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Changes in plasticity and cognition as a result of neurovascular disease and non-invasive brain stimulation

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Table of contents

Abbreviations

AH - Affected Hemisphere

BOLD - Brain Oxygen Level Dependent

DAN - Dorsal Attention Network

DMN - Default Mode Network

DLPFC - Dorsolateral Prefrontal Cortex

DTI - Diffusion Tensor Imaging

EEG - Electroencephalogram

ECM - Eigenvector Centrality Mapping

FA - Fractional Anisotropy

FC - Functional Connectivity

FLAIR - Fluid-attenuated Inversion Recovery

fMRI - Functional Magnetic Resonance Imaging

FPN - Frontoparietal Network

HC - Healthy Control

ICA - Internal Carotid Artery

IFG - Inferior Frontal Gyrus

MCA - Middle Cerebral Artery

MFV - Mean Flow Velocity

MRI - Magnetic Resonance Imaging

NIBS - Non-Invasive Brain Stimulation

PANAS - Positive and Negative Affect Scale

ROI - Region of Interest

RSFC – Resting State Functional Connectivity

SF - Structural Connectivity

TBSS - Tract-based Spatial Statistics

TCD - Transcranial Dopplersonography

tDCS - Transcranial Direct Current Stimulation

TMS - Trascranial Magnetic Stimulation

TPJ - Temporo-parietal Junction

UH - Unaffected Hemisphere

VMR - Vasomotor Reactivity

Abstract

Plasticity is a milestone in neuroscientific investigations into the biological mechanisms of learning. Plasticity is critically reduced in older adults and in individuals with neurovascular disorders that encompass changes in cerebral blood supply. Mechanisms underlying this altered plasticity are important to understand in order to develop future preventive and therapeutic strategies. Pathological and interventional changes in plasticity are thus the focus of the current thesis. In three interrelated studies, this thesis explores the underpinning of cognitive deterioration and the neural changes accompanying cognitive enhancement.

The first study tested the associations between cerebral autoregulation (vasomotor reactivity, VMR), magnetic resonance imaging (MRI) measurements of structural and functional connectivity, and cognitive performance in patients with unilateral occlusive processes of the internal cerebral artery (ICA, n=14) and matched controls (n=11). Patients revealed lower VMR in the affected hemisphere (AH), decrease in whole-brain microstructure and in inter- and intra-hemispheric functional connections. Moreover, reduced VMR in the AH correlated with reduced microstructure in frontal areas and connectivity in the default-mode network (DMN). This suggests that disconnections within critical brain networks might mediate the effects of impaired VMR on cognition.

The second study tested the short-term changes induced by transcranial direct current stimulation (tDCS) on language performance and the underlying neuronal mechanisms of these behavioral gains. Blood-oxygen level dependent (BOLD) activity and functional networks under real and sham tDCS were measured in 24 young healthy subjects. Here, tDCS over a core language area improved semantic fluency, decreased activity in the stimulated region and facilitated connections within the language network. The results point to a critical link between enhanced network connectivity and increased neural efficiency which might be necessary for improved task performance.

The third study investigated long-lasting behavioral gains induced by training in combination with tDCS during language learning. In this study, 40 young healthy subjects acquired a novel vocabulary during 5 consecutive days under real or sham tDCS and were tested in two memory

tasks on a daily basis. The results indicate that tDCS facilitated language acquisition and retention of the learned vocabulary.

Findings from the three interconnected studies thus lead to the suggestion that tDCS could assist patients with occlusion / stenosis of the ICA, in which loss of cognition might be mediated by structural and functional changes in connectivity. A potential preventive therapy with tDCS could target these regions in frontal and parietal cortices, with the aim to improve cognition in these patients.

Zusammenfassung

Plastizität stellt einen Meilenstein in der neurowissenschaftlichen Erforschung der biologischen Lernmechanismen dar. Plastizität ist bei älteren Menschen, sowie bei neurovaskulären Erkrankungen, welche mit Veränderungen im zerebralen Blutfluss einhergehen, deutlich reduziert. Ein besseres Verständnis der Plastizitätsmechanismen, könnte zukünftig dazu beitragen neue präventive und therapeutische Maßnahmen zu entwickeln. Der Fokus dieser Arbeit liegt daher auf pathologischen und interventionsbedingten Veränderungen der Plastizität. Innerhalb dieser Dissertationsschrift wurden in drei zusammenhängenden Studien die Ursachen kognitiven Abbaus, sowie die mit kognitiven Verbesserungen assoziierten neuronalen Veränderungen untersucht.

