Enhanced Rapid-Onset Cortical Plasticity in CADASIL as a Possible Mechanism of Preserved Cognition

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Ischemic small vessel disease (SVD) may lead to cognitive impairment, but cognitive deficits with a given burden of SVD vary significantly. The underlying mechanisms of impaired or preserved cognition are unknown. Here, we investigated the impact of ischemic SVD on rapid-onset cortical plasticity, as induced with a paired-associative stimulation protocol. To exclude concomitant effects of aging, we examined 12 middle-aged patients (48.3 ± 8.3 years) with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) who suffered from severe ischemic SVD and a group of 12 age-matched controls (49.9 ± 8.3 years). Cognitive status, motor performance and learning, and motor cortex excitability in response to cathodal transcranial direct current stimulation (ctDCS) were assessed. White matter integrity was analyzed by conventional magnetic resonance imaging and diffusion tensor imaging. We found that cognitive and motor functions were largely preserved in CADASIL patients, while rapid-onset cortical plasticity was significantly higher in the CADASIL group compared with controls (repeated measures analysis of variance [group × time] interaction: P = 0.03). This finding was even more pronounced in patients with higher white matter lesion load. ctDCS revealed no evidence of cortical dysplasticity. We conclude that increased rapid-onset cortical plasticity may contribute to largely preserved cognitive and motor function despite extensive ischemic SVD.

Keywords: diffusion tensor imaging, ischemic small vessel disease, LTP-like plasticity, transcranial magnetic stimulation

Introduction

Ischemic small vessel disease (SVD) accounts for approximately 20% of cases of dementia and is characterized by lacunar infarcts and focal as well as diffuse ischemic white matter hyperintensities (WMH) (Erkinjuntti and Gauthier 2009). WMH can also be found in normal aging (>70% in adults >60 years; O’ Sullivan 2008; Arsava et al. 2009), and the extent of WMH has been linked to cognitive impairment and increased risk of incident dementia (Viswanathan et al. 2009). However, severe ischemic SVD may be found in individuals with normal cognitive performance (Duning et al. 2005; Galluzzi et al. 2008), which has been attributed to compensatory brain plasticity (e.g., Stern et al. 2005; Stern 2006; Park and Reuter-Lorenz 2009).

One of the main factors underlying learning and memory formation at the cellular level is long-term potentiation (LTP) (Rioult-Pedotti et al. 2000; Frantseva et al. 2008). In humans, LTP-like cortical plasticity can be assessed using peripheral electric stimulation and subsequent transcranial magnetic stimulation (TMS). This so-called paired-associative stimulation (PAS; Stefan et al. 2000) is now a widely used protocol to noninvasively investigate rapid-onset cortical plasticity in healthy subjects and neurological patients (e.g., Morgante et al. 2006; Weise et al. 2006; Frantseva et al. 2008; Castel-Lacanal et al. 2009; Zeller et al. 2010). Rapid-onset cortical plasticity is known to be reduced in the aging brain (e.g., Müller-Dahlhaus et al. 2008), but the impact of acute or chronic brain damage like ischemic SVD is still unclear.

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an early-onset monogenic variant of ischemic SVD caused by a mutation in the NOTCH3 gene (Dichgans 2002; Chabriat et al. 2009). CADASIL is associated with progressive ischemic damage (WMH and subcortical lacunar infarcts). Clinical symptoms include mood changes, migraine, and cognitive deficits that usually begin in middle age (between 30 and 40 years of age; Adib-Samii et al. 2010). The earliest changes in white matter occur several years earlier and may be detectable on magnetic resonance (MR)-based diffusion tensor imaging (DTI), indicating metabolic and structural damage of white matter (Chabriat et al. 1999). Due to the early onset of the disease, comorbid conditions from age-related pathologies such as neurochemical changes (Floël et al. 2005; Burke and Barnes 2006) and shrinkage in frontal and hippocampal volume (Raz et al. 2005) are rare. This makes CADASIL a unique model for investigating mechanisms of neurophysiological and cognitive changes in ischemic SVD, including possible compensatory mechanisms. Adaptive functional reorganization has been suggested in CADASIL patients in studies using functional magnetic resonance imaging (MRI) (Reddy and Stefano 2002). However, the question whether enhanced rapid-onset cortical plasticity is seen in CADASIL patients with relatively preserved cognition has not been addressed.

In the present study, a group of CADASIL patients was recruited to assess the impact of extensive ischemic SVD on cognition, motor performance and learning, response to cathodal transcranial direct current stimulation (ctDCS), and rapid-onset cortical plasticity. SVD was quantified by the degree of WMH and number of lacunar lesions on conventional MRI, as well as integrity of white matter tracts on diffusion-weighted images. We hypothesized that cortical plasticity would be enhanced in patients who showed cognitive and motor functions within the normal age-corrected range, despite...
extensive ischemic SVD, thus serving as one possible mediator for preserved function.

Materials and Methods

Patients and Healthy Volunteers

Twelve patients with CADASIL (aged 48.3 ± 8.3 years, range 36–67 years, 8 females) from 8 different families and 12 healthy volunteers (HV) (49.9 ± 8.3 years, range 39–67 years, 8 females) were included in the present study.

HV, all with normal results on neurological examination, were chosen to match the patients with regard to age, sex, and years of education. For patients, inclusion criteria comprised a previous diagnosis of CADASIL by genetic testing (missense change on chromosome 19 of the NOTCH3 gene [Joutel et al. 1996]), presence of ischemic SVD as confirmed by previous MRI scans (see below), Mini Mental State Examination (MMSE; Folstein et al. 1975) ≥ 28 to exclude severe cognitive deficits, Beck’s depression inventory (BDI) (Hautzinger et al. 1994) to exclude severe depression, and absence of severe concomitant internal disease or other major psychiatric disorders (see below). Neurological examination excluded a motor paresis of the dominant hand. All participants were right handed according to the Edinburgh Handedness Inventory (Oldfield 1971), except 1 left-handed CADASIL patient (patient #5; for details, see Table 1).

