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Relationship between excitability, plasticity and thickness of the motor cortex in older adults

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ABSTRACT

The relationship between brain structure, cortical physiology, and learning ability in older adults is of particular interest in understanding mechanisms of age-related cognitive decline. Only a few studies addressed this issue so far, yielding mixed results. Here, we used comprehensive multiple regression analyses to investigate associations between brain structure on the one hand, i.e., cortical thickness (CT), fractional anisotropy (FA) of the pyramidal tract and individual coil-to-cortex distance, and cortical physiology on the other hand, i.e. motor cortex excitability and long-term potentiation (LTP)-like cortical plasticity, in healthy older adults (mean age 64 years, 14 women). Additional exploratory analyses assessed correlations between cortical physiology and learning ability in the verbal domain. In the regression models, we found that cortical excitability could be best predicted by CT of the hand knob of the primary motor cortex (CT-M1$_{HAND}$) and individual coil-to-cortex distance, while LTP-like cortical plasticity was predicted by CT-M1$_{HAND}$ and FA of the pyramidal tract. Exploratory analyses revealed a significant inverse correlation between cortical excitability and learning ability. In conclusion, higher cortical excitability was associated with lower CT and lower learning ability in a cohort of healthy older adults, in line with previous reports of increased cortical excitability in patients with cortical atrophy and cognitive deficits due to Alzheimer's Disease. Cortical excitability may thus be a parameter to identify individuals at risk for cognitive decline and gray matter atrophy, a hypothesis to be explored in future longitudinal studies.

1. Introduction

The combined use of imaging techniques with high spatial resolution like magnetic resonance imaging (MRI), and non-invasive measurements of brain physiology like transcranial magnetic stimulation (TMS) has greatly advanced understanding of human brain function (Siebner et al., 2009). With regard to cognitive neuroscience of aging, use of these techniques offers great potential to investigate mechanisms of age-related structural and functional decline. However, most studies focused on young healthy individuals, or on patients with advanced neurodegeneration like Alzheimer's Disease (AD). So far, comprehensive evaluations in elderly individuals without overt dementia, most likely to yield information about compensatory mechanisms with regard to cognitive function, have not been conducted.

Different electrophysiological protocols have been used in exploring cortical neurophysiology. Resting motor threshold (rMT) is generally defined as the lowest stimulus intensity of a single pulse TMS that elicits a predefined, small motor response in the contralateral targeted muscle (Rossini et al., 1994). RMT is a measure of the excitability of corticocortical neuronal structures that are directly activated by TMS and project onto corticospinal output neurons (Ziemann, 2004). While being inter-individually highly variable, rMT is relatively stable and thus reproducible in a given individual (Wassermann, 2002). rMT is critically dependent on coil-to-cortex-distance (CCD) in a given subject (Stokes et al., 2005), but might be also influenced by other functional and anatomical parameters, particularly inter-individual structural variability of the motor cortex (Hübers et al., 2012) and age-related anatomical and physiological brain changes (Silbert et al., 2006). For example, a study in healthy young subjects indicated that deep white matter microstructure, as measured by fractional anisotropy (FA) in diffusion tensor imaging (DTI) may be an important contributor (Klöppel et al., 2008). However, a subsequent study could not confirm these results (Hübers et al., 2012), and thus the association between white matter

Abbreviations: TMS, transcranial magnetic stimulation; MEP, motor evoked potential; APB, abductor pollicis brevis muscle; rMT, resting motor threshold; CCD, coil-to-cortex distance; LTP, long term potentiation; PAS, paired associative stimulation; CT, cortical thickness; FA, fractional anisotropy; PT, pyramidal tract; M1, primary motor cortex; AD, Alzheimer's Dementia; VD, vascular dementia; MRI, magnetic resonance imaging; TR, repetition time; TE, echo time; FLAIR, fluid attenuation inversion recovery; DTI, diffusion tensor imaging; ROI, region of interest; WMH, white matter hyperintensities; GLM, general linear model; TMT, trail making test; AVLT, auditory verbal learning test; GABA, gamma amino butyric acid; NMDA, N-methyl-D-aspartate.

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microstructure and cortical excitability remains to be fully explored in healthy individuals. With regard to pathological conditions, rMT was decreased in patients with predominantly white matter damage as seen in vascular dementia (VAD) (Pennisi et al., 2011) or cortical atrophy due to AD (e.g. (Di Lazzaro et al., 2004; Ferreri et al., 2011). However, a potential association between cortical thickness (CT) and cortical excitability has not been investigated in healthy individuals or in patients so far.

Long-term potentiation (LTP) is assumed to be the basis of learning and memory (Rioult-Pedotti et al., 2000). In humans, LTP-like plasticity can be induced in the primary motor cortex (M1) by the paired associative stimulation (PAS) protocol (Stefan et al., 2000). Here, inter-individual variability was influenced by age (Fathi et al. 2010; Müller-Dahlhaus et al., 2008; Tecchio et al., 2008), cognitive decline (Battaglia et al., 2007), and white matter damage (List et al., 2013). Recently, associations between LTP-like cortical plasticity and cortical thickness (CT) in young healthy individuals have been reported (Conde et al., 2012). The association of LTP-like cortical plasticity and CT in older individuals has not been determined so far.

In the present study, we investigated associations between measures of brain structure and cortical physiology in a well-defined cohort of healthy older adults. We further asked in an exploratory approach if cortical excitability and LTP-like cortical plasticity were correlated with learning ability.

2. Methods

Participants were recruited via newspaper and internet advertisements and via the intranet of the Charité Hospital in Berlin, Germany. 30 subjects (63.9 ± 6.2 years, range 50–75 years, 14 females, 16.7 ± 2.7 years of education) were included in the study. None of the subjects reported use of psychoactive medication or recreational drugs, and none of them had a history of neurological or psychiatric disorders. The Mini Mental State Examination (MMSE; (Folstein et al., 1975), cut off ≤ 28); and Beck's depression inventory (BDI; (Hautzinger et al., 1994), cut off ≥ 12) were used for screening of cognitive deficits or depressive symptoms. All participants were right-handed according to the Edinburgh Handedness Inventory (Oldfield, 1971).

The study was approved by the local ethics committee and conducted in accordance with the Helsinki Declaration on the use of human subjects in experiments. Written informed consent was obtained from all participants.

2.1. Magnetic resonance imaging

2.1.1. Image acquisition

Using a 3T system (Magnetom TIM Trio, Siemens, Erlangen, Germany) equipped with a 12-channel head coil, diffusion-weighted images (TR = 7500 ms, TE = 86 ms, 61 axial slices, voxel size of 2.3 × 2.3 × 2.3 mm^3; 64 directions with a b-value of 1000 s/mm^2 and one b0), high-resolution T1-weighted MPRAGE images (TR = 1900 ms, TE = 2.52 ms, 192 sagittal slices, voxel-size of 1.0 × 1.0 × 1.0 mm^3, flip angle = 9°), and fluid attenuation inversion recovery (FLAIR) images were acquired.