Die erste Studie analysierte Zusammenhänge zwischen zerebraler Autoregulation (Vasomotor-Reaktivität, VMR), kognitiver Leistungsfähigkeit, sowie Parametern struktureller und funktioneller Konnektivität, die mittels Magnetresonanztomographie (MRT) erhoben wurden, bei Patienten (n=14) mit unilateralen Verschlussprozessen der Arteria carotis interna (ICA) im Vergleich zu einer Kontrollgruppe (n=11). Die Patienten zeigten verminderte VMR in der betroffenen Hemisphäre (bH), verringerte Mikrostruktur im gesamten Gehirn sowie in der inter- und intrahemisphärischen funktionellen Konnektivität. Des Weiteren ging verminderte VMR innerhalb der bH mit verringerter Mikrostruktur in frontalen Arealen sowie verringerter Konnektivität innerhalb des Default-mode-Netzwerks (DMN) einher. Dies lässt vermuten, dass Störungen innerhalb aufgabenrelevanter Gehirnnetzwerke die Effekte einer beeinträchtigten VMR auf die Gedächtnisleistung vermitteln.

Die zweite Studie untersuchte mittels transkranieller Gleichstromstimulation (tDCS) induzierte Kurzzeitveränderungen auf die Sprachleistung, sowie deren zugrunde liegenden neuronalen Mechanismen. Die vom Blutsauerstoffgehalt abhängige Aktivität und funktionale Netzwerke wurden mittels tDCS- und Scheinstimulation bei 24 jungen gesunden Probanden gemessen. Eine Platzierung der tDCS-Elektroden oberhalb der Kernareale des Sprachzentrums führte zu verminderter Aktivität innerhalb der stimulierten Region, zu einer verbesserten semantischen Sprachkompetenz, sowie zu begünstigten Verbindungen innerhalb des Sprachennetzwerks. Die Ergebnisse weisen auf eine entscheidende Verbindung zwischen verstärkter Netzwerkkonnektivität und erhöhter neuronaler Effizienz hin, welche möglicherweise notwendig für verbesserte Gedächtnisleistungen sind.

Die dritte Studie untersuchte langfristige Gedächtnissteigerungen, die durch Kombination aus kognitivem Training und tDCS während einer Sprachlernaufgabe induziert wurden. In dieser Studie erlernten 40 junge gesunde Probanden innerhalb eines Zeitraums von fünf aufeinanderfolgenden Tagen einen neuen Wortschatz. Während zweimaligen täglichen Gedächtnisaufgaben wurde bei den Probanden entweder tDCS- oder Scheinstimulation angewendet. Die Ergebnisse legen nahe, dass tDCS eine begünstigende Wirkung auf Spracherwerb und Beibehaltung des erlernten Wortschatzes hat. Zusammenfassend weisen die Ergebnisse der drei vorliegenden Studien darauf hin, dass tDCS eine unterstützende Wirkung bei Patienten mit Gefäßverschlüssen/Stenosen der ICA haben könnte, deren Gedächtnisverlust über strukturelle und funktionelle Konnektivitätsveränderungen vermittelt sein könnte. Eine potentielle präventive tDCS-Therapie könnte speziell auf Regionen abzielen, die in Zusammenhang mit dem DMN im frontalen und parietalen Kortex stehen, um die kognitive Leistungsfähigkeit betroffener Patienten zu verbessern.

Introduction

The main principle of plasticity entails that "what fires together wires together" (1). In other words, plasticity signifies long term changes in the effectiveness of connections between two distinct parts of the central nervous system (2). These changes are reflected across multiple levels of the nervous system, going from cellular level to circuits and large-scale brain networks. Natural changes in plasticity happen in the brain through learning and experience and interact with a range of biological and environmental factors across the lifespan. Reduced or altered plasticity is considered to cause cognitive decline in aging (3) and to act as a biomarker of neuropsychiatric diseases (4).

Since the brain is highly interconnected, local changes in plasticity rely upon changes in large-scale brain networks, and vice versa (5). In recent years, MRI has been used to assess structural and functional intrinsic connectivity through fiber tracts (Diffusion tensor imaging, DTI) and BOLD signal correlations across brain regions (Resting state functional connectivity, RSFC), respectively. In neurovascular diseases, reduced cerebral autoregulation leaves its signature on structural and functional connectivity (6) and set in motion degenerative processes which affect cognition, leading to higher risk for developing dementia (7). Exanimating the association between reduced cerebral autoregulation, disruption in connectivity and declined cognition enables to understand the chain of events culminating to the cognitive symptoms observed in patients with stenosis or occlusion of the ICA. Importantly, due to the unilateral nature of the disease, it is possible to investigate within-subject whether cognitive deficits are associated with local changes in plasticity or disrupted functional networks.