The study was approved by the local Institutional Review Board and was conducted in accordance with the declaration of Helsinki on the use of human subjects in experiments. All participants gave written informed consent.

Study Outline

Patients were recruited from the outpatient unit of the Department of Neurology, University Hospital of Münster, between January 2009 and November 2009. In all CADASIL patients, ischemic SVD as WMH or lacunar lesions had been detected on MRI scans that were acquired prior to this study. HV were recruited via local newspaper advertisements. Each HV was selected to match a CADASIL patient with respect to age, gender, and years of education. Recruitment of HV was continued until matching was complete. None of the HV had a history of major neurological or psychiatric disease.

Before inclusion, all participants underwent a clinical interview and a medical and neurological examination. The modified Rankin Scale (van Swieten et al. 1988) and the NIH-Stroke Scale Score (Lydén et al. 1999) were administered to screen for neurological deficits. After inclusion, patients and HV participated in the neuropsychological testing and the PAS protocol was administered (see below). Neurological examination excluded a motor paresis of the dominant hand. All participants were right handed according to the Edinburgh Handedness Inventory (Oldfield 1971), except 1 left-handed CADASIL patient (patient #5; for details, see Table 1). The study was approved by the local Institutional Review Board and was conducted in accordance with the declaration of Helsinki on the use of human subjects in experiments. All participants gave written informed consent.

Experimental Procedures

Neuropsychological Testing

A comprehensive neuropsychological test battery (see Lezak 2004) was administered to each participant (Table 3). Processing speed as well as executive function/set shifting were assessed with the trail making test (TMT; versions A + B) and the color word interference subtest of the Stroop test. Verbal fluency (semantic/phonemic fluency) was assessed with the Regensburger Verbal Fluency Test. The German version of the Auditory Verbal Learning Test was used to assess verbal learning capacity across 5 trials and the retrieval from verbal memory by delayed recall (30 min) (Verbaler Lern- und Merkfähigkeitstest, Version A; Helmstaedter et al. 2001). Rey figure copy and recall examined visuospatial skills and nonverbal memory; digit span and block tapping (Revised Wechsler Memory Scale; Lezak 2004), both forward and backward, tested working memory performance.

Motor Tasks

a) Finger tapping task:

Subjects were instructed to press a key with the index finger as quickly as possible for a total of 10 s. The keypad was connected to a laboratory computer that recorded the frequency of taps. The task was repeated 3 times, with 1-min resting intervals between trials and executed with first the left and then the right index finger. Numbers of taps of each hand were then summed.

b) Motor sequence learning task: In this task (for detailed description, see e.g., Jimenez et al. 1996; Rösser et al. 2008), participants performed finger movements repeatedly without being aware of the sequential order underlying most of the presented stimuli. A modified version of the serial reaction time task was used, with a probabilistic instead of a deterministic sequence to ensure the procedural task nature mixing shorter and longer sequential segments with random ones (15%
random and 85% sequential elements in each block). The sequential structure of the 85% was generated by a so-called finite-state grammar that instantiated a set of rules that described the permissible transitions between successive stimuli. The advantage of a probabilistic sequence is that participants show no explicit knowledge of the learned sequences, even after several trials, and that an almost unlimited number of different sequences of equal complexity can be produced. Although improved performance during the whole task can be observed due to procedural motor learning and increasing task routine, differences in reaction times between random and sequential elements represent a measure of procedural motor learning only, the main outcome measure of our study (Rösser et al. 2008)

Participants performed the task while sitting in front of a monitor. Their dominant hand was placed on a special keypad with 5 different keys, one for each finger. Following the rules of the finite-state grammar, one of the black squares on the screen was replaced by an asterisk. The patients had to press the key corresponding to the given asterisk as fast as possible. The sequence (with intermixed random elements) was presented in 2 blocks of 500 key presses each. A new asterisk was presented 500 ms after every key press. Data were saved as a .log-file by the Presentation software. Implicit motor learning was defined as the difference in reaction times between random and sequential elements in the second block (see Rösser et al. 2008).

Neurophysiological Testing
Participants were seated comfortably in an armchair. Focal TMS was performed using a Magstim 200 stimulator (Magstim) connected to a figure-of-eight-shaped coil (9 cm outer diameter of each wing). The hand of the coil was pointing backward at an angle of 45° to the interhemispheric fissure, oriented to induce posterior-anterior current flow approximately perpendicular to the central sulcus over the hand area of motor cortex. Optimal position ("hot spot") of the coil was the cortical representation area of the abductor digiti minimi (ADM) muscle resulting in a visible abduction of the little finger at a moderately suprathreshold stimulation intensity. The hot spot was then marked on the scalp of the subject. The coil was placed over the left hemisphere in right-handed subjects or over the right hemisphere in left-handed subjects. Nerve stimulation was applied with a standard stimulation block (cathode proximal) with an intensity of 300% of the individual sensory threshold.

Peripheral nerve stimulation was performed on the right ulnar nerve at the wrist of right-handed subjects or on the left ulnar nerve at the wrist of the left-handed subject. Nerve stimulation was applied with a standard stimulation block (cathode proximal) with an intensity of 300% of the individual sensory threshold.

PAS consisted of 90 pairs of peripheral stimulation of the ulnar nerve at the wrist and a single TMS impulse over the hot spot of the ADM muscle on the contralateral hemisphere at a rate of 0.05 Hz. The TMS intensity was adjusted to produce MEP of at least 0.5–1 mV in the relaxed targeted muscle was determined.

Resting Motor Threshold
At optimal position of the coil, resting motor threshold (rMT) was determined as stimulator intensity required to produce an MEP of the ADM muscle of at least 50 μV in at least 5 of 10 consecutive trials. Intensity of stimulation was quoted as the percentage of maximal stimulator output. Thereafter, the stimulator intensity sufficient to evoke peak-to-peak amplitude of 0.5–1 mV in the relaxed targeted muscle was determined.