2.1.2. Coil-to-cortex distance (CCD)

CCD was determined on anatomic T1-weighted images. The location of the left hand knob was determined visually (Yousry et al., 1997), and the shortest distance between gray matter and the skull surface was defined as the CCD (according to Hübers et al., 2012). CCD_{left} denotes CCD on the left side.

2.1.3. Quantification of white matter hyperintensities

White matter hyperintensities (WMH) were identified on the FLAIR- and T2-weighted images, and severity was graded semiquantitatively on 4 levels using a modified version of the Fazekas scale (Pantoni et al., 2010): 0 = absence of WMH; 1 = punctuate foci below 10 mm, areas of grouped lesions must be smaller than 20 mm in diameter; 2 = single lesions between 10 and 20 mm, areas of "grouped" lesions more than 20 mm in any diameter; and 3 = large confluence of foci, single lesions of more than 20 mm in diameter.

2.1.4. Cortical thickness (CT) measurements

For CT measurements, we used FreeSurfer (Version 5.1.0), a set of automated tools for reconstruction of the brain cortical surface (Fischl et al., 1999), and is freely available for download online (http://surfer.nmr.mgh.harvard.edu). Technical details of these procedures are described in prior publications (Dale et al., 1999; Fischl et al., 1999, 2002; Han et al., 2006). The processing of T1-weighted images included removal of nonbrain tissue (Segonne et al., 2004), automated Talairach transformation and intensity normalization (Sled et al., 1998), as well as surface deformation to detect gray matter/white matter and gray matter/cerebrospinal fluid boundaries (Fischl et al., 2001; Segonne et al., 2007). The resulting representation of CT was then calculated as the distance between the above tissue boundaries (Fischl and Dale, 2000). The surface models of each subject were inspected visually for accuracy. For whole-brain cortical thickness analyses, we calculated cortical maps at the vertex-wise level by means of a general linear model (GLM) approach, which is implemented in QDEC from FreeSurfer. Individual CT maps were registered bilaterally to the standard template and smoothed with a Gaussian kernel of 25 mm FWHM.

2.1.5. Region of interest definition

A region of interest (ROI) comprising the hand area of left M1 (cortical area where rMT and LTP-like cortical plasticity were assessed, see section on "TMS measurements" below) was defined and subsequently drawn manually in the "knob-like" part of the precentral gyrus, which is further defined by the central sulcus and the superior frontal sulcus (Yousry et al., 1997). This hand drawn ROI had a size of 1775 vertices. The average thickness within this ROI in each subject was extracted for further multiple regression analyses.

2.1.6. Diffusion tensor imaging

2.1.6.1. Preprocessing of DTI data. We used FSL for preprocessing and fibertracking the left pyramidal tract (PT; tract containing the fibers originating from left M1), (http://www.fmrib.ox.ac.uk/fsl). A 3-dimensional rigid body registration was applied to correct for eddy currents and head motion, followed by brain extraction (Smith, 2002). Probability distributions were then calculated, allowing estimation of 2 dimensions per voxel (Behrens et al., 2006). Directional diffusivities were determined as lambda 1 > lambda 2 > lambda 3 and fractional anisotropy (FA) was calculated from those eigenvalues.

2.1.6.2. Probabilistic tractography of the pyramidal tract. In order to reconstruct the PT according to their cortical origins in $M1_{left}$, a ROI was drawn in the subcortical white matter on the individual FA maps in native space, as descried previously (Rüber et al., 2012). Further ROIs were placed in the posterior limb of the internal capsule and the basis pontis (Lindenberg et al., 2012). Using the brainstem ROI as the seed region and the ipsilateral internal capsule and subcortical ROIs as waypoint masks as well as a sagittal exclusion mask in the corpus callosum, we reconstructed the PT in the left hemisphere: fibers originating from M1, passing through the internal capsule and descending to the anterior pons. Probabilistic maps were generated by iterations of the streamline process. For every seed voxel in the brainstem ROIs, 5000 "particles" were propagated through the multi-tensor field. The resulting maps of streamline intensities were then constrained to voxels with more than 10% of the individual robust range in order to extract tract-specific FA values of the left PT ($FA\text{-}PT_{left}$) for regression analyses (see below).

2.2. TMS measurements

All participants were seated comfortably in an armchair. TMS was performed using a Magstim 200 stimulator (Magstim, Whitland, Dyfed, UK) connected to a figure-of-eight shaped coil (9 cm outer diameter of each wing) with the handle pointing backwards at an angle of 45° to the interhemispheric fissure. In optimal position ("hot spot") of the coil, a moderately suprathreshold stimulation intensity leads to a visible abduction of the thumb. This hot spot, i.e. the cortical representation area of the right adductor pollicis brevis (APB) muscle, was then marked with a soft pen on the subjects scalp. Motor evoked potentials (MEP) of the APB muscle were recorded by surface EMG activity using Ag/AgCl surface electrodes. The active electrode was placed on the muscle belly, the inactive electrode over the base of the metacarpophalangeal joint of the thumb. The MEP-signals were amplified and digitized and then stored on a laboratory computer for later offline analysis. The bandpass filter was 5 Hz to 5 kHz (Digitimer D360, Digitimer Ltd., Hertfordshire, UK). Data was digitized at an analog-to-digital rate of 5 kHz.

2.2.1. Resting motor threshold (rMT)

At the "hot spot", rMT was determined as stimulator intensity required to produce a MEP of the APB muscle of at least 50 μV in at least five of ten consecutive trails (Rossini et al., 1994; Wassermann, 2002). Intensity of stimulation was quoted as percentage of maximum stimulator output.

2.2.2. Paired associative stimulation (PAS)

A modified version of the PAS protocol first described by Stefan et al. (2000) was administered (Sale et al., 2007). PAS consisted of an electrical stimulation of the median nerve at the wrist, followed by a subsequent TMS-impulse over the "hot-spot". According to the modified version of PAS, 132 paired stimuli were delivered at a frequency of 0.2 Hz. The interstimulus interval was set to 25 ms, known as the excitatory PAS-protocol (Stefan et al., 2000). Nerve stimulation was applied with a standard stimulation block (cathode proximal) with an intensity of 300% of the individual sensory threshold. TMS pulse intensity was set at 130% of rMT to evoke MEPs with peak-to-peak amplitudes of 0.5 to 1 mV in the relaxed APB. In order to maintain a standardized level of attention during the PAS intervention, subjects were instructed to stay alert, voluntarily relax the APB of the dominant hand, and count the number of motor responses. Muscle relaxation was continuously monitored by visual feedback from the surface EMG. As PAS-induced MEP facilitation is substantially affected by diurnal cycles (Sale et al., 2008), PAS procedures were performed between 2 pm and 5 pm in all subjects. The complete PAS protocol comprised baseline MEP measurements followed by the PAS stimulation and subsequent MEP measurements immediately after stimulation. MEP amplitudes were measured peak-to-peak in each individual trial before and after intervention. Before and after PAS, MEP were induced 20 times with a random pulse interval between 4 and 6 s. MEP amplitudes after the PAS protocol were then averaged and normalized to the MEP amplitude before PAS for each subject. These PAS-induced MEP changes are referred to as "LTP-like cortical plasticity" throughout the manuscript.

