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2016-08

Julkunen , P , Määttä , S , Säisänen , L , Kallioniemi , E , Könönen , M , Jäkälä , P , Vanninen , R & Vaalto , S 2016 , ' Functional and structural cortical characteristics after restricted focal motor cortical infarction evaluated at chronic stage - Indications from a preliminary study ' , Clinical Neurophysiology , vol. 127 , no. 8 , pp. 2775-2784 . <https://doi.org/10.1016/j.clinph.2016.05.013>

<http://hdl.handle.net/10138/224536>

<https://doi.org/10.1016/j.clinph.2016.05.013>

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Functional and structural cortical characteristics after restricted focal motor cortical infarction evaluated at chronic stage – Indications from a preliminary study

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ARTICLE INFO

Article history:

Accepted 10 May 2016

Available online 26 May 2016

Keywords:

Navigated transcranial magnetic stimulation

Stroke

Infarction

Motor cortex

Motor evoked potential

Diffusion weighted imaging

HIGHLIGHTS

- TMS and DWI show changes in chronic stroke patients with minor motor symptoms.
- Affected hemisphere is less excitable to produce strong responses.
- At chronic stage, neuronal structure remains disrupted in the affected motor cortex.

ABSTRACT

Objective: To assess the inter-hemispheric differences in neuronal function and structure of the motor cortex in a small group of chronic stroke patients having suffered a restricted ischemic lesion affecting hand motor representation. GABAergic intracortical inhibition, known to be affected by stroke lesion, was also investigated.

Methods: Eight patients exhibiting little or no motor impairment were studied using transcranial magnetic stimulation (TMS) and diffusion weighted imaging (DWI) >15 months from diagnosis. Resting motor threshold (MT) for 50 μ V and 2 mV motor evoked potentials, and short-interval intracortical inhibition (SICI) were measured from hand muscles. Apparent diffusion coefficients (ADCs) were analyzed from the DWI for the primary motor cortex (M1), the supplementary motor area (SMA) and thalamus for reflecting changes in neuronal organization.

Results: The MTs did not differ between the affected (AH) and unaffected hemisphere (UH) in 50 μ V responses, while the MTs for 2 mV responses were higher ($p = 0.018$) in AH. SICI was weakened in AH ($p = 0.008$). ADCs were higher in the affected M1 compared to the unaffected M1 ($p = 0.018$) while there were no inter-hemispheric differences in SMA or thalamus.

Conclusions: Inter-hemispheric asymmetry and neuronal organization demonstrated abnormalities in the M1. However, no confident inference can be made whether the observed alterations in neurophysiological and imaging measures have causal role for motor rehabilitation in these patients.

Significance: Neurophysiological changes persist and are detectable using TMS years after stroke even though clinical symptoms have normalized.

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1. Introduction

Motor disability is among the most common consequences of ischemic stroke. Following focal ischemic damage, the reorganization of the cortical functions begins (Ward and Cohen, 2004). Some initial improvement after the acute phase occurs due to resolution of the perilesional edema and recovery of other tissue functions surviving the ischemia (Hallett, 2001). However, it is thought that the long-term recovery occurs primarily due to brain plasticity through functional and structural reorganization (Hallett, 2001; Hodics et al., 2006). For example, increased gray matter density and contralesional cortical thickness in the essential cortical motor areas has been correlated with improved arm function in chronic stroke (Gauthier et al., 2008; Sterr et al., 2013).

Lesions in the motor cortex, and the following recovery, cause functional changes in the cortex, in both the affected (AH) and unaffected hemisphere (UH) as well as in the interhemispheric connections (Chen and Schlaug, 2013; Cunningham et al., 2015; Li et al., 2015; Rossini et al., 2007; Ward and Cohen, 2004). In the acute phase after stroke, the cortical activity increases in the intact non-primary motor and non-motor areas of both hemispheres, whereas later on in well-recovering patients the activity is shifted towards the affected M1 and is reduced in other areas (Calautti et al., 2001a; Calautti et al., 2001b; Feydy et al., 2002; Ward et al., 2003; Ward and Cohen, 2004). The reorganization of the representation area of the paretic hand may occur during the first months after stroke and is usually seen as a mediolateral shift of representation in affected M1. Also an anteroposterior shift of representation towards sensory cortex and premotor areas may be seen (Fridman et al., 2004; Rossini et al., 2007; Traversa et al., 1997) indicating the recruitment of motoneurons from adjacent cortical areas. Interhemispheric inhibition is altered after stroke leading to increased activation in UH and increased inhibition in AH. Greater imbalance in interhemispheric inhibition and asymmetry in cortical activations has been related to poor recovery (Chen and Schlaug, 2013; Cunningham et al., 2015; Di Lazzaro et al., 1999; Li et al., 2015). Schaechter and Perdue (2008) demonstrated with functional MRI that in stroke patients with good recovery, cortical activity in the AH motor areas is enhanced depending on the demands of the motor task.