Externally induced changes in plasticity, also called neuromodulation, could be achieved using various methods of non-invasive brain stimulation (NIBS; 8,9). Among others, tDCS modifies patterns of ongoing activity by increasing or decreasing the mean firing rate of the neurons (10). In times when aging becomes the highest risk factor for developing neurovascular diseases, tDCS is a viable non-pharmacological option for cognitive protection and enhancement. The short-term immediate behavioural gains of tDCS have been extensively studied on a range of motor and cognitive functions (11). For example, in the language domain, modulation of learning has been demonstrated in healthy individuals (12,13) and patients with post-stroke aphasia (14–16).

Moreover, it has been recently reported that application of tDCS in the treatment of patients with neurovascular diseases is safe and efficient (17,18).

On the brain level, the combination of MRI and tDCS enables to explore the modulatory effects of tDCS on dynamic interactions between brain areas. For examples, analyzing low frequency BOLD fluctuations with graph theory (19) and measuring regional cerebral blood flow (20) showed that tDCS modulates activity in a widespread network of brain regions which are functionally related to the stimulated area. Specifically, Pena-Gomez and colleagues (21) reported that tDCS applied over the dorsolateral prefrontal cortex (DLPFC), as fundamental part of the dorsal attention network (DAN), increased connectivity between the DLPFC and parietal regions, which are part of the DMN; accordingly, the recruitment of parietal regions disturbed the connectivity within the DMN by disconnecting anterior and posterior parts. These findings indicate that changes in spatial and temporal synchronized activity are dynamically specific and complex. Given that cognitive performance is predicted by network dynamics (22), flexible balance between large-scale networks might be necessary for facing cognitive demands, particularly in older adults with neurological condition.

Although tDCS holds a considerable therapeutic promise, a variety of parameters such as sex, age and history of synaptic activity of the stimulated region needs further evaluation in order to optimize tDCS for clinical applications (23). Moreover, it is still unclear whether lasting changes in plasticity as a result of tDCS interact with natural learning in a way that can significantly support retention (12). Together, the relationship between local and network changes in plasticity and cognition will help elucidate the complexity of brain organization and learning mechanisms which are necessary for prevention and recovery. In the present thesis, these questions are explored in three interrelated studies using a variety of methods and a large corpus of subjects with the premise of advancing knowledge about brain efficiency and the plasticity changes required to reach it. Findings from these studies could contribute to the evolution of therapeutic avenues in aging and in age-related diseases, such as occlusion or stenosis of the ICA.

Objectives

In *Study 1*, we examined the relationship between structural (DTI) and functional (RSFC) brain connectivity, VMR and cognition in healthy subjects and in patients with occlusion / stenosis of the ICA. The aim of this cross-sectional study was thus to promote a better understanding of the plasticity mechanisms which mediate cognitive deterioration in neurovascular diseases.

In *Study 2*, we examined the immediate changes in RSFC accompanying enhanced task-related brain activity (BOLD) and language performance due to neuromodulation (tDCS). The aim of this interventional cross-over study was thus to promote a better understanding of the plasticity mechanisms of NIBS application to ameliorate cognition.

In *Study 3*, we examined whether accumulative application of neuromodulation (tDCS) facilitates language learning. The aim of this interventional cross-sectional study was thus to promote a better understanding of the long-term effects of NIBS application to maximize training-induced plasticity.

Methods

Subjects

A total of 89 right-handed native German speakers participated in the three studies. In *Study 1*, 14 patients with unilateral occlusive process of the ICA (65±11 years, range 50–80 years, 11 males, occlusion: n=10, high grade stenosis (>80%): n=4; diagnosis >1 year before enrolment in the study) were recruited from the database of the ultrasound laboratory of the Department of Neurology of the Charité University Hospital in Berlin. These patients were matched with11 healthy controls (HC) without stenosis or occlusion of the ICA or other extracranial vessels (67±6 years, range 55–74 years, 7 males). In *Study 2*, 24 young healthy subjects (26±4 years, range 19–34 years, 14 females) were recruited. In *Study 3*, 40 young healthy subjects (24±4 years, range 18–32 years, 24 females) were recruited. All subjects were recruited according to the relevant inclusion criteria and signed a consent form. The studies were approved by the ethics committee of the Charité University Hospital in Berlin and were conducted in accordance with the Helsinki declaration.

Neuropsychological Testing

The neuropsychological test battery in *Study 1* evaluated cognitive performance on a range of functions; the trail-making-test (TMT) was used to measure processing speed and to assess executive functions (24): the verbal fluency task was used to test phonemic and semantic fluency (25); the German version of the auditory verbal learning test (AVLT) was used to examine verbal learning capacity across five trials and the retrieval from verbal memory by delayed recall after 30 minutes (26); working memory performance was assessed using the forward and backward digit span (part of the revised Wechsler Memory Scale; (27)).