2.3. Neuropsychological tests

Each participant underwent a comprehensive neuropsychological test battery (see (Lezak et al., 2004) (Table 2). The German version of the Auditory Verbal Learning Test (Verbaler Lern- und Merkfähigkeitstest, Version A; (Helmstaedter et al., 2001) was used to assess learning ability in the verbal domain (sum of trials 1–5 of word list learning; "learning ability"). Retrieval from verbal memory was tested by a delayed recall task 30 min later. Processing speed and executive function/set shifting were assessed with the trail making test (TMT; version B) and the color word interference subtest of the Stroop test. Verbal fluency (semantic/phonemic fluency) was assessed using the Regensburger Verbal Fluency Test.

Table 1
Baseline characteristics and structural MRI data.

	Mean ± SD
Age (years)	63.93 ± 6.20
Years of education	16.67 ± 2.70
Intracranial volume (mm^3)	1,446,580 ± 113,528
Whole brain cortical volume (mm^3)	431,807 ± 32,151
$CT\text{-}M1_{HANDleft}$ (mm)	2.325 ± 0.242
$CT\text{-}M1_{HANDright}$ (mm)	2.141 ± 0.242
$FA\text{-}PT_{left}$	0.481 ± 0.026
$FA\text{-}PT_{right}$	0.467 ± 0.030

MRI = magnetic resonance imaging, SD = standard deviation, CT = cortical thickness, M1 = primary motor cortex, FA = fractional anisotropy; PT = pyramidal tract

2.4. Statistical analyses

2.4.1. Multivariate regression analyses

To quantify associations between brain structure and cortical physiology, we used multiple linear regression analyses with independent variables age, sex, years of education, CCD_{left}, $CT\text{-}M1_{HANDleft}$, $FA\text{-}PT_{left}$, and each of the two dependent variables (rMT and LTP-like cortical plasticity). All variables were checked for possible collinearity before multiple regression analyses by estimating the variance inflation factor (values > 10 may point towards collinearity) and tolerance (values < 0.1 may point towards collinearity).

2.4.2. Exploratory analyses

For whole-brain cortical thickness analyses within FreeSurfer, we focused on the main effects of rMT, LTP-like cortical plasticity, and learning ability. Corrections for multiple comparisons were addressed using the false discovery rate ($p < 0.05$).

We also assessed partial correlations between rMT and learning ability, as well as LTP-like cortical plasticity and learning ability, corrected for baseline characteristics (age, sex, years of education).

Statistical analyses outside FreeSurfer and FSL were performed using SPSS (Version 21). All data are reported as mean ± standard error of the mean (SEM), unless stated otherwise. Levels of statistical significance were set to $p < 0.05$.

3. Results

All patients tolerated the experiments well, and reported no adverse effects with regard to the TMS or MRI procedures.

3.1. Baseline assessments

Baseline characteristics and structural MRI data are provided in Table 1.

3.1.1. Neuropsychology

Subjects performed within age-adjusted range on neuropsychological tests (Table 1).

Table 2
Neuropsychological test results of the subjects, compared with a percentile Rank of a normative group.

Test /Domain	mean ± SD	Percentile rank of normative group (%)
Verbal learning (AVLT, sum of word lists1–5, # of words)	51.8 ± 10	65
Delayed verbal retrieval (AVLT, # of words)	10.1 ± 3.3	40–45
Verbal fluency, S-Words (# of words)	16.8 ± 5.3	50–75
Verbal fluency, GR-Words (# of words)	14.2 ± 4.6	25–50
Executive function TMT-B (# of words)	88.1 ± 49	>50

SD: standard deviation; AVLT: auditory verbal learning task (German version); TMT: Trail making test

3.1.2. TMS measurements

rMT at baseline was 42.6% ± 7.5% of the maximum stimulator output. MEP amplitude increased after PAS (33% ± 24%; diff. n.s.), similar to previous studies of our group and others in healthy older subjects (see Fig. 2A left in Kang et al., 2011; Fig. 2 in List et al., 2013).

3.1.3. Magnetic resonance imaging

No prior silent stroke, major structural lesions or severe WMH (Fazekas score of 3) were detected.

3.2. Multivariate regression analyses

Simple correlations between baseline parameters, neuropsychological test results, TMS measurements and MRI-deived structural parameters are provided in Table 3. To assess predictive values for individual rMT, we performed a multiple linear regression analysis with rMT as the dependent variable and independent variables age, sex, years of education, CCD_{left}, CT-$M1_{HANDleft}$, and FA-PT_{left}.

For our current model no indices of collinearity of the data was provided by collinearity statistics (variance inflation factor = 1.12 (values > 10 may point towards collinearity); tolerance = 0.90 (values < 0.1 may point towards collinearity)).

Using stepwise regression (backward elimination), CT-$M1_{HANDleft}$ and CCD_{left} remained in the model (linear regression: $F = 9.51$, $p = 0.001$, $R^2 = 0.40$), with beta coefficient = 0.485 for influence of CT-$M1_{HANDleft}$, and beta coefficient = 0.282 for influence of CCD_{left}. Adjusted R^2 likewise indicated that this model achieved the best fit (adjusted $R^2 = 0.36$ compared to adjusted $R^2 = 0.29$ for a model including all six predictors). Please note that estimation of adjusted R^2 takes into account the number of included predictors, thus providing additional information on the magnitude of a predictor's contribution with regard to the model.

Fig. 1 illustrates the partial correlation between rMT and CT-$M1_{HANDleft}$ ($p < 0.01$, $r = 0.51$), after correction for CCD_{left}. rMT and CCD_{left} were not correlated when corrected for CT-$M1_{HANDleft}$ ($p = 0.084$, $r = 0.33$).

To assess predictive values for LTP-like cortical plasticity, we performed a multiple linear regression analysis with LTP-like cortical plasticity as dependent variable, and independent variables age, sex, years of education, CCD_{left}, CT-$M1_{HANDleft}$, and FA-PT_{left}. Using stepwise regression (backward elimination), only FA-PT_{left} and CT-$M1_{HANDleft}$ remained in the model ($F = 6.42$, $p = 0.005$, $R^2 = 0.32$), with beta coefficient = −0.540 for influence of FA-PT_{left} on LTP-like cortical plasticity, and beta coefficient = 0.339 for influence of CT-$M1_{HANDleft}$. Fig. 2 illustrates the partial correlation between PAS-induced MEP-changes and FA-PT_{left} ($p < 0.01$, $r = -0.54$), after correction for CT-$M1_{HANDleft}$.

As a control, we also ran the analysis using rMT and PAS-induced plasticity as dependent variables, and age, sex, years of education as well as FA-PT_{right}, CCD_{right} and CT-$M1_{HANDright}$ as independent variables.