Paired-Associative Stimulation
For all CADASIL patients and HV, the PAS protocol similar to the protocol of Stefan et al. (2000) was administered. Previous activity was controlled for by a standardized pretesting protocol including a period of motor rest (interview, baseline testing on MMSE, and BDI) followed by the PAS protocol. In addition, none of the subjects reported specific hand training on the day of PAS testing. In order to maintain a standardized level of attention during the PAS intervention, subjects were instructed to stay alert, voluntarily relax the ADM of the dominant arm, and count the number of ulnar nerve stimulations. 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 10 AM and 3 PM in all subjects.

Peripheral nerve stimulation was performed on the right ulnar nerve at the wrist of right-handed subjects or the left ulnar nerve at the wrist of the left-handed subject. Nerve stimulation was applied with a standard stimulation block (cathode proximal) with an intensity of 300% of the individual sensory threshold.

PAS consisted of 90 pairs of peripheral stimulation of the ulnar nerve at the wrist and a single TMS impulse over the hot spot of the ADM muscle on the contralateral hemisphere at a rate of 0.05 Hz. The TMS intensity was adjusted to produce MEP of at least 0.5–1 mV. The interstimulus interval between peripheral nerve stimulation and TMS pulse was set to 25 ms, which has been shown previously to induce an
LTP-like increase in MEP amplitude (Stefan et al. 2000; Zeller et al. 2010). The complete PAS protocol comprised baseline MEP measurements followed by the PAS stimulation and subsequent MEP measurements at time points 0 (T0, i.e., immediately after stimulation), 15 (T15), 30 (T30), and 60 (T60) min after PAS. A schematic overview of PAS is given in Figure 1. MEP amplitudes were measured at peak to peak in each individual trial before and after intervention. For each time point, MEP were induced 20 times with a frequency of 0.1 Hz. MEP amplitudes of each time point were then averaged and normalized to the MEP amplitude at baseline for each subject. Additionally, PAS-induced plasticity of each subject was further assessed by the grand average of normalized MEP amplitudes measured at time points T0, T15, T30, and T60. Values >1 indicate responders to PAS, whereas values <1 indicate nonresponders to PAS (for details, see Müller-Dahlhaus et al. 2008).

Cathodal Transcranial Direct Current Stimulation Stimulation was delivered using a battery-driven direct current (DC) stimulator (Elithid, Neuroconn) via 2 conductive rubber electrodes placed in saline-soaked sponges (active electrode: 5 cm, reference electrode: 10 x 10 cm) and positioned over the representational field of the right ADM (active electrode) and over the contralateral supraorbital area (reference electrode) (for overview, see also Nitsche and Paulus 2001). DC flow was administered continuously for 10 min at an intensity of 1 mA.

To detect current-driven changes of cortical excitability, MEP of the right ADM were recorded following TMS of its motor cortical representational field. MEP were elicited by single-pulse TMS before (baseline) and immediately after ctDCS (post-ctDCS). For each time point, MEP were elicited 20 times with a frequency of 0.1 Hz. MEP amplitudes of each time point were averaged and MEP amplitude post-ctDCS normalized to the MEP amplitude at baseline for each subject.

Magnetic Resonance Imaging MRI data acquisition Imaging data were obtained on a 3-T system (Magnetom TIM Trio; Siemens). High-resolution structural $T_1$ and $T_2$-weighted and fluid attenuation inversion recovery (FLAIR) scans were acquired. MRI data acquisition was performed with the following parameters: $T_1$, 3D magnetization-prepared rapid gradient echo sequence (repetition time [TR] 1900 ms, echo time [TE] 2.52 ms, NEX 1, flip angle 9°, matrix 256 x 256 x 160 over an field of view of 256 x 256 x 176 mm³, cubic voxel edge length of 1.0 mm), $T_2$, TR = 4220, TE = 102 ms; and FLAIR imaging, TR = 9000 ms, TE = 93 ms, time to inversion = 2500 ms. For DTI, we employed echo planar imaging (EPI) with 20 diffusion directions (2 b-factors, 0 s/mm², and 1000 s/mm², TR = 7 s/TE = 104 ms, voxel size: 1.80 x 1.80 x 3.6 mm, 2 averages) according to Jones et al. (1999).

MRI data analysis. Quantification of WMH WMH were identified on the FLAIR- and $T_2$-weighted images, and severity was graded semi-quantitatively on 4 levels using a modified version (Pantoni et al. 2010) of the Fazekas scale (Fazekas et al. 1987): 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. Examples for the modified Fazekas scale are provided in Figure 2.

Quantification of lacunar lesions The burden of lacunar lesions was determined by assessing parenchymal defects not extending to the cortical gray matter, with signal intensity corresponding to that of cerebrospinal fluid and a diameter between 2 and 15 mm on $T_2$-weighted scans (Liem et al. 2007). Due to the heterogeneous quality of MRI data acquired previously as part of routine clinical examination, the lacunar lesion load was only calculated in patients who participated in an additional study-specific high-resolution $T_2$-weighted scan (i.e., not in patients 1, 5, 11, and 12, see Table 1).

Global volumetric measurements Brain tissue volumes, normalized for subject head size, were calculated from $T_2$-weighted images using the cross-sectional version of the Structural Imaging Evaluation of Normalized Atrophy (SIENA) software (SIENAx) (Smith et al. 2002). This method is an automated brain volume analysis tool that has been validated in healthy controls and patient data (Chen et al. 2004). SIENAx first segments the image volume into brain and skull. The brain-only volume is then spatially normalized to the Montreal Neurological Institute (MNI) stereotaxic space using an affine registration. The skull-only volume is used to obtain an appropriate scaling factor to limit normalization error due to atrophy. Then, tissue classification including partial volume estimation is performed, from which estimates of gray matter, white matter, and cerebrospinal fluid volumes are calculated. Finally, atrophy is assessed by calculating a brain parenchyma fraction as the ratio of the (normalized) brain parenchyma volume to the sum of the parenchyma and cerebrospinal fluid volume, that is, as percentage of intracranial volume.