Semantic Verbal Fluency

The task used in *Study 2* was an adaptation to fMRI of the semantic verbal fluency task (28). Accordingly, subjects were asked to generate different exemplars of a given semantic category (e.g., food, sport equipment, insects etc.). The semantic categories were selected to comprise a large range of category exemplars according to a German norming study (29). The fMRI task consisted of 90 trials of 6 seconds and lasted in total for 10.4 minutes. Each semantic category was visually presented at the centre of the screen for 3.8 seconds in blocks of 10 consecutive trials using Presentation® software (Version 14.8, www.neurobs.com). These blocks alternated with a baseline condition (saying the word "rest"; five consecutive trials) controlling for modulation in verbal response following stimulation. Verbal responses were recorded using a microphone (the t.bone SC450, http://www.tbone-mics.com/) and analyzed using Audacity sound editing software (http://audacity.sourceforge.net/). The dependent variable was the amount of correct responses during each session.

Learning Paradigm

The explicit new word learning paradigm in *Study 3* included 120 black and white pictures of objects, 60 standardized pictures of familiar objects (30) and 60 novel objects (31), as well as 120 non-words in different lengths. During the acquisition phase, pictures and non-words appeared simultaneously on a black screen for four seconds. Following the acquisition phase, which lasted 12-14 minutes, subjects performed two memory tasks; first, in the recall task subjects had to type

the non-word that appeared with a given picture; secondly, in the recognition task subjects had to choose the correct non-word out of two choices, while in 50% of the trials the correct non-word was presented with a non-word that did not appear in the acquisition phase. Presentation® software was used for stimulus presentation and recording of responses. The dependent variable was the percentage of correct responses during each daily testing session.

Data Acquisition

MRI images were obtained on a 3-T system (Magnetom TIM Trio, Siemens, Erlangen, Germany) using a 12-channel head coil. The parameters for the task-related BOLD fMRI in *Study 2* (T2*-sensitive echo-planar imaging) were: resolution, 3 × 3 × 3 mm^3; TR=6000; TA=2000; TE=30; flip angle, 90°; 32 transverse slices; gap, 0.75 mm; slice thickness, 3 mm; interleaved acquisition, FOV, 192×192; acquisition matrix, 64×64, 104 functional whole-brain volumes per session. The parameters for the continuous resting-state acquisition in *Study 1 and 2* were: resolution, 3 × 3 × 4 mm^3; TR/TA = 2300; TE = 30; flip angle, 90°; 34 transverse slices, no gap; interleaved acquisition, FOV, 192 × 192; acquisition matrix, 64 × 64, 150 functional whole-brain volumes per session. The parameters for DTI in *Study 1* (diffusion-weighted images in a spin-echo EPI sequence) were: resolution, 2.3 × 2.3 × 2.3 mm^3; TR = 7500 ms; TE = 86 ms; 61 axial slices, 64 directions with a b-value of 1000 s/mm^2 and one or ten b0. The parameters for high-resolution T1-weighted MPRAGE and fluid-attenuated inversion recovery (FLAIR) images *in Study 1 and 2* were: resolution, 1 × 1 × 1 mm^3; TR = 1900 ms; TE = 2.52 ms; flip angle, 9°; 192 sagittal slices.

Diffusion Tensor Imaging (DTI)

Structural connectivity in *Study 1* was measured using DTI (32). Data analyses were performed using the Diffusion Toolbox package and tract-based-spatial statistics (TBSS) from the FMRIB Software Library FSL 4.1 (http://www.fmrib.ox.ac.uk/fsl). Individual fractional anisotropy (FA) maps were obtained after pre-processing and fitting of a diffusion tensor model. All FA images were registered to the study-specific template, smoothed with a Gaussian kernel of sigma = 2 (~ 4.6 mm full-width-half-maximum), and averaged to get a mean FA image. This mean FA image was thinned ("skeletonised") and thresholded at > 0.2 to create a mean FA skeleton. Mean FA values were extracted separately to test for differences between groups. In order to obtain clusters associated with perfusion, voxel-wise correlations across the skeleton between FA values and VMR

measures were performed in each group separately. Permutation-based statistical analyses were conducted with correction for multiple comparisons (threshold-free cluster enhancement, TFCE; $p < 0.05$) and data demeaning (–D-option) using the program "randomise" implemented in FSL (33).

Functional Magnetic Resonance Imaging (fMRI)

Task-related BOLD activity in *Study 2* was measured using event-related fMRI (34). Data analyses were performed using Statistical Parametric Mapping (SPM5; Wellcome Department of Imaging Neuroscience, London, UK). The effects of the conditions were determined in a single statistical model at the individual subject level after data was pre-processed and filtered (cutoff period 128 seconds). The model comprised co-variants for the two conditions (semantic category and baseline trials), nuisance co-variants related to motion, and co-variant marking excluded trails for incorrect responses. For region of interest (ROI) analysis, the three regions which were chosen, one target region (left ventral inferior frontal gyrus, vIFG) and two control regions (left dorsal IFG and right vIFG), were created using the WFU pick-atlas (35). To assess the specificity of task-related activity differences between the stimulation conditions (tDCS vs. Sham), mean beta activity (semantic category vs. baseline) was extracted from the respective ROIs and compared using paired t-tests.