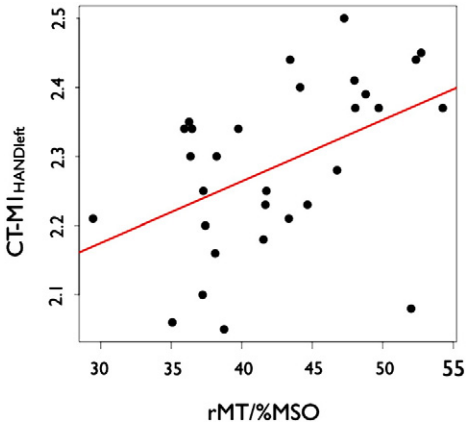

Fig. 1. Partial correlation between rMT and CT-$M1_{HANDleft}$, corrected for CCD_{left}. rMT = resting motor threshold; MSO = maximum stimulator output; CT-$M1_{HANDleft}$ = mean cortical thickness in the left hand knob. CCD left = Coil-to-Cortex distance left; $r = 0.51$; $p < 0.01$.

Here, we found no significant model to predict the dependent variables (all p's > 0.05).

3.3. Exploratory analyses

Whole brain analysis revealed an association between rMT and a cluster of 915 vertices ($p < 0.05$, corrected for multiple comparisons by false discovery rate; see Fig. 3). This cluster was located centrally within the hand knob of M1 of the left hemisphere (Talairach coordinates: $x = -35$, $y = -24.1$, $z = 52.4$; (Talairach and Tournoux, 1988), including the area of the lowest distance between scalp surface and cortex within the left hand knob (i. e., where CCD_{left} was taken). No significant associations were detected between LTP-like cortical plasticity and CT. No correlation between electrophysiological parameters and age were found. A whole brain analysis revealed no effects of age on CT.

Moreover, we assessed potential partial correlations between rMT or LTP-like cortical plasticity, and verbal learning, after correcting rMT/LTP and verbal learning for age, sex and years of education. Here, a positive partial correlation emerged between rMT and verbal learning (Fig. 4; $r = 0.38, T = 2.15, p = 0.04$) but not between LTP-like cortical plasticity and verbal learning ($r = -0.14, T = -0.75, p = 0.46$). No correlations between FA-PT_{left} and verbal learning ($p = 0.22$, $r = 0.01$) were found. A whole brain analysis revealed no associations between verbal learning and CT.

4. Discussion

In the present study, we found that cortical excitability could best be predicted by cortical thickness (CT) of the hand knob of the

Table 3
Correlation matrix for intercorrelations between rMT, MRI-derived structural parameters, demographic variables, verbal learning and PAS-induced cortical plasticity (Pearson's correlation coefficients).

	CCDleft	CT-$M1_{HANDleft}$	CT-$M1_{HANDright}$	FA-PT_{left}	FA-PT_{right}	age	sex	education	AVLT, sum 1-5	PAS-incued plasticity
rMT	**0.44***	**0.58****	0.35	0.22	−0.06	−0.34	0.045	0.02	**0.45***	
CCDleft		0.33	0.11	−0.03	−0.32	−0.35	−0.14	0.11	0.19	0.18
CT-$M1_{HANDleft}$			**0.58****	0.23	−0.08	−0.32	0.30	0.14	0.35	0.21
CT-$M1_{HANDright}$				0.16	−0.02	−0.30	0.18	0.13	0.13	0.12
FA-PT_{left}					**0.46***	−0.13	0.05	−0.17	0.01	**−0.46***
FA-PT_{right}						0.14	0.05	−0.17	−0.31	−0.26
Age							−0.14	0.01	−0.31	0.12
Sex								−0.29	0.10	−0.20
Education									0.03	0.15
AVLT, sum 1–5										−0.14

rMT = resting motor threshold; MRI = magnetic resonance imaging; CCD = Coil-to-cortex distance, CT = cortical thickness, PT = pyramidal tract, FA = fractional anisotropy, AVLT = auditory verbal learning task, PAS = paired associative stimulation, * significance level < 0.05, ** significance level < 0.01.
Significant correlations are marked in bold.

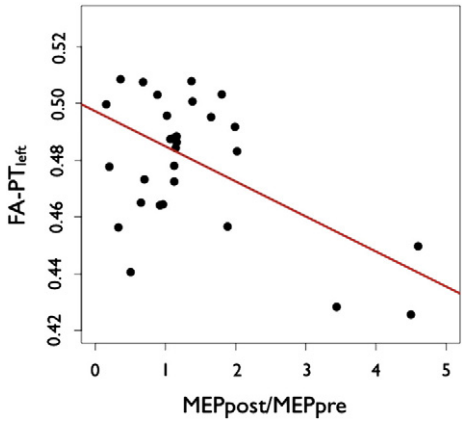

Fig. 2. Partial correlation between normalized PAS-induced MEP changes (i.e. LTP-like cortical plasticity) and FA-PT$_{left}$, corrected for CT-M1$_{HANDleft}$. PAS = paired associative stimulation, MEP = motor evoked potential, FA = fractional anisotropy, PT = pyramidal tract, CT = cortical thickness.

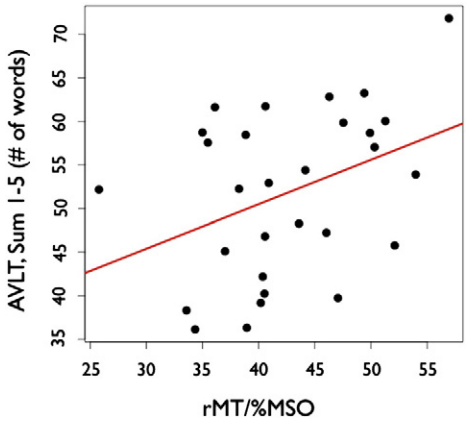

Fig. 4. Correlations between rMT and verbal learning ability (as assessed by the word list learning of the AVLT), corrected for age, sex and years of education. rMT = resting motor threshold; MSO = maximum stimulator output; AVLT: auditory verbal learning task. r = 0.38, p = 0.04.

respective primary motor cortex (M1$_{HANDleft}$) and coil-to-cortex distance (CCD). These findings were supported by independent whole-brain analyses, showing that individual differences in cortical excitability were selectively associated with CT of a cluster within the left hand knob, but no other cortical areas.

Moreover, higher LTP-like cortical plasticity could best be predicted by lower fibre coherence of the pyramidal tract (PT) and CT in M1$_{HANDleft}$. Finally, exploratory analyses revealed a correlation between cortical excitability and learning ability: The higher the excitability, the lower the learning ability.