Diffusion tensor imaging For DTI analyses, the EPI images were spatially normalized to the MNI coordinate system after correction for eddy currents, following an optimized procedure on the basis of multiple image contrasts (Mohammadi et al. 2007). Fractional anisotropy (FA) is reported to be the most valid and reliable parameter when quantifying microstructural white matter changes (Hagemann et al. 2006; Deppe et al. 2007; Mukherjee et al. 2008). The MRI analysis of this study is focused on subtle white matter changes and its clinical relevance. Thus, in order to display the most established parameter that renders the clearest results,

Figure 2. Examples of WMH severity rating according to the “modified Fazekas scale” (Pantoni et al. 2010): score 1 = punctuate foci below 10 mm. Areas of grouped lesions must be smaller than 20 mm in diameter. Score 2 = single lesions between 10 and 20 mm, areas of “grouped” lesions more than 20 mm in diameter. Score 3 = large confluence of foci, single lesions of more than 20 mm in diameter.
we calculated only FA values from the MRI data sets and omitted to display additional parameters (e.g., mean diffusivity or trace) with less validity.

First, the diffusion tensor and FA field maps (Bihan et al. 2001) of all participants were calculated from spatially normalized images. In a second step, all FA images were normalized to an FA template image also corresponding to the MNI coordinate system. FA images were smoothed with an 8-mm isotropic Gaussian filter. These steps and the voxel-based analysis of FA were performed using Matlab software (Mathworks Inc.) and statistical parametric mapping (SPM5; Wellcome Department; http://www.fil.ion.ucl.ac.uk/spm/).

To assess the magnitude of regional FA alterations between CADASIL patients and HV and the relationship between regional FA alterations and clinical parameters (PAS-induced plasticity, rMT, and neuropsychological data), we performed quantitative region-of-interest (ROI) analyses. The definition of ROIs was described in detail elsewhere (Deppe et al. 2007). Briefly, we used an averaged and symmetric (x-axis) mask of all HV with FA values >0.4. The ROIs were derived from this preliminary FA mask by deleting voxels not associated with the respective structures (see illustrations in Fig. 3). Mean FA values in all ROIs were calculated for all CADASIL patients and HV.

In addition, the regression tool of the SPM5 software was used to correlate FA values across the entire brain on a voxel-by-voxel basis with the normalized MEP values following PAS as well as with neuropsychological data. This approach allows for a data-driven analysis of associations between indicators of brain integrity (i.e., FA values) and clinical variables that does not require an a priori definition of ROIs.

**Statistical Analysis**

Demographic characteristics, MMSE, and BDI scores were compared between CADASIL patients and HV using unpaired t-tests.

**Neuropsychological Testing**

Single-test results were $Z$ transformed in correlation to the control group (mean score of 0 and standard deviation of 1). To determine meaningful composite scores, each neuropsychological test was assigned to one cognitive domain (see Table 3; adapted after Peters et al. 2005; Knecht et al. 2008). Mean $Z$ scores of cognitive domains were then calculated by using the mean of the individual test’s $Z$ scores. For timed tests, the sign of the $Z$ score was reversed so that improved performance resulted in a higher score in all tests. Due to the small number of subjects, we additionally compared mean results of each neuropsychological test with standardized normal values, if available (see Table 3). Differences between CADASIL patients and HV in neuropsychological test results were assessed with unpaired t-tests.

**Motor Tasks**

Differences between CADASIL patients and HV in motor tasks were assessed with unpaired t-tests.

**PAS-Induced Plasticity**

To test for the effects of PAS-induced plasticity, repeated measures analyses of variance (ANOVA) were used with time as the repeated measure (pre, T0, T15, T30, and T60) and the between-subject factor group (CADASIL and HV). In case of significance, post hoc testing was conducted using unpaired t-tests to compare the groups at the different time points.

**Figure 3.** Defined regions of interest (ROIs; in green) superimposed on an average FA template of 160 healthy individuals (not shown: ROI of the parietal lobes and the thalamus).


**Catodical Transcranial Direct Current Stimulation**

Differences between CADASIL patients and HV in ctDCS-induced motor cortex excitability changes were assessed with unpaired t-tests.

**Association between MRI Data, Neuropsychological/ Motor Testing, and PAS-Induced Plasticity**

To quantify associations between PAS and imaging data (ROI-FA values, brain volumes, lacunar lesions, and WMH), as well as PAS and neuropsychological/motor test scores, Pearson's correlations, Spearman rank correlations, and partial correlations were calculated to assess the relationship between PAS-induced plasticity and the modified Fazekas scale in CADASIL patients.

The regression tool of the SPM5 software was used to correlate maps of decreased FA with the grand average of MEP following PAS. Statistical threshold for the correlation analysis was set at $P < 0.005$, corrected for multiple comparison using the false discovery rate method (correction, Genovese et al. 2002), which is a standard approach in SPM. Additionally, voxelwise differences in FA values between the CADASIL patients and HV were statistically evaluated by analysis of covariance (ANCOVA), modeling age as a covariate to account for the age dependency of FA ($P < 0.001$, uncorrected; minimum cluster size 25 voxels).

Statistical analyses outside of SPM5 were performed using the statistical software R (R Development Core Team, 2010; http://www.r-project.org). All data are reported as mean ± standard error of the mean, and $P$ values for statistical significance were set to $P < 0.05$, unless stated otherwise.

**Results**

Demographic and clinical characteristics of the CADASIL patients and HV are summarized in Table 1. Patients and HV did not differ significantly with regard to age, gender, years of education, MMSE, and BDI scores (see Table 2). No focal neurological deficits were observed in the HV. Patient #5 had dysarthria and mild spastic hemiparesis on the right side, patient #8 had a persistent hypesthesia on the right side with recurrent pain in the right arm. Both patients did not report reduced use of their dominant hand in everyday life. Note that the dominant hand was used for PAS-induced plasticity (left hand in patient #5).