Resting-state Functional Connectivity (RSFC)

Functional connectivity was measured through continuous low frequency fluctuations in a resting state. In *Study 1*, AFNI (http://afni.nimh.nih.gov/afni) and FSL (http://www.fmrib.ox.ac.uk) were used to process functional and anatomical data using customized scripts from the 1000 Functional Connectomes Project (www.nitrc.org/projects/fcon_1000) (36). To test functional changes in the DMN, the frontoparietal network (FPN) and the DAN, 14 seed regions were defined (6). Mean time series for each seed were extracted from the data then correlated with each voxel in the brain to produce individual-level correlation maps. These maps were then converted to Z-value maps using Fisher's r-to-z transformation for subsequent analyses. Individual inter-hemispheric and intra-hemispheric FC strength between ROI pairs was calculated (17 ROI pairs). Group-level analyses were carried out using a mixed-effects model (FLAME) as implemented in FSL (37). In the patients group, one sample t-test was computed separately for each hemisphere to find clusters within the

respective networks that associated with VMR. Gaussian random field theory was used to correct for multiple comparisons at the cluster-level ($Z>2.3$; $p<0.05$, corrected).

In *Study 2*, data was processed and analyzed using LIPSIA software in a graph based approach, i.e., eigenvector centrality mapping (ECM; 38). Individual voxel wise spectral coherence analysis was conducted for four spectral bands (0.03, 0.04, 0.05, and 0.06 Hz) separately for each stimulation condition. A Gaussian normal distribution for each stimulation condition was obtained through z-transformation and mean spectra. The individual mean images were compared by a whole-brain paired t-test. Clusters were considered significant at $p<0.05$, corrected for multiple comparisons using a Monte-Carlo simulation (39). To test functional changes in the frontal language network as a result of tDCS, two seed regions in the left hemisphere were defined (40). The time series was averaged across voxels within the vIFG and dIFG seeds (33 voxels each) and correlated with all other voxels in the gray matter mask. The resulting voxel wise correlation coefficients were normalized using the Fisher's r-to-z transformation (41).

Transcranial Direct Current Stimulation (tDCS)

Direct current was provided through a battery-driven stimulator (DC-Stimulator Plus, neuroConn GmbH, Ilmenau, Germany) using two electrodes covered by synthetic sponge and soaked in saline. A current of 1mA was applied while impedance was controlled at 5-10 Ω. The anodal electrode (5×7 cm^2) was positioned on the left IFG in *Study 2* and on the left posterior temporo-parietal Junction (TPJ) in *Study 3*. The reference electrode (10×10 cm^2) was always positioned on the contralateral supraorbital cortex in line with previous tDCS studies of our group (12,15) which used this site as reference site for stimulation over parietal and frontal areas. Both stimulation sites were determined using the EEG-10-20 system (IFG= at the center of line connecting the intersection of T3-F3 and midpoint between F7-F3; posterior TPJ= Cp5). In both studies subjects were given the Positive and Negative Affect Scales (PANAS; 42) in order to control for possible emotional modulation as a result of brain stimulation; In *Study 3*, subjects were also given the Adverse Effects questionnaire (43) in order to control for tolerability and safety of tDCS application.

Vasomotor Reactivity (VMR)

Transcranial Dopplersonography (TCD) was used to assess VMR in *Study 1*. Subjects were measured in a lying position in a quiet room. Two TCD dual 2MHz transducers were fitted on a

headband and placed on the temporal bone windows. Mean flow velocity (MFV) was recorded over the middle cerebral artery (MCA) at rest (MFV-$MCA_{BASELINE}$) and after 2 minutes of carbogen-inhalation (5%CO2 + 95% oxygen) (MFV-MCA_{CO2}). VMR was then obtained using the following formula (44):

$$\mathbf{VMR = (MFV\text{-}MCA_{CO2} - MFV\text{-}MCA_{BASELINE})/MFV\text{-}MCA_{BASELINE}}$$

Statistics

Study 1: Statistical analyses outside FSL were carried out using SPSS, Version 20. Shapiro-Wilk-test was used to test for normality (set to $p>0.05$) prior to data analysis. Unpaired t-tests were employed to compare baseline characteristics, cognitive scores, VMR, ventricle volume, cortical volume, FA and FC between patients and HC. Paired sampled t-tests were used to compare VMR, FC and FA between the hemispheres in the patient group and in HC. Pearson's correlation coefficients were used to probe associations between VMR and MRI measurements, as well as associations between VMR and cognitive scores in patients. All statistical tests were considered significant at a level of $p<0.05$.