4.1. Brain structure and cortical excitability

Cortical excitability was examined by rMT, which is defined as the lowest stimulus intensity at which single-pulse TMS to M1 of one hand elicits a predefined, small motor response in the contralateral target muscle (Rossini et al., 1994). RMT reflects the trans-synaptic excitability of corticospinal output neurons that are directly activated by TMS (Ziemann, 2004). Sodium and calcium channel blockers that reduce axon excitability elevate rMT, indicating that rMT largely reflects excitability of the axonal membrane rather than transsynaptic neurotransmission (Chen et al., 1997; Ziemann et al., 1996). rMT is critically sensitive to the individual CCD, based on the known properties of magnetic field distributions generated in TMS (McConnell et al., 2001; Stokes et al., 2005, 2013), a finding reproduced in the present study. Moreover, it is influenced by several properties of the motor system including geometry of precentral gyrus and orientation of the PT relative to the induced current in the brain, fibre coherence of stimulated axons and spinal cord excitability level (Hübers et al., 2012). However, a large amount of inter-individual variability remains unexplained (Siebner and Ziemann, 2007). Klöppel et al. (2008) reported associations between microstructure of the subcortical white matter, as estimated by FA, and rMT in younger subjects, indicating higher cortical excitability to be linked with higher white matter microstructural integrity. However, the associations between rMT and FA were not seen in the FA of the PT, but in the subcortical white matter outside motor pathways, and no associations between rMT and FA of the PT could be found in a subsequent study (Hübers et al., 2012). Significance of cerebral white matter microstructural integrity on cortical excitability thus remains unclear. In the present study, FA-PT$_{left}$ was not predictive for rMT in our linear regression model. Rather, rMT could best be predicted by CT-M1$_{HANDleft}$ and CCD$_{left}$, explaining 40% of variance in our group of healthy older adults.

The underlying physiological mechanisms of the association of increased cortical excitability with cortical atrophy (as indicated by lower CT measurements) might lie in an altered balance of different cortical neurotransmitter systems. Here, a modulation of Gamma amino butyric acid (GABA)-ergic and possibly also cholinergic tone, following neuronal degeneration and functional reorganization has been proposed (Ferreri et al., 2003; Koliatsos et al., 2006). Cortical thinning may reflect a decrease in GABA-mediated intracortical inhibition, following degeneration of GABAergic cortical interneurons (Ferreri et al., 2003), and may result in a decreased threshold for a given TMS pulse to elicit an action potential in corticospinal output neurons. Furthermore, a dysbalance of N-methyl D-aspartate (NMDA) and non-NMDA glutamatergic transmission has been suggested to account for hyperexcitability in neurodegenerative diseases such as AD (Di Lazzaro et al., 2004).

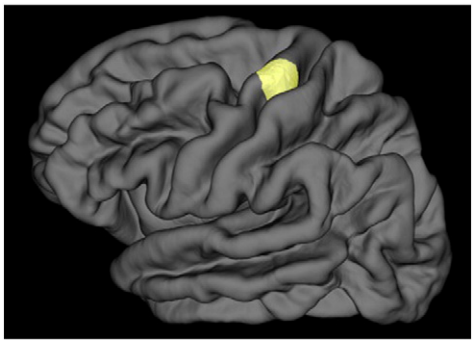

Fig. 3. Composite pial representation of the statistically significant clusters of cortical thickness (915 vertices, Talairach coordinates: x = −35, y = −24.1, z = 52.4), that were positively correlated with rMT in the hand knob of the left primary motor cortex, p-values corrected with multiple comparisons by false discovery rate, $p < 0.05$.

4.2. Brain structure and LTP-like cortical plasticity

LTP-like cortical plasticity was increased in individuals with lower white matter fibre coherence in the PT, similar to previous results in both middle-aged and older individuals (List et al., 2011, 2013). PAS-induced LTP-like cortical plasticity shares distinct properties with synaptic LTP and is assumed to be the synaptic basis of learning and memory (Bliss and Collingridge, 1993; Ziemann et al., 2004). Even though several factors influencing the ability to induce LTP-like cortical plasticity in humans are known, e.g. aging (Fathi et al. 2010; Müller-Dahlhaus et al., 2008; Tecchio et al., 2008), genetic factors (Cheeran et al., 2008; Witte et al., 2012), brain metabolism (List et al., 2012), and white matter microstructural integrity (List et al., 2011, 2013), a large part of inter-individual variability remains to be elucidated. Using linear regression, FA in the PT and CT-M1$_{HANDleft}$ were able to predict 32% of inter-individual variability in LTP-like cortical plasticity. Interestingly, lower structural fibre coherence of the PT$_{left}$ but higher CT in M1 was associated with higher LTP-like cortical plasticity.

Similar to previous studies of our group (List et al., 2011, 2013), the present data further support an active role of LTP-like cortical plasticity to counteract structural decline of the PT, as seen in the negative associations between LTP-like cortical plasticity and FA, in older adults with preserved cognition. As reported previously (Conde et al., 2012), CT-$M1_{HANDleft}$ also remained in our linear regression model.

Thus, our results help to further elucidate factors that contribute to inter-individual variability in LTP-like cortical plasticity. Moreover, they support the hypothesis of a compensatory increase in cortical plasticity in cognitively normal older adults that show, within a group of elderly individuals (List et al., 2011, 20113), lower white matter microstructural integrity.

4.3. Brain structure and cortical physiology in the aging brain

Age-related changes in brain structure are well documented, including not only an overall cortical atrophy due to aging (Fjell and Walhovd, 2010), but also a distinct pattern of cortical thinning involving predominantly the frontal and occipital lobes, with prominent atrophy of M1 (Salat et al., 2004). The latter may eventually lead to an increase in CCD in advancing age, as reported (albeit as a trend) in Freitas et al. (2011). With regard to neurophysiology in animal models, aging has been associated with a reduction in synaptic plasticity (Barnes 2003), which was even more pronounced in the presence of neurodegenerative disease (Battaglia et al., 2007). Using TMS-based neurophysiological measurements in humans, an age-related decrease of LTP-like cortical plasticity (Müller-Dahlhaus et al., 2008; Tecchio et al., 2008), with further reduction in older individuals with cognitive decline (Battaglia et al., 2007; Frantseva et al., 2008) has likewise been reported. Investigations of age-related modifications of cortical excitability, on the other hand, revealed conflicting results. Some studies showed an increase in rMT in healthy older compared to young individuals (McGinley et al., 2010; Rossini et al., 1992), whereas other studies reported no differences in rMT between different age groups (Oliviero et al., 2006; Pitcher et al., 2003). In the present study, we did not examine a control group of young subjects and therefore cannot directly address this question. However, over the (rather narrow) age range of our study, we did not observe associations between age and cortical excitability or LTP-like plasticity.

Motor cortex and global cortical hyperexcitability has been shown in AD (Di Lazzaro et al., 2004; Rossini et al., 2007) and VAD (Pennisi et al., 2011), and may even correlate with cognitive decline in these conditions (Alagona et al., 2001; Bella et al., 2013; Khedr et al., 2011). As discussed above, increased cortical excitability in patients suffering from dementia may reflect a dysbalance of cortical transmitter systems following degeneration of cortical neurons and synapses. In fact, neuroimaging studies reported a widespread trend towards cortical thinning in virtually all parts of the brain in patients with AD (Lehmann et al., 2010), even though most pronounced in the parieto-occipital cortex (Ridgway et al., 2012). The ubiquitous impact of the neuropathological processes underlying AD onto the entire human brain is supported by neuropathological studies, which revealed AD-typical neurofibrillary tangles and senile plaques also in the motor cortex, equivalent to other brain areas (Selden et al., 1998; Suvà et al., 1999).