**Neuropsychological Testing**

Detailed results of the neuropsychological tests of the CADASIL patients and HV are shown in Table 3. In general, performance of the patients was slightly below average as assessed by percentile rank compared with a normative group (Tombaugh 2004), with most pronounced reductions on one of the measures of executive functions (TMT-B; percentile rank of 0--10%). Compared with the scores of the matched HV, in CADASIL patients, there was a slight overall reduction of performance for all tests, but significant differences emerged only for 1 of the 5 cognitive domains tested (verbal memory; $t = 3.99, P = 0.0009$). Even though the patients performed

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**Table 2**

Comparison of baseline characteristics of CADASIL patients and HV

<table>
<thead>
<tr>
<th>CADASIL (mean ± SD)</th>
<th>HV (mean ± SD)</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 48.3 ± 8.3</td>
<td>49.9 ± 8.3</td>
<td>-0.49</td>
<td>0.60</td>
</tr>
<tr>
<td>Gender (4 males, 8 females)</td>
<td>4 males, 8 females</td>
<td>0.49</td>
<td>0.60</td>
</tr>
<tr>
<td>YoE 12.5 ± 2.5</td>
<td>13.9 ± 3.6</td>
<td>1.11</td>
<td>0.25</td>
</tr>
<tr>
<td>MMSE 29.3 ± 0.9</td>
<td>29.4 ± 0.8</td>
<td>0.49</td>
<td>0.62</td>
</tr>
<tr>
<td>BDI 8.4 ± 9.4</td>
<td>6.5 ± 5.1</td>
<td>0.62</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Note: SD, standard deviation; YoE, years of education. Groups were compared using unpaired t-tests.

**Table 3**

Neuropsychological testing

<table>
<thead>
<tr>
<th>Domain</th>
<th>Tests</th>
<th>CADASIL patients</th>
<th>HV</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Percentile rank of normative group (%)</td>
<td>Mean ± SD</td>
<td>Percentile rank of normative group (%)</td>
</tr>
<tr>
<td>Processing speed, executive functions,</td>
<td>Sroop</td>
<td>54.1 ± 24.3</td>
<td>41 ± 11.6</td>
<td>0.05</td>
</tr>
<tr>
<td>set shifting</td>
<td>TMT-A</td>
<td>34.8 ± 15.3</td>
<td>23.8 ± 6.8</td>
<td>0.05</td>
</tr>
<tr>
<td>(time to completion)</td>
<td>TMT-B</td>
<td>88.3 ± 45.8</td>
<td>67.2 ± 17.9</td>
<td>0.02</td>
</tr>
<tr>
<td>Verbal memory</td>
<td>AVLT, sum of trials 1-5</td>
<td>45.5 ± 7.9</td>
<td>59.9 ± 6.2</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>(# correctly recalled items)</td>
<td>AVLT, trial 7</td>
<td>9.1 ± 3.0</td>
<td>12.8 ± 1.7</td>
<td>0.002</td>
</tr>
<tr>
<td>Working memory</td>
<td>WMS-digit-span, forward</td>
<td>7.9 ± 2.1</td>
<td>8.1 ± 2.2</td>
<td>0.85</td>
</tr>
<tr>
<td>(# correctly recalled items)</td>
<td>WMS-digit-span, backward</td>
<td>6.8 ± 1.8</td>
<td>7.8 ± 1.7</td>
<td>0.17</td>
</tr>
<tr>
<td>Verbal fluency</td>
<td>WMS-block-span, forward</td>
<td>7.5 ± 1.8</td>
<td>8.8 ± 1.5</td>
<td>0.06</td>
</tr>
<tr>
<td>(# of exemplars)</td>
<td>RWT-S-words</td>
<td>26 ± 7.6</td>
<td>26.8 ± 7.9</td>
<td>0.81</td>
</tr>
<tr>
<td>RVT-W and R-words</td>
<td>20.82 ± 7.1</td>
<td>24.31 ± 6.4</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>RWT-food</td>
<td>32.5 ± 8.6</td>
<td>41.5 ± 9.1</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>RWT-clothes-flowers</td>
<td>21.1 ± 3.6</td>
<td>23.8 ± 5.3</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Visuospatial skills</td>
<td>ROCF-figure</td>
<td>33.1 ± 3.2</td>
<td>34.4 ± 1.6</td>
<td>0.08</td>
</tr>
<tr>
<td>or recalled parts</td>
<td>ROCF-recall</td>
<td>14.8 ± 7.3</td>
<td>19 ± 3.7</td>
<td>0.06</td>
</tr>
<tr>
<td>Intelligence (IQ points)</td>
<td>HAWIE</td>
<td>106.7 ± 13.7</td>
<td>111.7 ± 11.3</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Note: SD, standard deviation; RWT, Regensburger Verbal Fluency Test; ROCF, Rey–Osterrieth Complex Figure; WMS, Wechsler Memory Scale; HAWIE, Hamburger Wechsler Intelligenztest für Erwachsene (German intelligence test); AVLT, Auditory Verbal Learning Test (German Version). Groups were compared using unpaired t-tests. Significant differences between groups in single test results are marked in bold.
worse on 1 of the 4 verbal fluency tests (simple semantic fluency), overall, there was no significant difference between the groups in this domain. Moreover, no significant differences in neuropsychological test scores were detected between CADASIL patients with higher (Fazekas 3) and lower lesion load (Fazekas 1 + 2) (all $t$ values $< 0.9$, all $P$ values $> 0.41$) except for executive functions ($t = 2.9, P = 0.01$), see Table 4.

### Motor Tasks

#### Motor Performance

Performance in motor tasks did not differ significantly between patients and controls, except for the 9-hole peg-board task; here, time to completion was decreased in CADASIL patients for the left hand compared with HV ($t = 2.78, P = 0.02$) (see also Table 5).

#### Motor Sequence Learning Task

In both groups, analysis of reaction times between random and sequential elements revealed implicit motor learning (CADASIL: $36 \pm 9$ ms; HV: $26 \pm 6$ ms) without a significant difference between groups ($t = -1.0, P = 0.33$) (see also Table 5).