Significant effort has been committed to understanding the neurophysiological mechanisms of functional recovery from brain lesions such as stroke. The region adjacent to lesion demonstrates modified plasticity through remapped sensorimotor functions (Brown et al., 2009; Clarkson et al., 2010; Dijkhuizen et al., 2003). Stroke-related plasticity consists of mechanisms such as early dendritic branching and synaptogenesis, initial increase of cortical excitability via decreased GABA activity enabling use-dependent plasticity, and later on decrease in hyperexcitability (Hagemann et al., 1998; Jones and Schallert, 1992; Neumann-Haefelin et al., 1998; Shimizu et al., 2002; Stroemer et al., 1995; Ward et al., 2003). Stroke is known to increase tonic GABAergic transmission in perilesional cortical area, and a reduction of this inhibition produces early motor recovery after stroke (Clarkson et al., 2010). Counteracting the increased GABAergic inhibition by administering inverse agonist specific for GABA receptors or genetically lowering the number of GABA receptors have been reported to be beneficial for the recovery of motor function after stroke (Clarkson et al., 2010). Hence, the role of the changes in cortical excitability and inhibition in chronic stroke patients with restricted focal lesions and almost complete clinical recovery should be studied more precisely.

Transcranial magnetic stimulation (TMS), a painless non-invasive method, is suitable for investigating the neurophysiological effects of stroke due to its ability to probe corticospinal excitability as well as facilitatory and inhibitory mechanisms of the motor cortex (Rossini et al., 2015). The motor threshold (MT)

based on the occurrence of motor evoked potentials (MEPs) induced by TMS is considered to be a common measure of general cortical excitability (Rossini et al., 2015). MT in the affected motor cortex has been shown to increase demonstrating a lowered level of excitation caused by the motor cortex lesion following stroke in the early state (Prashantha et al., 2013). In the long-term recovery, the cortical excitability in AH has been shown to exhibit a decrease in MT values approaching normal (Takechi et al., 2014; Traversa et al., 2000).

Primary motor cortex disinhibition is a characteristic sign of reorganization in the subacute stage after stroke enabling the recruitment of adjacent motoneurons and facilitating activity-dependent plasticity (Liepert et al., 2000). The intracortical GABA-related inhibition, called short-interval intracortical inhibition (SICI), can be studied by paired-pulse TMS using short inter-stimulus intervals (ISIs) (Chen et al., 1998; Kujirai et al., 1993). Intracortical facilitation (ICF) can be assessed with longer ISIs (Kujirai et al., 1993). Previously, SICI and ICF have been studied at different stages of stroke recovery (Cicinelli et al., 2003; Liepert et al., 2000; Malcolm et al., 2015) and a general finding is that SICI is decreased in the AH and normalization of inhibition is associated with successful recovery (Eliassen et al., 2008; Manganotti et al., 2002; Swayne et al., 2008). Interhemispheric inhibition is often imbalanced in unilateral stroke leading to disinhibition of UH and increased inhibition of AH through transcallosal fibers (Bütefisch et al., 2003).

In diffusion weighted imaging (DWI) apparent diffusion coefficient (ADC) map reflects the local diffusion of water molecules at each point with a single value (Le Bihan et al., 1986). Thereby in the ADC map, areas with restricted diffusion appear dark while unrestricted diffusion can be seen as bright. The mean ADC values in infarcted neuronal regions change over time; in the acute phase the ADC decreases, in the subacute phase the ADC returns near to normal values and in the chronic stage the ADC is higher than in the healthy tissue (Shen et al., 2011). Similar change has been observed in thalamus after middle cerebral artery infarcts (Hervé et al., 2005).

The aim of the present study was to understand the chronic phase characteristics of the neurophysiological motor cortical excitability and inhibition in patients with almost complete clinical recovery after restricted focal cortical infarction in the “hand knob” of the primary motor cortex. For this purpose, we studied the excitability, intracortical inhibition and facilitation of the motor cortex using navigated TMS (nTMS) over 15 months after stroke diagnosis in a small group of patients. To gain insight into the relation with parallel cortical anatomical changes, we also assessed ADC to study the local organization of the cortical neurons on the M1, where the lesions were located, and in the supplementary motor area (SMA) and thalamus.