In disease conditions like AD or VAD, cortical hyperexcitability and decreased LTP-like cortical plasticity might therefore be related to the degree of structural deterioration, as seen in the present study in our group of healthy older individuals. However, note that previous studies did not directly assess the association between cortical excitability and CT of specific cortical regions, but rather cortical excitability related to the neurodegenerative process of the entire brain. Our study is therefore the first to probe the association of cortical excitability with quantitative measures of microstructural integrity in cortical areas and the PT in healthy older subjects. We provide evidence that higher cortical excitability might be related to structural decline in cognitively (still) intact healthy older individuals.

4.4. Limitations

Although we showed clear associations between brain structure and cortical physiology, only rMT and LTP-like cortical plasticity were assessed. Other parameters analyzing cortical physiology, like long intracortical inhibition and short intracortical inhibition to assess GABA-activity, short afferent inhibition to assess cholinergic tone, and paired pulse protocols (e.g. intracortical facilitation) as well as MEP recruitment curves to assess additional aspects of cortical excitability, may yield additional information on the association of brain structure with human cortical physiology. Furthermore, factors that might additionally contribute to inter-individual variability in rMT and LTP-like cortical plasticity, such as genetic polymorphisms in learning relevant genes (Cheeran et al., 2008; Witte et al., 2012), or cerebral perfusion (List et al., 2012) were not determined in our cohort.

Although several lines of evidence support the hypothesis that changes in motor cortex physiology are associated with more general changes in brain function and plasticity, including cognitive functions (Battaglia et al., 2007; De Beaumont et al., 2011; Frantseva et al., 2008; List et al., 2011, 2012; Witte et al., 2012), additional assessments of motor function and learning, possibly more directly reflecting changes in motor cortex physiology, should be included in future studies. To address the question whether rMT might serve as a biomarker for neurodegenerative changes, further multimodal longitudinal studies are required.

Note also that the number of subjects investigated in this study was small, and we only included cognitively healthy individuals of a rather narrow age range. This might explain why we did not find associations between electrophysiological parameters and age.

4.5. Conclusion

We demonstrate that rMT and LTP-like plasticity may in part be explained by CT of the hand motor cortex and white matter fibre coherence of the PT in healthy older adults. Thus, the present study further contributes to elucidating inter-individual variability of TMS-based assessments of cortical physiology in humans.

Moreover, our results indicate that higher cortical excitability, noted previously in patients with cortical atrophy and cognitive deficits due to AD, may already occur in healthy elderly individuals that show, within a group of elderly individuals, lower cortical thickness and learning ability. Thus, rMT might be a parameter to identify individuals at risk for cognitive decline and gray matter atrophy, a hypothesis to be evaluated in future longitudinal studies.

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Conflict of Interest

None declared.

References

Alagona, G., Bella, R., Ferri, R., Carnemolla, A., Pappalardo, A., Costanzo, E., Pennisi, G., 2001. Transcranial magnetic stimulation in Alzheimer disease: motor cortex excitability and cognitive severity. Neurosci. Lett. 314, 57–60.

Battaglia, F., Wang, H.-Y., Ghilardi, M.F., Gashi, E., Quartarone, A., Friedman, E., Nixon, R.A., 2007. Cortical plasticity in Alzheimer's disease in humans and rodents. CORD Conference Proceedings, 62 1405–1412.

Barnes, C.A., 2003. Long-term potentiation and the ageing brain. Philos Trans R Soc Lond B Biol Sci. 358, 765–772.

Behrens, T.E., Johansen-Berg, H., Jbabdi, S., Rushworth, M.F., Woolrich, M.W.M., 2006. Probabilistic diffusion tractography with multiple fibre orientations: What can we gain? NeuroImage 34, 12-12.

Bella, R., Ferri, R., Lanza, G., Cantone, M., Pennisi, M., Puglisi, V., Vinciguerra, L., Spampinato, C., Mazza, T., Malaguarnera, G., Pennisi, G., 2013. TMS follow-up study in patients with vascular cognitive impairment-no dementia. Neurosci. Lett. 534, 155–159.

Bliss, T.V., Collingridge, G.L., 1993. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361, 31–39.

Cheeran, B., Talelli, P., Mori, F., Koch, G., Suppa, A., Edwards, M., Houlden, H., Bhatia, K., Greenwood, R., Rothwell, J.C., 2008. A common polymorphism in the brain-derived neurotrophic factor gene (BDNF) modulates human cortical plasticity and the response to rTMS. Available at: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=18845611&retmode=ref&cmd=prlinks.

Chen, R., Samii, A., Caños, M., Wassermann, E.M., Hallett, M., 1997. Effects of phenytoin on cortical excitability in humans. Neurology 49, 881–883.

Conde, V., Vollmann, H., Sehm, B., Taubert, M., Villringer, A., Ragert, P.P., 2012. Cortical thickness in primary sensorimotor cortex influences the effectiveness of paired associative stimulation. NeuroImage 60, 864–870.

Dale, A., Fischl, B., Sereno, M.I., 1999. Cortical Surface-Based Analysis: I. Segmentation and Surface Reconstruction. NeuroImage 9, 179–194.

De Beaumont, L., Tremblay, S., Poirier, J., Lassonde, M., Theoret, H., 2011. Altered Bidirectional Plasticity and Reduced Implicit Motor Learning in Concussed Athletes. Cereb. Cortex 22, 112–121 (Available at: message:%3C3E42557F0EB5B94696094FA941455E4EEFFACACF94@EXCHANGE21.charite.de%3E).

Di Lazzaro, V., Oliviero, A., Pilato, F., Saturno, E., Dileone, M., Marra, C., Daniele, A., Ghirlanda, S., Gainotti, G., Tonali, P.A., 2004. Motor cortex hyperexcitability to transcranial magnetic stimulation in Alzheimer's disease. J. Neurol. Neurosurg. Psychiatry 75, 555–559.

Fathi, D., Ueki, Y., Milma, T., Koganemanu, S., Nagamine, T., Tawfik, A., Fukuyama, H., 2010. Effects of aging on the human motor cortical plasticity studied by paired associative stimulation. Clin Neurophysiol. 121, 90–93.

Ferreri, F., Pauri, F., Pasqualetti, P., Fini, R., Dal Forno, G., Rossini, P.M., 2003. Motor cortex excitability in Alzheimer's disease: a transcranial magnetic stimulation study. Ann. Neurol. 53, 102–108.

Ferreri, F., Pasqualetti, P., Määttä, S., Ponzo, D., Guerra, A., Bressi, F., Chiovenda, P., Del Duca, M., Giambattistelli, F., Ursini, F., Tombini, M., Vernieri, F., Rossini, P.M., 2011. Motor cortex excitability in Alzheimer's disease: a transcranial magnetic stimulation follow-up study. Neurosci. Lett. 492, 94–98.