### Neurophysiological Testing

#### TMS

$rMT$ at baseline was $45.6 \pm 7.7\%$ of maximum stimulator output in CADASIL patients and $42.5 \pm 4.4\%$ in HV, with no significant difference between groups ($P = 0.25$). For changes in MEP amplitude induced by PAS, ANOVA revealed a significant interaction on group $\times$ time (linear trend, $F_{1,4} = 3.23, P = 0.03$). Post hoc analyses showed significant differences in MEP size for T0 ($t = 2.25, P = 0.037$), T15 ($t = 3.40, P = 0.004$), and T60 ($t = 2.33, P = 0.035$) (Fig. 4).

Changes in MEP amplitudes induced by PAS showed a high intersubject variability. In CADASIL patients, significant PAS-induced plasticity was found in all posttests at time points T0, T15, and T60. No significant PAS-induced plasticity was noted in HV; in fact, even a decrease emerged in all posttests and reached significance at T15 ($t = 3.39, P = 0.006$) (Fig. 4).

Calculating the grand average of the individual posttests, PAS-induced plasticity could be detected in 10 of 12 CADASIL patients (10 responders to PAS and 2 nonresponders to PAS) but only in 3 of 12 HV (3 responders to PAS and 9 nonresponders to PAS) (Fig. 5).

Post hoc tests showed a significant difference between CADASIL patients and HV in the grand average of normalized MEP changes (unpaired $t$-test, $t = 2.97, P = 0.009$). The averages of normalized MEP changes following PAS of all CADASIL patients increased by 37% (paired $t$-test, $t = 2.50, P = 0.03$), whereas a decrease of 12% ($t = 1.65, P = 0.13$) was observed in HV. MEP changes after PAS were not age dependent (Pearson’s correlation coefficient—CADASIL: $r = 0.09, P = 0.78$; HV: $r = -0.17, P = 0.59$).

### Tables

#### Table 4

Differences in neuropsychological test scores between CADASIL patients with higher (Fazekas 3) and lower lesion load (Fazekas 1 + 2) and HV.

<table>
<thead>
<tr>
<th>Fazekas 3 (mean ± SD)</th>
<th>Fazekas 1 + 2 (mean ± SD)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Executive function ($z$ score)</td>
<td>$-2.55 \pm 1.86$</td>
<td>$-0.13 \pm 0.67$</td>
</tr>
<tr>
<td>Verbal memory ($z$ score)</td>
<td>$-0.71 \pm 1.36$</td>
<td>$-0.16 \pm 1.29$</td>
</tr>
<tr>
<td>Working memory ($z$ score)</td>
<td>$-2.13 \pm 0.74$</td>
<td>$-1.59 \pm 1.07$</td>
</tr>
<tr>
<td>Verbal fluency ($z$ score)</td>
<td>$-0.60 \pm 0.91$</td>
<td>$-0.69 \pm 0.72$</td>
</tr>
<tr>
<td>Visuospatial skills ($z$ score)</td>
<td>$-0.60 \pm 2.25$</td>
<td>$-0.17 \pm 1.41$</td>
</tr>
</tbody>
</table>

Note: SD, standard deviation. Groups were compared using unpaired $t$-tests.

#### Table 5

Motor learning and motor performance in CADASIL patients and HV.

<table>
<thead>
<tr>
<th></th>
<th>CADASIL</th>
<th>HV</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motor learning (mean ± SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Implicit motor learning (ms)</td>
<td>36 ± 9</td>
<td>26 ± 6</td>
<td>0.33</td>
</tr>
<tr>
<td>Motor skills (mean ± SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9-hole peg-board</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left, time to completion (s)</td>
<td>15.5 ± 1.37</td>
<td>11.5 ± 0.42</td>
<td>0.02</td>
</tr>
<tr>
<td>Right, time to completion (s)</td>
<td>13 ± 0.78</td>
<td>12.9 ± 1.1</td>
<td>0.93</td>
</tr>
<tr>
<td>Grooved peg-board</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left, time to completion (s)</td>
<td>83.6 ± 6.51</td>
<td>70.5 ± 3.55</td>
<td>0.10</td>
</tr>
<tr>
<td>Right, time to completion (s)</td>
<td>78.9 ± 4.82</td>
<td>68.8 ± 4.13</td>
<td>0.13</td>
</tr>
<tr>
<td>Finger tapping</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finger tapping left (# of taps in 3 s)</td>
<td>113.25 ± 7.97</td>
<td>123.75 ± 5.71</td>
<td>0.30</td>
</tr>
<tr>
<td>Finger tapping right (# of taps in 3 s)</td>
<td>135.125 ± 9.82</td>
<td>159.375 ± 6.59</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Note: SD, standard deviation. Groups were compared using unpaired $t$-tests.
Associations between TMS-Related Measurements and Cognitive Performance/Motor Tasks

No significant correlations were found between PAS-induced plasticity and neuropsychological test scores or PAS-induced plasticity and motor task scores.

Associations between TMS-Related Measurements and MRI Data

Mean PAS-induced plasticity was significantly increased in patients with higher burden of WMH as assessed by the modified Fazekas scale (Spearman rank correlation Rho, \( P = 0.02, \) Rho = 0.67). Furthermore, patients with high burden of WMH (modified Fazekas scale score 3, \( n = 7 \)) showed significantly more PAS-induced plasticity, as compared with CADASIL patients with comparatively low WMH (modified Fazekas scale scores 1 and 2, \( n = 4 \)) (unpaired \( t \)-test, \( t = 2.51, P = 0.04 \)). Also, PAS-induced plasticity was higher in patients with larger number of lacunar lesions, but this association failed to reach significance (\( R = 0.62, t = 1.97, P = 0.10 \)).

Pearson’s correlation showed no significant association between grand averages of PAS-induced plasticity and mean FA values in any of the predefined ROIs.