2. Methods

2.1. Patients

The study was approved by the Research Ethics Committee of Kuopio University Hospital (95/2010). Patients were selected retrospectively from the patient register of Kuopio University Hospital from the years 2005–2009. Criteria for the first selection were the treatment period in the neurology clinic, ICD-10 diagnosis number I63 (stroke) and year of birth 1940 or later. After this, episcrisis from the neurology department and statements of CT- and MR images of selected stroke patients were read. CT and MRIs from patients having first ever unilateral stroke in the immediate vicinity of the anatomical primary hand motor area causing unilateral paresis symptoms in the acute phase were selected for re-evaluation by neuroradiologist. This criterion was set to enable the study of long-term plasticity in patients who had clearly intact areas in the close proximity of the lesion site. Selected patients

were informed about the study and volunteers gave written consent. Altogether eight patients (age: 48–68 years, 6 male) exhibiting little or no motor impairment were recruited for the study 15–43 months after having suffered an infarction. The patients were evaluated during a clinical evaluation after diagnosis using modified Rankin Scale (mRS) (van Swieten et al., 1988) and National Institutes of Health Stroke Scoring (NIHSS). None of the patients displayed significant permanent disability (mRS ≤ 1 , NIHSS ≤ 1). Due to a retrospective nature of the patient recruitment, no specific tests were used to analyze hand-motor function after the diagnosis.

Patients with epilepsy, metallic implants, a pacemaker, artificial heart valves, a cochlear implant or electronic drug pumps were excluded from the recruitment. Patient demographics are presented in Table 1.

2.2. Study protocol

All patients underwent MRI prior to nTMS experiments. Structural T1-weighted 3D MRI and DWI were acquired from all the patients (Philips Achieva 3.0T TX, Philips, The Netherlands) using a SENSE-head-8 coil. The 3D T1-weighted images were obtained with the following parameters: TR = 8.2 ms, TE = 3.7 ms, flip angle 8°, voxel size = $1 \times 1 \times 1 \text{ mm}^3$, slice orientation = sagittal, total = 190 slices. DWI was scanned with an echo planar sequence using the following parameters: TR = 11953 ms, TE = 55 ms, flip angle = 90°, voxel size = $2 \times 2 \times 2 \text{ mm}^3$, slice orientation = transverse, total = 70 slices, b -values = 0 and 1000 mm^2/s , number of diffusion gradient directions = 16.

The nTMS study was conducted using Magstim Bistim stimulator and a figure-eight coil with monophasic waveform (Magstim Company Ltd, Whitland, UK) utilizing the structural T1-weighted MRIs with the neuronavigation (eXimia 3.1, Nexstim Plc, Helsinki, Finland). During stimulation, electromyography (EMG) was recorded from abductor pollicis brevis (APB) muscle with eXimia EMG device (Nexstim Plc, Helsinki, Finland) at 3000 Hz (filtered to 10–500 Hz) using disposable Ag–AgCl surface electrodes.

First, the location of the optimal APB muscle representation (hotspot) was mapped, and the optimal coil rotation angle in the tangential plane was selected at the hotspot (Julkunen et al., 2009). This was set as the stimulation target for the following stimulation sequences. At the target, the resting motor threshold (MT50) for the APB was computed with the Rossini-Rothwell method (Rothwell et al., 1999) using acceptance criteria of 50 μV for the MEP amplitude. Then, 10 MEPs were induced using 120% of the MT50 intensity with an inter-trial interval (ITI) of over 5 s to characterize the mean MEP amplitudes (Julkunen et al., 2012b). Subsequently, a recruitment curve sequence was run with stimulation intensities between 100% and 150% of MT50 at 10%

intervals with 10 MEPs induced at each intensity to construct a threshold curve (Julkunen et al., 2011) and the recruitment curve (Devanne et al., 1997; Pitcher et al., 2003). The used ITI was 5–10 s (Julkunen et al., 2012b). The threshold curve was used to analyze the higher amplitude thresholds with an acceptance criteria of 2 mV (MT2000). Provided that the maximum amplitude of the subject was less 2 mV, MT2000 was set at maximum stimulator output, 100%-MSO.

To study the SICI and ICF we used paired-pulse stimulation with 70% of MT50 as conditioning stimulation intensity and 120% of MT50 as test pulse intensity (Vaalto et al., 2011). The conditioning pulse was given before the test pulse at three inter-stimulus intervals (ISIs); 2 ms (SICI2), 3 ms (SICI3) and 10 ms (ICF10) (Kujirai et al., 1993). 10 MEPs were recorded with each ISI. The order of the stimulated hemispheres was randomized. Also, the order of the applied paired-pulse sequence (the three different ISIs) was randomized within the investigated hemisphere.

2.3. Analyses

MEP amplitudes were analyzed offline using eXimia software by marking the peak-to-peak amplitudes of the MEPs. Peak-to-peak muscle activity of $\geq 20 \mu\text{V}$ in EMG within 1 s preceding the MEPs resulted in the rejection of the trial from further analyses. For SICI and ICF effects, MEP amplitudes were normalized to the mean of single-pulse MEPs.

