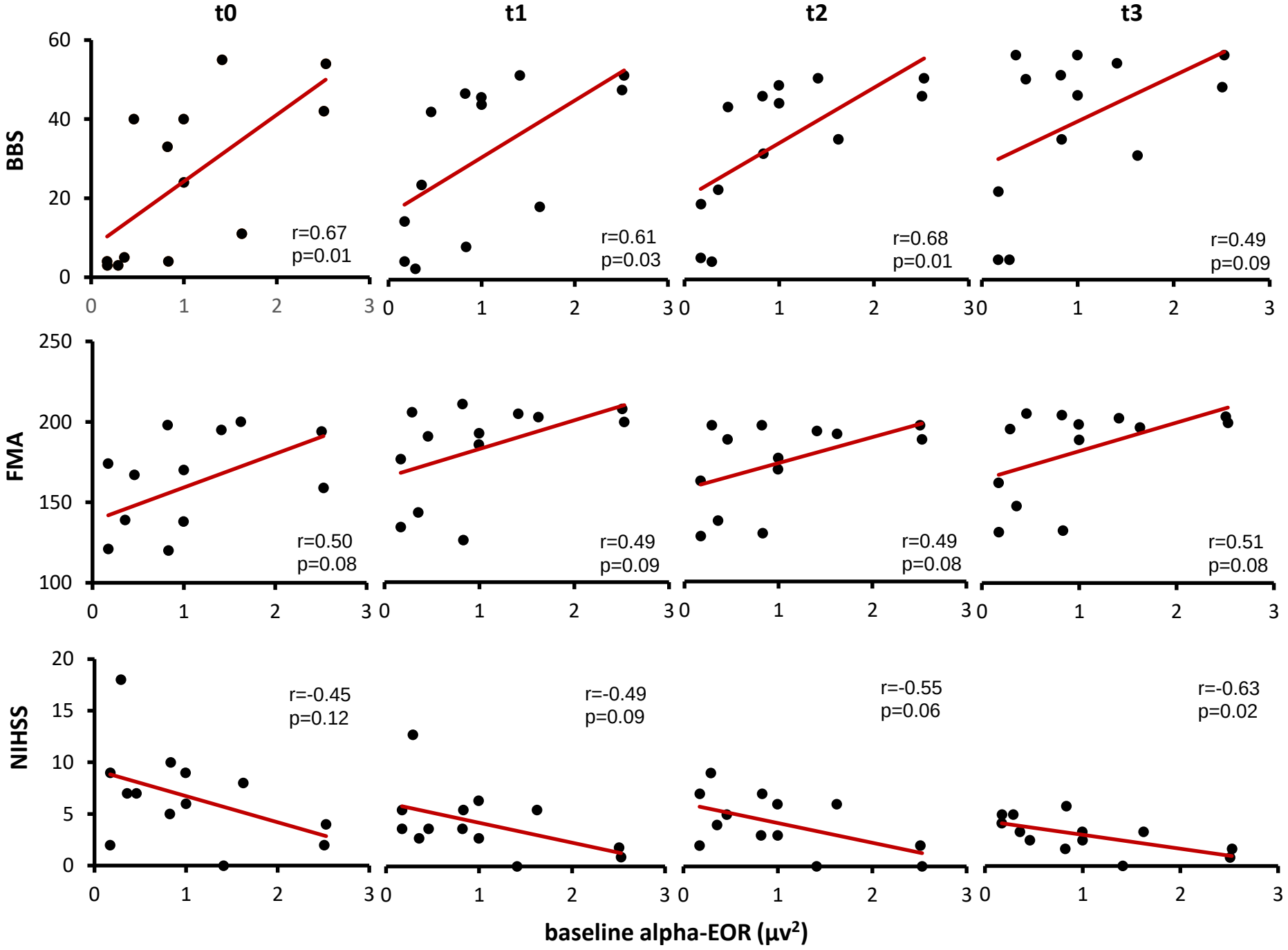

Amplitude(µV)
Peak 1
Peak 2
Peak 3
AH
UH
3,5
3
2,5
2
1,5
1
0,5
0
HS
SP t0
SP t1
SP t2
SP t3

M1


HS
t0
SP
AH
UH
t0
t1
t2
t3
Frequency (Hz)
30
25
20
15
10
5
-100
TMS
100
200
300
400
0
2
4 µV²
β
α
θ
δ
Time (msec)
-0.1 µV²
2 µV²

PPC


HS
t0
Frequency (Hz)
30
25
20
15
10
5
-100
TMS
100
200
300
400
0
1
2
μV²
β
α
θ
δ
SP
AH
UH
t0
t1
t2
t3
Time (msec)
0.1 μV²
1 μV²


t0
t1
t2
t3
BBS
FMA
NIHSS
r=0.67
p=0.01
r=0.61
p=0.03
r=0.68
p=0.01
r=0.49
p=0.09
r=0.50
p=0.08
r=0.49
p=0.09
r=0.49
p=0.08
r=0.51
p=0.08
r=-0.45
p=0.12
r=-0.49
p=0.09
r=-0.55
p=0.06
r=-0.63
p=0.02
baseline alpha-EOR (μv²)