Voxelwise regression analysis of FA values and the grand average of MEP changes following PAS in CADASIL patients revealed significant clusters in the frontal part of the corpus callosum and bilaterally in the brain stem and the medulla oblongata (Fig. 7) (\( P < 0.005, 200 \) continuous voxels), indicating negative correlations (i.e., more pronounced white matter damage in these areas predicted increased rapid-onset cortical plasticity). No positive correlations were found. Thus, analysis of FA values supports the finding from the modified Fazekas scale that more damage to white matter tracts (as indicated by low FA values) is associated with higher PAS-induced plasticity. In particular, an affection of fronto-subcortical brain damage. In line with previous studies (for overview, see Dichgans 2009), cognition in our sample was relatively preserved in CADASIL patients, compared with the healthy matched control group. Likewise, motor performance and motor learning were preserved. Most strikingly and in line with our a priori hypothesis, the magnitude of enhanced plasticity in the patients was positively correlated with the degree of white matter lesions as assessed with the Fazekas score. Moreover, the subgroup of patients with more pronounced lesions evidenced larger cortical plasticity compared with the subgroup with less severe brain pathology. Similarly, voxelwise regression analysis of FA values and magnitude of rapid-rate cortical plasticity in CADASIL patients showed specific clusters of a significantly negative correlation, particularly for the frontal part of the corpus callosum. Thus, our findings might point toward one adaptive mechanism to...
compensate for severe and recurrent injury due to ischemic SVD, preventing more pronounced functional decline.

Very little is known about the neurophysiological mechanisms underlying compensatory brain plasticity in ischemic SVD. Previous studies failed to obtain parallel information on behavioral outcomes, quantitative brain imaging, and quantitative measurements of brain plasticity. To avoid these shortcomings, we assessed cognitive and motor functions and rapid-onset cortical plasticity and obtained additional objective measures for the extent of ischemic SVD. DTI is sensitive to subtle white matter abnormalities, even in early stages of a disease with unsuspicious conventional MR images, thus providing a better index of tissue damage than conventional MRI (Jones et al. 1999; O’Sullivan et al. 2004; Naggara et al. 2006; Ciccarelli et al. 2008; Zhan et al. 2009). These structural changes have functional relevance because they correlate with clinical symptoms in early stages of neurodegenerative diseases and are closely associated with cognitive dysfunction (O’Sullivan et al. 2004; Nitkunan et al. 2008). Thus, we were able to detect specific brain areas (corpus callosum) where subtle white matter abnormalities (decrease in FA) were negatively correlated with the degree of rapid-onset cortical plasticity.

Compensatory Cortical Plasticity in SVD

To assess potential mechanisms underlying preserved cognitive functioning in ischemic SVD, we used the rapid-onset cortical plasticity protocol (Stefan et al. 2000). Cortical plasticity has been identified as one of the initial and rate-limiting steps of more slowly evolving large-scale reorganization in response to learning and to recovery from brain damage (Kleim and Jones 2008). In line with our a priori hypothesis, plasticity was enhanced in the patient sample compared with the control group. Moreover, we observed more pronounced cortical plasticity in patients with more severe SVD (modified Fazekas scale 3 vs. 1–2). Across the entire patient group, a higher degree of lesion load on DTI imaging was correlated with more pronounced cortical plasticity. Our findings are also in line with the results of a recent study by Zeller et al. (2010) who assessed performance on functional motor tasks and cortical plasticity in patients with multiple sclerosis (MS). The authors found enhanced plasticity in the
subgroup of patients described as having “good hand function” and “high central nervous system (CNS) injury” compared with patients with “low hand function” and high CNS injury. Although there are substantial differences in the pathophysiology of MS and ischemic SVD, these results provide converging evidence for an adaptive function of rapid-onset cortical plasticity to compensate for recurrent brain damage in MS (Zeller et al. 2010) and ischemic SVD (current study).

Previous studies in patients with focal dystonia had likewise noted enhanced rapid-onset cortical plasticity compared with healthy individuals (e.g., writer’s cramp; Quartarone et al. 2003; Weise et al. 2006; Kang et al. 2010; for review, see Quartarone and Pisani, 2010). However, given that motor function in these patients was below that of healthy controls and a paradoxical response to ctDCS was noted, as well as a loss of the normal homeostatic response pattern (Quartarone et al. 2005), enhanced plasticity in these individuals was interpreted as dysplasticity. To assess this possibility, we also examined the response to ctDCS in our patients and found no evidence for a paradoxical response. These results, together with almost preserved motor function despite severe cerebral white matter damage, further point to the compensatory role of enhanced rapid-onset plasticity in the CADASIL patients.

However, these findings in dystonia and severe ischemic SVD again demonstrate that enhanced plasticity is not per se beneficial for a given function but needs to be tightly regulated within existing cortical networks.

Figure 7. SPM “glass brain” representation (upper row) and statistical FA maps that were superimposed on an averaged FA template showing clusters of correlation between MEP changes after PAS and decreased FA values in CADASIL patients (\( P < 0.005 \), minimum of 200 continuous voxels; corrected for multiple comparison by false discovery rate method). Low FA values in clusters of the frontal part of the corpus callosum were associated with higher MEP after PAS in CADASIL patients (also in fiber tracts in the brain stem and the medulla oblongata). Colored bars represent \( t \) values; display threshold is set at \( t \) value >3.71.
Interindividual Variability of Rapid-Rate Cortical Plasticity

Previous studies found cortical plasticity in about 50–75% of participants in healthy younger adults (e.g., Stefan et al. 2000; Müller-Dahlhaus et al. 2008; Cirillo et al. 2009). Compared with these studies, the degree of cortical plasticity was lower in our control group, with only 3 of the 12 HV responded to the stimulation. However, most previous studies have assessed cortical plasticity in younger subjects. In advanced age, cortical plasticity was markedly reduced (Müller-Dahlhaus et al. 2008), and no significant increase of MEP following PAS could be shown in a recent study of Kang et al. (2010) in a group of middle-aged healthy controls (mean age 47 years). Moreover, given that our patient sample was carefully matched for demographic variables, including age, the highly significant enhanced plasticity in our patient sample compared with the control group is unlikely to be affected by the absence of cortical plasticity in the control group. Note that other factors that might influence the degree of cortical plasticity like gender, attention to the task, time of day, and previous activity of the hand tested (e.g., Stefan et al. 2006; Nitsche et al. 2007; Sale et al. 2008; Tecchio et al. 2008; Fathi et al. 2010) were held constant between the groups. Our findings are also corroborated by the fact that the degree of lesion load was positively correlated with the degree of plasticity in the patient sample. This may point to one possible compensatory process that allows the patients to perform at levels within the normal range, or only slightly below, despite extensive brain damage. As neuropsychological functions were similarly preserved in patients with lower and higher white matter lesion load, no linear relationship between test scores and PAS-induced plasticity across the group was found.