From the DWI data, ADC maps were automatically generated. From the ADC maps, the mean ADC values were calculated with the region of interest (ROI) evaluation of the white matter. Separate ROIs were drawn in M1 for the hand (“hand knob”), SMA and thalamus on both hemispheres (Fig. 1). M1 ROI was placed in the “hand knob” area in the precentral gyrus. SMA ROI was situated on the anterior portion of the paracentral lobule. ROIs were located in the middle slice of the visually estimated slices where the structure, i.e. “hand knob” or SMA, could be detected. Thalamus ROI was located on the slice in which the thalamus reached its maximal size in the axial orientation. Thus, ADC values were evaluated locally from subcortical locations.

2.4. Statistics

Interhemispheric differences between AH and UH were analyzed using Wilcoxon signed rank test for paired-samples. Indications of potential correlation between time-from-diagnosis (TFD) and interhemispheric differences in measured TMS parameters or ADC were tested using Spearman's ρ . Due to a small number of subjects, bootstrap resampling with 10,000 repetitions was used to estimate the 95% confidence interval (CI) for the significant correlations found. The level for statistical significance was set at $p < 0.05$. Analyses were performed using SPSS (IBM SPSS Statistics, version 22.0, Somers, NY, USA).

3. Results

In all patients, the hotspots were located in precentral gyri in both hemispheres and most often in the close vicinity of the “hand knob” lesion in AH (Fig. 2). The MT50s did not differ between the AH and UH hemispheres ($p > 0.999$), while the MT2000 was higher ($p = 0.018$) in the AH (Figs. 3B, 4A). Five patients exhibited MT2000 of over 100%-MSO in the AH, while one patient exhibited MT2000 of over 100%-MSO in the UH. The threshold curves indicated that the threshold for inducing high amplitude MEPs increased in the AH. MEP amplitudes induced at 120%rMT were lower ($p = 0.012$) in the AH (Figs. 3A, 4A).

At the group level, SICI was apparent in the UH, i.e. MEPs were lower in amplitude compared with single-pulse MEPs ($p = 0.012$

Table 1
Patient demographics.

Id	Age (years)	Gender	Affected hemisphere	Time from diagnosis (months)	Carotid stenosis (side)	mRS	NIHSS
1	65	Male	Right	31	Yes (right)	1	0
2	66	Male	Left	15	Yes (left)	0	0
3	56	Female	Right	28	No	0	0
4	65	Male	Right	42	No	1	1
5	68	Female	Left	42	No	0	0
6	59	Male	Right	38	No	0	0
7	48	Male	Right	43	No	0	0
8	55	Male	Right	37	No	1	0

Abbreviations: mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scoring.

Fig. 1. Placement of ROIs to analyze neuronal organization. Separate ROIs were manually placed on M1 “hand knob”, on SMA and on thalamus of both hemispheres. SMA was estimated to locate in the anterior part of the paracentral lobule. Regions with small apparent diffusion coefficient (ADC) values appear dark, whereas tissues with high ADC values are bright. Location of the lesion in the right hemisphere “hand knob” has been indicated.

for SIC12 and $p = 0.012$ for SIC13) (Fig. 4B). Instead, the SIC1 was often absent in the AH indicated by a non-significant difference compared with the single-pulse MEPs ($p = 0.161$ for SIC12 and $p = 0.263$ for SIC13). At the group level, ICF10 effect was non-significant in either hemisphere ($p \geq 0.327$). At the individual level, all patients exhibited SIC12 and SIC13, but only 3 exhibited ICF10 in the UH. In the AH, 6 patients exhibited SIC12, 5 patients exhibited SIC13 and 6 patients exhibited ICF10. Individual values for the TMS parameters are shown in Table 2.

DWI was successfully conducted in 7/8 patients. The locations of the ROIs are reported in Table 3. The ADC values in the AH M1 were higher than in the UH M1 ($p = 0.018$), while there were no significant differences between the hemispheres in SMA ($p = 0.176$) or thalamus ($p = 0.498$) (Fig. 4C). The ADC in the AH side thalamus correlated negatively with the MT2000 ($\rho = -0.906$, $p = 0.005$) and positively with MEP ($\rho = 0.786$, $p = 0.036$) in the AH. The correlations were verified by bootstrap resampling with MT2000 (95% CI for $\rho = -1.000$ to -0.624 , $p = 0.010$) and MEP

Fig. 2. Locations of motor “hotspots” and lesions. Location of the lesion (outlined by hand with red line) in the AH and the TMS target for the APB muscle in both hemispheres (yellow dots). The locations have been overlaid on axial slice of the structural MRI at the depth of the lesion. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(95% CI for $\rho = 0.059$ –1.000, $p = 0.048$). No other significant correlations were found between the mean ADC and any of the TMS-measures. Individual ADC values are shown in Table 4.