Fischl, B., Dale, A.M., 2000. Measuring the thickness of the human cerebral cortex from magnetic resonance images. Proc. Natl. Acad. Sci. U. S. A. 97, 11050–11055.

Fischl, B., Sereno, M.I., Dale, A., 1999. Cortical Surface-Based Analysis: II: Inflation, Flattening, and a Surface-Based Coordinate System. NeuroImage 9, 195–207.

Fischl, B., Liu, A., Dale, A.M., 2001. Automated manifold surgery: constructing geometrically accurate and topologically correct models of the human cerebral cortex. IEEE Trans. Med. Imaging 20, 70–80.

Fischl, B., Salat, D.H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., van der Kouwe, A., Killiany, R., Kennedy, D., Klaveness, S., Montillo, A., Makris, N., Rosen, B., Dale, A.M., 2002. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron 33, 341–355.

Fjell, A.M., Walhovd, K.B., 2010. Structural brain changes in aging: courses, causes and cognitive consequences. Rev. Neurosci. 21, 187–221.

Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. "Mini-mental state." A practical method for grading the cognitive state of patients for the clinician. J. Psychiatr. Res. 12, 189–198.

Frantseva, M.V., Fitzgerald, P.B., Chen, R., Möller, B., 2008. Evidence for impaired long-term potentiation in schizophrenia and its relationship to motor skill leaning. Cereb. Cortex 18, 990–996.

Freitas, C., Perez, J., Knobel, M., Tormos, J.M., Oberman, L., Eldaief, M., et al., 2011. Changes in cortical plasticity across the lifespan. Front. Aging Neurosci. 3, 5. http://dx.doi.org/10.3389/fnagi.2011.00005.

Han, X., Jovicich, J., Salat, D., van der Kouwe, A., Quinn, B., Czanner, S., Busa, E., Pacheco, J., Albert, M., Killiany, R., Maguire, P., Rosas, D., Makris, N., Dale, A., Dickerson, B., Fischl, B., 2006. Reliability of MRI-derived measurements of human cerebral cortical thickness: The effects of field strength, scanner upgrade and manufacturer. NeuroImage 32, 180–194.

Hautzinger, M., Bailer, M., Worall, H., Keller, F., 1994. Beck-Depressions-Inventar (BDI). Huber, Bern.

Helmstaedter, C., Lendt, M., Lux, S., 2001. VLMT.

Hübers, A., Klein, J.C., Kang, J.-S., Hilker, R., Ziemann, U., 2012. The relationship between TMS measures of functional properties and DTI measures of microstructure of the corticospinal tract. Brain Stimul. 5, 297–304.

Kang, J.-S., Terranova, C., Hilker, R., Quartarone, A., Ziemann, U., 2011. Deficient homeostatic regulation of practice-dependent plasticity in writer's cramp. Cereb. Cortex 21, 1203–1212.

Khedr, E.M., Ahmed, M.A., Darwish, E.S., Ali, A.M., 2011. The relationship between motor cortex excitability and severity of Alzheimer's disease: a transcranial magnetic stimulation study. Neurophysiol. Clin. 41, 107–113.

Klöppel, S., Bäumer, T., Kroeger, J., Koch, M.A., Büchel, C., Münchau, A., Siebner, H.R., 2008. The cortical motor threshold reflects microstructural properties of cerebral white matter. NeuroImage 40, 10-10.

Koliatsos, V.E., Kecojevic, A., Troncoso, J.C., Gastard, M.C., Bennett, D.A., Schneider, J.A., 2006. Early involvement of small inhibitory cortical interneurons in Alzheimer's disease. Acta Neuropathol. 112, 147–162.

Lehmann, M., Douiri, A., Kim, L.G., Modat, M., Chan, D., Ourselin, S., Barnes, J., Fox, N.C., 2010. Atrophy patterns in Alzheimer's disease and semantic dementia: a comparison of FreeSurfer and manual volumetric measurements. NeuroImage 49, 2264–2274.

Lezak, Muriel D., Howieson, Diane B., Loring, David W., 2004. Neuropsychological Assessment, In: Lezak, Muriel D., Howieson, Diane B., Loring, David W. (Eds.), 4th ed. Oxford University Press, USA (Hardcover).

Lindenberg, R., Zhu, L.L., Rüber, T., Schlaug, G., 2012. Predicting functional motor potential in chronic stroke patients using diffusion tensor imaging. Hum. Brain Mapp. 33, 1040–1051.

List, J., Duning, T., Meinzer, M., Kürten, J., Schirmacher, A., Deppe, M., Evers, S., Young, P., Flöel, A., 2011. Enhanced rapid-onset cortical plasticity in CADASIL as a possible mechanism of preserved cognition. Cereb. Cortex 21, 2774–2787.

List, J., Albers, J., Kürten, J., Schwindt, A., Wilbers, E., Flöel, A., 2012. Reperfusion does not improve impaired rapid-onset cortical plasticity in patients with severe stenosis of the internal carotid artery. PLoS One 7, e41004.

List, J., Duning, T., Kürten, J., Deppe, M., Wilbers, E., Flöel, A., 2013. Cortical plasticity is preserved in nondemented older individuals with severe ischemic small vessel disease. Hum. Brain Mapp. 34, 1464–1476.

McConnell, K.A.K., Nahas, Z.Z., Shastri, A.A., Lorberbaum, J.P.J., Kozel, F.A.F., Bohning, D.E.D., George, M.S.M., 2001. The transcranial magnetic stimulation motor threshold depends on the distance from coil to underlying cortex: a replication in healthy adults comparing two methods of assessing the distance to cortex. Biol. Psychiatry 49, 454–459.

McGinley, M., Hoffman, R.L., Russ, D.W., Thomas, J.S., Clark, B.C., 2010. Older adults exhibit more intracortical inhibition and less intracortical facilitation than young adults. Exp. Gerontol. 45, 671–678.

Müller-Dahlhaus, J.F.M., Orekhov, Y., Liu, Y., Ziemann, U., 2008. Interindividual variability and age-dependency of motor cortical plasticity induced by paired associative stimulation. Exp. Brain Res. 187, 467–475.

Oldfield, R.C., 1971. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9, 97–113 (Available at: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=5146491&retmode=ref&cmd=prlinks).

Oliviero, A., Profice, P., Tonali, P.A., Pilato, F., Saturno, E., Dileone, M., Ranieri, F., Di Lazzaro, V., 2006. Effects of aging on motor cortex excitability. Neurosci. Res. 55, 74–77.

Pantoni, L., Pescini, F., Nannucci, S., Sarti, C., Bianchi, S., Dotti, M.T., Federico, A., Inzitari, D., 2010. Comparison of clinical, familial, and MRI features of CADASIL and NOTCH3-negative patients. Neurology 74, 57–63.

Pennisi, G., Ferri, R., Cantone, M., Lanza, G., Pennisi, M., Vinciguerra, L., Malaguarnera, G., Bella, R., 2011. A review of transcranial magnetic stimulation in vascular dementia. Dement. Geriatr. Cogn. Disord. 31, 71–80.