Study 2: Statistical analyses outside SPM and LIPSIA were carried out using SPSS, Version 20. Paired t-tests were used to compare correct responses, responses times, mood ratings (PANAS), and mean beta activity in ROIs between the two stimulation conditions. All statistical tests were considered significant at a level of $p<0.05$.

Study 3: Statistical analyses were carried out using SPSS, Version 20. Percentage of correct responses on the recall and recognition tasks were separately calculated using linear mixed models with fixed factor TIME (level-one) and SUBJECT (level-two) and random factor STIMULATION (tDCS, sham) which included OBJECT TYPE as covariate (familiar objects; novel objects). Additionally, a squared centred time variable (TIME2) was used to test if there was a curvilinear learning curve. The same models were used to assess differences between the stimulation groups on long-term maintenance (last day and follow-up), separately for recall and recognition tasks. Differences between stimulation groups on mood ratings (PANAS) along the training sessions were also tested using linear mixed models. Unpaired t-tests were used for post hoc comparisons. All statistical tests were considered significant at a level of $p<0.05$.

Results

Study 1 – ***Reduced cerebral autoregulation induces changes in structural and functional brain networks which might mediate cognitive decline.*** VMR of the AH differed significantly from VMR in the UH in patients ($t=-7.14$, $p<0.001$) and from VMR of the right hemisphere in controls ($t=-2.11$, $p=0.047$). Patients also showed poorer results on several cognitive tests compared to HC, most notably with regard to verbal fluency and memory, as well as cognitive flexibility and executive functions. MRI in the patients group revealed silent infarctions in five patients and significantly more white matter lesions, compared to HC ($t=2.56$, $p=0.02$). Whole-brain mean FA was significantly lower in patients than in HC ($t=-2.63$, $p=0.016$). Additionally, voxel-wise permutation testing showed that decreased VMR was significantly associated with decreased FA in specific white matter tracts of the AH, mainly in the frontoparietal areas. RSFC analysis revealed significant decreases in the long-distance inter-hemispheric FC within the bilateral DMN, FPN and DAN. The DMN was most notably affected as changes in intra-hemispheric connections were also observed. In the AH, lower VMR was correlated with lower FC from parietal seeds to precuneus, cingulate and frontal cortices.

Study 2 – ***Enhanced task performance as a result of neuromodulation is associated with less activity and more connectivity.*** Although subjects produced significantly more correct responses under tDCS over the left vIFG ($t=2.94$, $p=0.008$), they were not faster to respond ($t=0.43$, $p=0.67$). Furthermore, mood was not affected by the stimulation condition. These behavioural results bring further support to the claim that the cognitive gains induced by tDCS are task-specific and not driven by changes in arousal or affect. This was also observed in reduced mean beta activity of the stimulated area ($t=2.58$, $p=0.02$), while other interconnected areas were not affected by the excitatory neuromodulation. ECM analysis revealed that tDCS increased connectivity in major hubs which are part of the bilateral language network with the strongest increase in the left IFG and anterior insula. In seed-based analysis, increase in connections was found not only within the language network but also in connections to the motor network (e.g., supplementary motor area, basal ganglia, and cerebellum).

Study 3 – ***Neuromodulation during acquisition facilitates learning and supports retention***. Analysis of linear mixed models revealed a significant effect of TIME (recall task: $\beta=0.14$, $SE=0.006$, $t(354)=22.28$, $p<0.0001$; recognition task: $\beta=0.08$, $SE=0.003$, $t(346)=25.59$, $p<0.0001$) pointing to a successful acquisition of novel vocabulary across the five learning sessions. A significant effect of OBJECT TYPE (recall task: $\beta=0.27$, $SE=0.02$, $t(354) = 11.84$, $p < 0.0001$; recognition task: $\beta=0.14$, $SE=0.01$, $t(346)=13.05$, $p<0.0001$) indicated that learning of familiar objects was more pronounced than learning of novel objects. Most importantly, the analysis yielded a significant effect of STIMULATION in the recognition task ($\beta=0.06$, $SE=0.02$, $t(346)=3.06$, $p=0.002$), showing more overall learning success under stimulation and a significant TIME x STIMULATION interaction in the recall task ($\beta=0.04$, $SE=0.009$, $t(354) =4.35$, $p<0.0001$), showing that the learning curve was steeper under stimulation. Post hoc comparisons confirmed better acquisition of novel vocabulary on the last day of the training in the stimulation group for both types of objects. Despite a significant decrease in performance on the follow-up sessions, the advantage observed on the last day of the training in the stimulation group was maintained one week after the training (recall task: $\beta=0.17$, $SE =0.07$, $t(116)=2.41$, $p=0.017$; recognition task: $\beta=0.06$, $SE=0.02$, $t(116)=3.49$, $p=0.0007$).