Correlations were observed between SIC12 and the TFD in both AH ($\rho = -0.874$, $p = 0.005$) and UH ($\rho = -0.762$, $p = 0.028$). Bootstrap resampling verified our finding in the AH (95% CI for $\rho = -1.000$ to -0.333 , $p = 0.005$) and UH (95% CI for $\rho = -1.000$ to -0.062 , $p = 0.022$). Hence, the difference in SIC12 between the AH and UH tended to normalize over time, i.e. difference in the SIC12 became smaller ($\rho = 0.881$, $p = 0.004$, Fig. 5) again verified by bootstrap resampling (95% CI for $\rho = 0.392$ –1.000, $p = 0.003$). Other TMS-parameters or the mean ADC did not exhibit correlation with TFD.

4. Discussion

In this study, the functional and structural properties of affected and unaffected motor cortices were studied in chronic stroke patients with unilateral restricted cortical infarctions located in

anatomical hand motor representation. We found significant differences between the AH and UH in cortical excitability, intracortical inhibition as well as in the neuronal organization of M1. The lesions of these patients were small and focused in the immediate vicinity of the anatomical hand motor representation (“hand knob”) and little or no symptoms remained at the moment of the study, over 15 months after diagnosis. Therefore, the recovery at the chronic stage, while potentially symptom-free, still seems to remain incomplete, as indicated by the great imbalance in MT2000s between the AH and UH despite overall good interhemispheric symmetry in MT50s. Although no follow-up studies were conducted and the group of patients studied was small, indications of recovery were observed in the inter-individual comparison through SIC12, which showed at the group-level that the intracortical inhibition may be advancing towards normal when more time had passed from diagnosis. However, it must be kept in mind that a low number of subjects were studied and therefore these results are only indications.

Fig. 3. Recruitment characteristics from TMS. (A) Recruitment curves (mean $\pm$ SEM) for the AH and UH. The gray areas indicate significant differences between the hemispheres. (B) MEP threshold curves (mean $\pm$ SEM) for the AH and UH. The threshold curve has been constructed by computing the MT using different MEP amplitude threshold criteria after stimulation with several different stimulation intensities (Julkunen et al., 2011). In the AH, not all subjects demonstrated above 2 mV responses meaning that their threshold value MT2000 was 100%-MSO. Thus, a low SEM value at high threshold amplitudes in the AH reflects reaching maximal motor threshold-value of 100%-MSO.

The increase in MT is common in the AH in stroke. While we found no significant difference in the conventional resting MT (MT50), we found that the high amplitude MT (MT2000) was significantly higher. This indicates that normal recruitment of cortical motoneurons in suprathreshold ($>$ MT50) stimulation intensities cannot be achieved at the chronic stage, even though the normalization of the low amplitude threshold MT50 had already occurred. Lower MEP amplitudes in the AH agree well with the findings on MTs, and may potentially be explained by different aspects of the tissue damage and plasticity related changes: (1) partial motoneuron loss in cortical motor representation preventing to produce high-amplitude MEPs, (2) disorganization of the cortical structure affecting the proneness to TMS-effects, (3) affected and reorganized subcortical parts of the corticospinal tract, or (4) scattered reorganization of the hand motor representation. Even if motoneuron loss could purely explain increased MT and lower MEP amplitudes in the AH, the SICI findings potentially suggest the downregulation of the GABA_A receptor activity, which is known to allow the recruitment of additional motoneurons and reorganization of the motor function (Cicinelli et al., 2003; Hickmott and Merzenich, 2002; Mittmann et al., 1994). Thus, MT and MEP-amplitude findings are probably also affected by various plasticity mechanisms. Alternatively, the observed changes in SICI in the AH could be due to changes in the composition of the corticospinal volley evoked by TMS after stroke.

Measures of motor cortical excitability (e.g. MT) are influenced also by anatomical factors, such as the coil-to-cortex distance affected by distance of scalp from the stimulated cortex (Danner et al., 2012; Julkunen et al., 2012a). In addition, the anatomical neuronal organization and macrostructure may affect the measures of excitability (Janssen et al., 2013, 2014; Kallioniemi et al., 2015b). Hence, stroke-induced changes in any of these anatomical factors may also affect the measured corticospinal excitability and are reflected in the results of the present study.