Compensatory Large-Scale Network Reorganization

Functional imaging studies have provided evidence for large-scale brain reorganization to compensate for diffuse brain damage (for review, see Cabeza 2002; Park and Reuter-Lorenz 2009). In CADASIL patients, Reddy and Stefano (2002) found increased ipsilateral cortical recruitment during a simple motor task when they compared functional activity patterns of middle-aged CADASIL patients and healthy subjects. Similar to the present study, a higher degree of brain damage (axonal injury as indicated on FLAIR MRI and MR spectroscopy) in the patients was associated with more pronounced bilateral activation, while function was preserved, pointing to an adaptive functional mechanism in response to lesion load.

With regard to the impact of ischemic SVD on adaptive changes in the brain, our study for the first time demonstrated that the degree of ischemic SVD is associated with increased cortical plasticity in middle-aged individuals. As ischemic SVD leads to dysfunction of existing neural networks, formation of new brain networks and strengthening of existing synapses will be required to maintain function (Kleim and Jones 2008). The mechanisms mediating these changes may include increased rapid-onset cortical plasticity, as demonstrated in the present study. Enhanced plasticity might be an active coping mechanism and may at least in part account for the largely preserved cognitive function in patients. This interpretation is further corroborated by the finding that cortical plasticity was even larger in patients with more pronounced lesions of white matter tracts. However, the slight decrease in verbal memory and executive functions in these patients may indicate that enhanced brain plasticity was not sufficient to counteract all damage, similar to what has been observed in patients with MS (Zeller et al. 2010).

This assumption is also supported by more recent functional imaging studies in healthy aging that found compensatory recruitment outside of the network activated by younger subjects, which was sufficient to maintain performance during relatively easy tasks (Cabeza 2002). However, this compensatory activity may be less efficient or even detrimental to task performance during more challenging tasks that draw heavily on working memory or executive functions (e.g., Meinzer et al. 2009). Thus, enhanced plasticity may possibly account for the largely preserved functioning seen in early stages of ischemic SVD (Reddy and Stefano 2002). However, if additional neurodegenerative changes are superimposed or task demands are increased, compensation is exhausted and severe cognitive decline ensues, an issue to investigate in upcoming studies.

Potential Mechanisms Supporting Enhanced Plasticity in CADASIL

A number of endogenous compensatory mechanisms have been discussed as potential contributors to functional recovery after ischemic injury. For example, retrospective studies have demonstrated that the outcome after stroke was better in patients with previous transient ischemic attacks compared with patients with first-time strokes (Weih et al. 1999). Experimentally, hypoxic preconditioning stimuli that induce neuroprotection are thought to increase neural plasticity (Dinnagl et al. 2009). Thus, chronic hyperperfusion, as in CADASIL, might induce compensatory brain plasticity. The mechanisms underlying this increased plasticity may include release of neurotrophic factors. For example, activated astrocytes around lacunar lesions increase their release of Brain-derived neurotrophic factor (BDNF) (Sato et al. 2009), known to enhance synaptic plasticity (Mattson et al. 2004). Recent studies from Castel-Lacanal et al. (2007, 2009) demonstrated enhanced cortical plasticity during the recovery phase from lacunar infarctions. As CADASIL patients suffer recurrent, mostly silent, lacunar strokes, increased BDNF levels triggered by these episodes may possibly underlie enhanced cortical plasticity in these patients. This hypothesis remains to be tested in future studies though.

Limitations

First, even though our results show clear evidence of enhanced cortical plasticity in CADASIL patients, the number of patients investigated in this study was small, and all of them showed relatively preserved cognition. However, the group is relatively homogeneous with regard to age, all patients showed at least moderate ischemic SVD, and patient numbers were comparable to previous studies using rapid-onset cortical plasticity (e.g., Weise et al. 2006; Cirillo et al. 2009). Second, the hypothesized upregulation of BDNF in the brain was not investigated more closely, as brain levels of BDNF cannot be measured in living humans. In particular, the more local release of BDNF expected to occur after small ischemic lesions is unlikely to be captured peripherally (e.g., by serum levels). Molecular imaging may at some point be able to capture these locally enhanced releases noninvasively. Third, within the group of patients with most severe SVD, we did not observe better neuropsychological or motor scores in those with higher PAS responses compared
with those with lower PAS responses. Thus, other compensatory mechanisms are likely to account for preserved function as well and should be investigated in future studies.

**Conclusions and Clinical Implications**

The present findings suggest that in middle-aged individuals, the brain uses endogenous compensatory mechanisms, as indicated by increased rapid-onset cortical plasticity, to successfully counteract damage. In the aging brain, particularly in the presence of additional degenerative changes, these mechanisms may at some point be exhausted, and decline in function will ensue, a hypothesis to be investigated in upcoming studies.

Thus, strategies aiming at enhancing plasticity in the brain may contribute significantly to counteract functional decline. Such strategies include, among others, physical activity, dietary interventions, or cognitive stimulation (for review, see van Praag 2009), which have been shown to increase learning ability and cortical as well as hippocampal plasticity in animal (Mattson et al. 2004; Rasmussen et al. 2009) and human models (Colcombe et al. 2004; Winter et al. 2007; Ruscheweyh et al. 2009; Flöel et al. 2010). Future studies should now assess the ability of these interventions to enhance rapid-onset cortical plasticity as well as cognitive functions in elderly individuals.

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**Notes**

Conflict of Interest None declared.

**References**