Discussion

Study 1: This study tested the association between cerebral autoregulation and brain networks as well as cognition, in patients with unilateral stenosis or occlusion of the ICA. Cerebral autoregulation was tested by VMR. In line with previous findings (45,46), ICA patients showed reduced VMR in the AH, compared with the UH and with HC. Reduced VMR of the AH was associated with lower FA in the entire brain and in the AH. Correlational analysis revealed for the first time that decreased integrity of white matter microstructure in tracts passing through the frontal lobe are most affected by reduced VMR. This comes in support of the hypothesis that chronically impaired cerebral hemodynamics render mainly the frontal lobe vulnerable (47), and may therefore be responsible for lower functions in the respective cognitive domains (48). We extend this finding by demonstrating that decreased cerebral hemodynamics, as measured by reduced VMR, induce both ipsilateral and bilateral alterations in FC between major nodes of the DMN and the FPN. Of

note, the bilateral DMN plays an important role in memory performance (49). Thus, reduced VMR in the AH modifies not only white matter microstructure distal of the stenosis/occlusion, but changes RSFC throughout the entire brain with similar patterns to patients with cognitive complaints and with mild cognitive impairment (50). Our findings also suggest that changes in white matter microstructure and functional networks as a result of impaired cerebral hemodynamics are associated with lower cognitive abilities, mostly in verbal learning and verbal fluency tasks. Future studies on larger cohorts using specialized tasks for each hemisphere should now be conducted to replicate and extend the findings of the current study.

Study 2: This study tested the relationship between neural changes and cognitive gains induced by tDCS. The novelty of this study was based on testing high cognitive function, such as language, while measuring immediate changes in task-related activity and RSFC. Neuromodulation improved word-retrieval as shown in previous behavioral studies (51). Moreover, it reduced task-related activity in the vIFG, probably as a sign of more efficient processing in the region which is critical for the task (52). The use of modern neuroimaging techniques also enables to investigate the large-scale effects of tDCS on brain networks. Here, the centrality of the vIFG within the language network increased under tDCS, indicating that the functional interaction between the vIFG and other parts of the vast language network went through reorganizational processes to achieve higher efficiency. Congruently, the whole-brain analysis revealed that areas related to attention and memory which are functionally connected with the IFG showed increased centrality. The neurobiological interpretation of these results is that functional network architecture is dynamic and could be modulated by NIBS. Future studies should test other cognitive tasks and functional networks in order to determine that increased centrality of core regions is a key element in large-scale changes promoting neural efficiency and improved task performance.

Study 3: This study tested the beneficial effects of multiple tDCS sessions on novel words learning. Here, tDCS during training resulted in better learning and retention as measured by two memory tasks. Success in learning regardless of object types shows that tDCS generally facilitates the creation of novel associations between objects and words. Moreover, the beneficial effects of stimulation over TPJ suggest that this region, which overlaps with “Wernicke’s area”, and is involved with word production and phonological retrieval processes (53), is necessary for lexical

acquisition. Our results demonstrated that superior learning induced by tDCS is maintained over time, contrary to the effects obtained by a single session (12). Indeed, long-term maintenance of superior learning during tDCS was observed in training of motor skills (54) and numerical abilities (55). Still, further studies with longer follow-up periods are required in order to indicate whether the stimulation effect is stable for more than one week. Of note, neuromodulation by tDCS, which is safe and function-related, reached similar results to studies using pharmacological intervention (56). To summarize, the superior learning achieved by repeated training sessions combined with stimulation supports future investigations into the potential of tDCS for long-term enhancement of language rehabilitation in post-stroke aphasia and language acquisition in developmental disorders. Additional MRI analyses of changes induced by long-term stimulation will provide significant insight into plasticity processes involved in learning.

General Discussion

The effects of metabolic factors, such as cerebral blood supply, on diseases of the central nervous system have been increasingly investigated with the aim to prevent the occurrence of dementia and stroke (57). Evidence from *Study 1* suggests that chronic carotid stenosis has a significant effect on cognitive dysfunction and brain connectivity. Moreover, evidence from the interventional studies (*Study 2* and *3*) proposes that tDCS application induces neural efficiency and enhances cognitive functions in healthy young subjects, which may persist over time. In light of the three studies, tDCS on frontoparietal regions ipsilateral to the occlusion/stenosis might offer a potential tool to increase FC in ICA patients and to preserve cognition. These regions play an important role in functional networks which are necessary for high levels of attention and memory functions. Specifically, parietal regions which seem to have a more flexible connectivity profile, dynamically changing connections within and between networks based on task demands, might be crucial for proper functioning of the DMN (19). Indeed, the reorganization of the language network during stimulation of the core language area, as shown in *Study 2*, might suggest a new approach to promote plasticity changes in cases of aberrant FC. Accordingly, measuring RSFC prior to tDCS application could target core regions in the altered networks and therefore induce plasticity changes more efficiently. Similarly to *Study 3*, tDCS could be also implemented during cognitive training, in which repetition

of tasks under stimulation might facilitate performance on respective functions, with immediate but also long-term beneficial effects. These interventions could supplement strategies to increase VMR such as physical activity (58) and statins (59).