In the UH, the SICI was observed both with 2 and 3 ms ISI, but in the AH it did not appear in the group level indicating a state of disinhibition in the AH. The SICI effect size in the UH was comparable to those measured previously from a healthy population (Kujirai et al., 1993; Säisänen et al., 2011). The observation of the decreased SICI supports the hypothesis that molecular and cellular events of neuronal plasticity are affected perilesionally, and disinhibition is facilitating functional recovery even in the chronic phase (Clarkson et al., 2010). Furthermore, the SICI2 demonstrated inverse correlation with TFD at the group level, providing some indications that the difference between the UH and AH may normalize with time (Fig. 5). Such a relation was not observed with any other parameter. This suggests that cortical disinhibition may be facilitating functional plasticity at least a few years after stroke although the disinhibition could be affected at the corticospinal level after stroke. However, as these correlations were observed with a small group of patients, this should be confirmed with larger patient population. Furthermore, in case of subject 5 (Table 2), SICI2 was 0.00 and SICI3 was 4.56, which might suggest possible interference of short-interval intracortical facilitation (SICF) with SICI, which could appear at ISIs of around 3 ms, but not at an ISI of 2 ms (Peurala et al., 2008). This was, however, not observed in the other subjects, and requires further study.

We observed that the recruitment curves of the AH remained at lower amplitude levels than the curves of the UH due to restricted focal lesions (Fig. 3A) similarly to previous stroke studies (Bütefisch et al., 2003; Ward et al., 2006). In the current study, the changes were significant at greater stimulation intensities and higher response amplitudes. This implies that the AH is unable to recruit as large neuronal population to produce a high amplitude response or powerful movements. Besides motoneuron loss, one explanation could be the nature of reorganization of the motor cortex in a manner that the overlapping representation areas with connecting interneurons and shared descending pathways are not as efficiently activated via focal cortical stimulation based on more scattered and less dense representation areas.

Following stroke, focal reductions in the neuronal tract integrity are observed as the interhemispheric asymmetry of tissue anisotropy. Previous studies have shown that interhemispheric connections between the motor cortices are impaired (Cunningham et al., 2015; Li et al., 2015). A few studies have investigated both TMS and DWI aspects in stroke. Callosal FA has been shown to correlate with motor improvement detected after repetitive-TMS (rTMS) therapy, and higher FA values were associated with a better motor outcome (Demirtas-Tatlidede et al., 2015). In a recent study, Cunningham et al. (2015) reported that patients with greater interhemispheric asymmetry in FA showed greater corticospinal output in the UH. To compare those results with ours, we computed the asymmetry index for ADC and found a similar phenomenon with MEP amplitudes induced at 120% of MT50 from the recruitment curve ($\rho = 0.857$, $p = 0.014$). This confirms that higher MEP amplitudes evoked by stimulation of UH were associated with greater asymmetry in ADC values. Greater hemispheric asymmetry in the organization of neuronal fibers may be related to a greater imbalance of interhemispheric inhibition which usually results in decreased inhibition in UH and increased inhibition in AH. Decreased inhibition and increased activation in the contralesional

Fig. 4. Group-wise TMS and DWI characteristics. (A) Resting MTs determined with two MEP threshold criteria: 50 μ V (MT50) and 2 mV (MT2000) presented for the AH and UH as group mean and SEM. MEP amplitudes induced at 120% of MT50 are presented on the right. (B) Normalized MEP amplitudes are presented for SICI2, SICI3 and ICF10. SICI appeared significantly only on the UH. Significant ICF effect was not observed in the group-level. (C) Apparent diffusion coefficients (ADCs) determined from three ROIs, M1 “hand knob”, SMA and thalamus. ADCs are presented for the AH and UH as a group mean and SEM. * indicates a significant ($p < 0.05$) difference between the hemispheres according to the Wilcoxon signed rank test.

motor cortex could explain the finding of more efficient recruitment of motoneurons in AH than in UH. Interestingly, we found that ADC measured from AH side thalamus correlated positively with the ipsilateral MEP amplitude and negatively with MT2000. It has been shown previously that mean diffusivity in thalamus is greater in healthy controls than in stroke patients in the acute stage while it tends to recover especially on the AH side (Hervé et al., 2005). If we consider that greater MEP amplitude and lower MT2000 are indicative of good recovery, then our results agree. In accordance with previous studies on healthy subjects, we found no

other significant correlations between the DWI-derived ADC and the TMS parameters in stroke patients.

Previously, carotid artery stenosis has been shown to affect DWI-weighted MRI and MTs (Avirame et al., 2015; List et al., 2014). In the present study, two patients were observed with hemodynamically relevant carotid artery stenosis (Table 1). Although, we found that stenosis did not clearly affect the MTs or ADC-values (Tables 3 and 4), we made an observation that the two patients diagnosed with relevant stenosis displayed no SICI2 or SICI3. In SICI2, these patients were the only two patients not

Table 2
Measured TMS characteristics.