Pitcher, J.B., Ogston, K.M., Miles, T.S., 2003. Age and sex differences in human motor cortex input-output characteristics. J. Physiol. 546, 605–613.

Ridgway, G.R., Lehmann, M., Barnes, J., Rohrer, J.D., Warren, J.D., Crutch, S.J., Fox, N.C., 2012. Early-onset Alzheimer disease clinical variants: multivariate analyses of cortical thickness. Neurology 79, 80–84.

Rioult-Pedotti, M.S., Friedman, D., Donoghue, J.P., 2000. Learning-induced LTP in neocortex. Science 290, 533–536.

Rossini, P.M., Desiato, M.T., Caramia, M.D., 1992. Age-related changes of motor evoked potentials in healthy humans: non-invasive evaluation of central and peripheral motor tracts excitability and conductivity. Brain Res. 593, 14–19.

Rossini, P.M., Barker, A.T., Berardelli, A., Caramia, M.D., Caruso, G., Cracco, R.Q., Dimitrijević, M.R., Hallett, M., Katayama, Y., Lücking, C.H., 1994. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. Electroencephalogr. Clin. Neurophysiol. 91, 79–92.

Rossini, P.M., Rossi, S., Babiloni, C., Polich, J., 2007. Clinical neurophysiology of aging brain: From normal aging to neurodegeneration. Prog. Neurobiol. 83, 26-26.

Rüber, T., Schlaug, G., Lindenberg, R.R., 2012. Compensatory role of the cortico-rubrospinal tract in motor recovery after stroke. Neurology 79, 515–522.

Salat, D.H., Buckner, R.L., Snyder, A.Z., Greve, D.N., Desikan, R.S.R., Busa, E., Morris, J.C., Dale, A.M., Fischl, B., 2004. Thinning of the cerebral cortex in aging. Cereb. Cortex 14, 721–730.

Sale, M.V., Ridding, M.C., Nordstrom, M.A., 2007. Factors influencing the magnitude and reproducibility of corticomotor excitability changes induced by paired associative stimulation. Exp. Brain Res. 181, 615–626 (Available at: http://www.springerlink.com/index/R1782V7448N47066.pdf).

Sale, M.V., Ridding, M.C., Nordstrom, M.A., 2008. Cortisol inhibits neuroplasticity induction in human motor cortex. CORD Conference Proceedings, 28 8285–8293.

Segonne, F., Dale, A.M., Busa, E., Glessner, M., Salat, D., Hahn, H.K., Fischl, B., 2004. A hybrid approach to the skull stripping problem in MRI. NeuroImage 22, 1060–1075.

Segonne, F., Pacheco, J., Fischl, B., 2007. Geometrically accurate topology-correction of cortical surfaces using nonseparating loops. IEEE Trans. Med. Imaging 26, 518–529.

Selden, N.R., Gitelman, D.R., Salamon-Murayama, N., Parrish, T.B., Mesulam, M.-M., 1998. Trajectories of cholinergic pathways within the cerebral hemispheres of the human brain. Brain 121 (Pt 12), 2249–2257.

Siebner, H.R., Ziemann, U., 2007. Das TMS-Buch. Springer.

Siebner, H.R., et al., 2009. Consensus paper: combining transcranial stimulation with neuroimaging. Brain Stimul. 2, 58–80.

Silbert, L.C., Nelson, C., Holman, S., Eaton, R., Oken, B.S., Lou, J.S., Kaye, J.A., 2006. Cortical excitability and age-related volumetric MRI changes. Clin. Neurophysiol. 117, 1029–1036.

Sled, J.G., Zijdenbos, A.P., Evans, A.C., 1998. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. IEEE Trans. Med. Imaging 17, 87–97.

Smith, S.M., 2002. Fast robust automated brain extraction. Hum. Brain Mapp. 17, 143–155.

Stefan, K., Kunesch, E., Cohen, L.G., Benecke, R., Classen, J., 2000. Induction of plasticity in the human motor cortex by paired associative stimulation. Brain 123, 699–708.

Stokes, M.G., Chambers, C.D., Ian, G., Tracy, H., Natasha, J., Nicholas, A., Jason, M., 2005. Simple Metric For Scaling Motor Threshold Based on Scalp-Cortex Distance:

Application to Studies Using Transcranial Magnetic Stimulation. J. Neurophysiol. 94, 4520–4527.
Stokes, M.G., Barker, A.T., Dervinis, M., Verbruggen, F., Maizey, L., Adams, R., Chambers, C.D., 2013. Biophysical Determinants of Transcranial Magnetic Stimulation: Effects of Excitability and Depth of Targeted Area. J. Neurophysiol. 109, 437–444.
Suvà, D., Favre, I., Kraftsik, R., Esteban, M., Lobrinus, A., Miklossy, J., 1999. Primary motor cortex involvement in Alzheimer disease. J. Neuropathol. Exp. Neurol. 58, 1125–1134.
Talairach, J., Tournoux, P., 1988. Co-planar stereotaxic atlas of the human brain.
Tecchio, F., Zappasodi, F., Pasqualetti, P., De Gennaro, L., Pellicciari, M.C., Ercolani, M., Squitti, R., Rossini, P.M., 2008. Age dependence of primary motor cortex plasticity induced by paired associative stimulation. Clin. Neurophysiol. 119, 675–682 (Available at: http://books.google.com/books?hl=en&lr=&id=YeKleGrKwC4C&oi=fnd&pg=PR5&dq=wassermann+variation+in+the+response+to+transcranial&ots=DZv75QCDPl&sig=V0mGXOPqAFI21tK1nMdERz3PjSQ).
Wassermann, E., 2002. Variation in the response to transcranial magnetic brain stimulation in the general population. Clin. Neurophysiol. 113, 1165–1171.
Witte, A.V., Kürten, J., Jansen, S., Schirmacher, A., Brand, E., Sommer, J., Flöel, A., 2012. Interaction of BDNF and COMT Polymorphisms on Paired-Associative Stimulation-Induced Cortical Plasticity. J. Neurosci. 32, 4553–4561.
Yousry, T.A., Schmid, U.D., Alkadhi, H., Schmidt, D., Peraud, A., Buettner, A., Winkler, P., 1997. Localization of the motor hand area to a knob on the precentral gyrus. A new landmark. Brain 120 (Pt 1), 141–157.
Ziemann, U., 2004. TMS and drugs. Clin. Neurophysiol. 115, 1717–1729.
Ziemann, U., Lönnecker, S., Steinhoff, B.J., Paulus, W.W., 1996. Effects of antiepileptic drugs on motor cortex excitability in humans: a transcranial magnetic stimulation study. Ann. Neurol. 40, 367–378.
Ziemann, U., Ilić, T.V., Iliać, T.V., Pauli, C., Meintzschel, F., Ruge, D., 2004. Learning modifies subsequent induction of long-term potentiation-like and long-term depression-like plasticity in human motor cortex. J. Neurosci. 24, 1666–1672.