Outlook

The three studies lay down a complex portrait of the plastic brain and its intrinsic flexibility which enables it to change in response to lesions as well as environmental demands (60). Our abilities to support functioning and to increase learning in the healthy aging and the impaired human brain significantly progress, given the accumulating knowledge of several decades of intensive research into neuromodulation (61). Particularly, cognitive enhancement becomes an important topic of research in modern life due to the increase of aging individuals that face an ever growing demand for learning and memory formation both in the workplace and in their social life. In light of ethical doubts which have been raised in regard to pharmacological interventions for enhancing cognition in healthy individuals, NIBS offers a viable choice to promote and protect cognitive functioning into old age. When combined with brain imaging techniques such as MRI, NIBS could be applied to beneficially modulate altered network dynamics that occur in various disease states such as impaired cerebral blood supply and stroke (62). Future studies using NIBS should therefore take into account the connectivity profile of the individuals undergoing stimulation protocols in order to augment the effects of neuromodulation.

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Publications

Keren Avirame had the following share in the following publications:

Publication 1: Avirame K*, Lesemann A*, List J*, Witte AV, Schreiber SJ, Flöel A. "Cerebral autoregulation and brain networks in occlusive processes of the internal carotid artery". *Journal of Cerebral Blood Flow & Metabolism* (2014); **Contribution:** Acquisition of data, analysis and interpretation of data, writing the paper, submission and revision of the manuscript.

Publication 2: Meinzer M,* Antonenko D*, Lindenberg R*, Hetzer S, Ulm L, **Avirame K**, Flaisch T, Flöel A. "Electrical brain stimulation improves cognitive performance by modulating functional connectivity and task-specific activation". *The Journal of Neuroscience* (2012); **Contribution:** Acquisition of data, analysis and interpretation of data, critical revision of the manuscript for important intellectual content.

Publication 3: Meinzer M,* Jähnigen S*, Copland DA, Darkow R, Grittner U, **Avirame K**, Rodriguez AD, Lindenberg R, Flöel A. "Transcranial direct current stimulation over multiple days improves learning and maintenance of a novel vocabulary". *Cortex* (2014); **Contribution:** Study design and preparation, acquisition of data, critical revision of the manuscript for important intellectual content.

Signature, Date and Stamp of Prof. Dr. med. Agnes Flöel

…….

Signature of Keren Avirame

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Publication 1:

Avirame K*, Lesemann A*, List J*, Witte AV, Schreiber SJ, Flöel A. “Cerebral autoregulation and brain networks in occlusive processes of the internal carotid artery”. *Journal of Cerebral Blood Flow & Metabolism* (2014); http://dx.doi.org/10.1038/jcbfm.2014.190

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Publication 3:

Meinzer M,* Jähnigen S*, Copland DA, Darkow R, Grittner U, Avirame K, Rodriguez AD, Lindenberg R, Flöel A. "Transcranial direct current stimulation over multiple days improves learning and maintenance of a novel vocabulary". *Cortex* (2014); http://dx.doi.org/10.1016/j.cortex.2013.07.013

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

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Cohen-Maximov T, **Avirame K**, Flöel A, Lavidor M. Modulation of gestural-verbal semantic integration by tDCS. Brain Stimulation. 2014;

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Ben-Soussan TD*, **Avirame K***, Glicksohn J, Goldstein A, Harpaz Y, Ben-Shachar M. Changes in cerebellar activity and inter-hemispheric coherence accompany improved reading performance following Quadrato Motor Training. Frontiers in systems neuroscience. 2014;8.

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Alexander T*, **Avirame K***, Lavidor M. Improving emotional prosody detection in the attending ear by cathodal tDCS suppression of the competing channel. Neuroscience letters. 2012;508(1):52–5.

Poster

Avirame K, Meinzer M, Flöel A. Facilitation of semantic retrieval by sensorimotor pre-activation. SMCLC, December 2011, Düsseldorf, Germany.

Affidavit

I, Keren Avirame certify under penalty of perjury by my own signature that I have submitted the thesis on the topic „Changes in plasticity and cognition as a result of neurovascular disease and non-invasive brain stimulation“, I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The sections on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) correspond to the URM (s.o) and are answered by me. My contributions in the selected publications for this dissertation correspond to those that are specified in the following joint declaration with the responsible person and supervisor. All publications resulting from this thesis and which I am author of correspond to the URM (see above) and I am solely responsible.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

. . . .

Date Keren Avirame

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