Id	MT50 (%-MSO)		MT2000 (%-MSO)		MEP (μ V)		SICI2 (normalized)		SICI3 (normalized)		ICF10 (normalized)	
	AH	UH	AH	UH	AH	UH	AH	UH	AH	UH	AH	UH
1	46	45	77	54	610	2346	1.25	0.75	1.34	0.40	1.13	0.71
2	73	54	100	100	469	481	1.67	0.41	1.57	0.36	1.68	0.66
3	51	57	100	90	335	621	0.55	0.52	0.78	0.71	2.47	3.73
4	62	32	100	49	489	895	0.00	0.28	0.00	0.41	1.57	0.91
5	45	56	100	72	119	3207	0.00	0.11	4.56	0.11	3.13	0.75
6	36	40	100	52	424	1262	0.44	0.44	0.24	0.69	1.18	1.97
7	60	63	99	91	681	1161	0.04	0.08	0.02	0.00	0.08	1.18
8	61	61	93	92	973	1060	0.34	0.36	0.03	0.17	0.80	0.70

Abbreviations: AH, affected hemisphere; UH, unaffected hemisphere; MEP, motor evoked potential; MT50, motor threshold with 50 μ V MEP amplitude threshold; MT2000, motor threshold with 2 mV MEP amplitude threshold; SICI2, short-interval intracortical inhibition with 2 ms interstimulus interval; SICI3, short-interval intracortical inhibition with 3 ms interstimulus interval; ICF10, intracortical facilitation with 10 ms inter-stimulus interval.

Table 3
Individual MNI-coordinates (lateral–medial, anterior–posterior, superior–inferior) in mm for centroids of the ROIs used for the computation of ADC.

Id	M1		SMA		Thalamus	
	AH	UH	AH	UH	AH	UH
1	30, –20, 54	–32, –16, 52	12, –10, 58	–10, –8, 58	16, –14, –2	–16, –14, –2
2	–34, –18, 54	32, –18, 42	–10, –6, 54	6, –4, 54	–16, –14, –4	16, –14, –4
3	32, –18, 50	–34, –22, 60	6, –14, 56	–14, –12, 56	16, –14, –2	–14, –14, –2
4	32, –16, 48	–34, –18, 62	10, –8, 58	–10, –6, 58	16, –14, 0	–16, –14, 0
6	34, –16, 52	–36, –20, 52	8, –6, 52	–14, –12, 52	14, –12, –2	–14, –14, –2
7	30, –20, 54	–36, –16, 54	14, –6, 54	–14, –6, 54	16, –14, 0	–16, –14, 0
8	26, –20, 54	–26, –22, 54	10, –8, 58	–8, –8, 58	14, –14, 0	–14, –14, 0

Abbreviations: AH, affected hemisphere; UH, unaffected hemisphere; ADC, apparent diffusion coefficient; M1, primary motor cortex; SMA, supplementary motor area.

Table 4
Measured ADC values from DWI.

Id	ADC on M1 (–)		ADC on SMA (–)		ADC on thalamus (–)	
	AH	UH	AH	UH	AH	UH
1	0.886	0.851	0.835	0.849	0.719	0.696
2	1.403	0.851	0.792	0.862	0.703	0.692
3	0.818	0.764	0.802	0.727	0.663	0.704
4	1.406	0.737	0.729	0.702	0.697	0.716
6	0.877	0.694	0.746	0.699	0.701	0.720
7	0.846	0.712	0.745	0.727	0.711	0.691
8	1.085	0.853	0.852	0.769	0.712	0.740

Abbreviations: AH, affected hemisphere; UH, unaffected hemisphere; ADC, apparent diffusion coefficient; M1, primary motor cortex; SMA, supplementary motor area.

displaying inhibition. While we cannot make a conclusion that stenosis is the reason for the remaining disinhibition, it may be of future research interest.

Previous studies have emphasized the sensitive impact of TMS coil placement and rotation angle to stimulate specific neuronal circuits (Kallioniemi et al., 2015a; Schmidt et al., 2015; Volz et al., 2015). Volz et al. reported that different coil orientations stimulate different neuronal circuits, meaning that the choice of the optimal orientation angle might bias the neuronal circuits investigated. In the present study we utilized nTMS with patient-specific structural MRIs to enable clear anatomical localization of the primary motor cortex and the “hand knob”. The optimal coil rotation angle for stimulation was selected based on maximizing the MEP amplitude (Julkunen et al., 2009), and therefore neuronal circuits most directly involved in the execution of muscle movement action were most likely targeted. In most cases the coil rotation angle followed the expected angle across the underlying gyrus, perpendicularly to the closest sulcus. The findings reported may include the limitation that they represent only properties of the neuronal circuits directly involved in the execution of the muscle

Fig. 5. Group-level recovery of interhemispheric difference in SICI2 over time. Scatter plot between time from diagnosis and interhemispheric difference in SICI effect at 2 ms ISI. As the time from diagnosis at the time of the study varied between patients, we were able to estimate at the group-level how SICI could recover over time. While the time from diagnosis becomes longer, the SICI effect difference between hemispheres becomes smaller, i.e. the affected side SICI2 tends to normalize. Due to small number of subjects, bootstrap resampling was conducted for the correlation (95% CI for $\rho = 0.392 - 1.000$, $p = 0.003$).

movements, while the other circuits potentially not activated by TMS were not assessed.

Although the characteristics of stroke in terms of lesion location and size as well as induced symptoms were strictly defined in the present study, the demographics of the patients were otherwise heterogeneous. The age of the subjects at the time of measurement varied between 48 and 68 years. Aging may have some effect on the rMT (Säisänen et al., 2008), while contrasting results have been reported (Mills and Nithi, 1997; Wassermann, 2002). The studied population also included female and male subjects. It has been

reported that there may or may not be differences between genders in rMTs (Mills and Nithi, 1997; Säisänen et al., 2008; Wassermann, 2002), while no significant gender differences have been reported for SICI or ICF (Säisänen et al., 2011; Wassermann, 2002). We also observed large variation in rMT, SICI and ICF in the AH side. Previous reports on inter-hemispheric differences in healthy populations show that no clear inter-hemispheric differences exist in rMT, SICI or ICF (Maeda et al., 2002; Mills and Nithi, 1997; Säisänen et al., 2008, 2011). A recent large-scale study on diffusion tensor imaging on healthy adult subjects reported no gender differences or age-dependency in M1 neuronal arrangement, while mild differences between hemispheres were reported (Jang and Seo, 2015). None of the aforementioned effects alone are such that they can be detected with confidence on the individual level. Therefore, we acknowledge that such factors induce some additional variation to our data and may have prevented some effects caused by stroke recovery from appearing.

The number of patients in the present study was low due to strict criteria set for the patients to specifically study focused motor cortical lesion, and more patients are required to generalize the results. Therefore, the validation of the correlation findings was conducted using the bootstrap resampling, which provides additional confidence in the results. Homogeneity of the patient population studied may have helped to identify some aspects of the motor cortex function and structure in the chronic stage otherwise unidentifiable in a more heterogeneous population, as indicated by the sensitive correlations found between SICI2 and TFD. In addition, the neurological characterization performed using NIHHS and mRS, do not specifically describe the hand function, which limit the analyses of neurophysiological parameters measured with TMS and those experienced by the patients. This was due to the retrospective nature of the patient recruitment, for which reason we had no baseline measures of the hand motor function. Nevertheless, based on the scales used to assess symptom severity, little or no residual motor symptoms were present at the time of study. Due to low number of subjects no confident inference can be made based on this study whether the observed alterations in electrophysiological and imaging measures have causal role for motor rehabilitation in these patients.

The indications provided by the present study motivate for future studies, which should include: (1) subjects which have suffered from focal lesions in the primary motor cortex but have either undergone only partial motor recovery or full motor recovery to enable comparison between the two in progression of motor cortical function and structure, (2) measures able to discriminate between subtle motor impairments (e.g. Action Research Arm Test, ARAT) in order to enable correlating changes in neurophysiological measures with motor function, and (3) study on the specificity of the neurophysiological alterations by comparing different types of impairments (e.g. in speech). Some measurements could also be conducted during a motor task.

5. Conclusions

In conclusion, clear changes in the excitability of the AH were observed in this preliminary study with nTMS in a small group of patients expressing little or no remaining symptoms from restricted focal motor cortical infarction. The ability of the AH motor cortex to respond to activation with higher stimulus intensities was significantly attenuated suggesting that powerful and fine motor movements of the hand were still suffering from the stroke. Functional changes were accompanied by increased diffusion indicating impaired neuronal organization at the site of the lesion. Considering that these changes were observed at chronic stage at least 15 months after diagnosis and that the lesions were

focused in M1, the resilient plasticity of the motor cortex to transfer the necessary action functions can be assumed to have occurred. The disinhibition in AH observed even at this stage indicates that excess facilitation of activity-dependent plasticity may still be present, but the normalization of inhibition in a few years' time-window after stroke may potentially be associated to the end of stroke-related functional plasticity. While these findings are indicative of long-term stroke-induced changes in cortical inhibition, proving clinical significance of these findings may require further validation with larger number of patients.

Acknowledgements

The authors acknowledge the Research Committee of the Kuopio University Hospital Catchment Area for the State Research Funding (projects 5041726, 5041730 and 5041749, Kuopio, Finland).

Conflicts of interest: PJ and SV have received consulting pay from Nexstim Plc., unrelated to this study. The other authors have nothing to disclose.

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