Aus der Klinik für Neurologie
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DISSERTATION

Influence of anodal transcranial direct current stimulation combined with cognitive training on visuospatial episodic memory in healthy young adults, healthy elderly adults and patients with Mild Cognitive Impairment

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Abbreviations

OLM	Object-location memory
MCI	Mild Cognitive Impairment
CT	Cognitive training
tDCS	Transcranial direct current stimulation
atDCS	Anodal transcranial direct current stimulation
HY	Healthy young
HE	Healthy elderly
DMN	Default mode network
MRI	Magnetic resonance imaging
SD	Standard deviation
CERAD	Consortium to Establish a Registry for Alzheimer's Disease
MMSE	Mini-Mental State Examination
IR	Item recognition
AFC	Alternative forced-choice
BDI	Beck's Depression Inventory
PANAS	Positive and Negative Affect Schedule
AVLT	Auditory Verbal Learning Test
BSKE	Ger. Befindlichkeitsskalierung anhand von Kategorien und Eigenschaftswörtern
ROCF	Rey–Osterrieth Complex Figure
WHOQoL	WHO Quality of life
SVF120	Stress coping strategies
PSQI	Pittsburgh sleep quality
NFC	Need for Cognition
TMT	Trail Making Test
TAP	Test of attentional performance
MWT	Multiple-Choice Vocabulary Intelligence Test
fMRI	Functional magnetic resonance imaging
LMM	Linear mixed models
ANOVA	Analysis of variance
PFC	Prefrontal cortex

Abstract

Background: Object-location memory (OLM) plays an essential role in orientation in the environment and is of high relevance for functioning of daily life. However, this function declines early in the course of aging and is even more accelerated in conditions like amnestic Mild Cognitive Impairment (MCI) and dementia due to Alzheimer's disease. Therefore, strategies for promoting improvements on cognitive functions like visuospatial memory become increasingly important.

Objectives: Evaluate the efficacy of a repeated combined intervention of anodal transcranial direct current stimulation (atDCS) and cognitive training to boost visuospatial memory performance in healthy individuals and in individuals with MCI. Neuronal (study 1) and behavioral (study 1, 2 and 3) effects of atDCS applied during an OLM training task were investigated.

Method: Subjects were trained over 3 days to learn the correct object-location pairings on a street map while receiving 20 minutes-atDCS or a sham stimulation. In study 1, effects of the combined approach were tested in a between-subject design in 20 healthy young (HY) and 20 healthy elderly (HE) subjects. Recall performance in trained and untrained memory tasks was assessed immediately after training, 1 day and 1 month later. Resting state functional magnetic resonance imaging was conducted at baseline and at 1-day follow-up to examine the influence of OLM training simultaneously to atDCS on functional coupling in default mode network. In study 2 and 3, the impact of the combined approach was tested using a within-subject design. Training success and delayed memory after 1 month were assessed in 32 HE (study 2) and compared to the performance of 16 MCI patients (study 3).

Results: In study 1 HY performed better than HE, but both groups showed beneficial effects of stimulation. Increased functional connectivity of neural networks was positively related to enhanced memory performance 1 day later, and atDCS improved trained and untrained tasks 1 month later. In study 2, atDCS combined with OLM training did not enhance training success, performance in 1-month delayed memory, or transfer tasks. In study 3, MCI patients showed enhanced performance at the end of training, but no positive effect was found regarding delayed memory 1-month. Exploratory analyses suggested positive impact on online performance and negative effect on offline performance in MCI patients.

Conclusion: atDCS may modulate behavior and has potential to counteract memory decline, but individual variability and heterogeneity between studies seems to substantially impact performance, calling for the development of individualized protocols in the future.

Zusammenfassung

Hintergrund: Das Objekt-Lokalisierungs-Gedächtnis (kurz OLM) trägt zur Orientierung in der Umwelt und Funktionsfähigkeit im Alltag bei. Im normalen Alterungsprozesses kommt es zur Abnahme der OLM-Leistung, was unter pathologischen Bedingungen, z.B. amnestischer leichter kognitiver Beeinträchtigung (engl. Mild cognitive impairment MCI) und Demenz bedingt durch die Alzheimer Erkrankung, akzeleriert sein kann. Dementsprechend wichtig ist die Entwicklung von Strategien zur Förderung und Aufrechterhaltung kognitiver Funktionen - wie die des visuell-räumlichen Gedächtnisses.
Ziele: Auswirkung einer mehrtägigen kombinierten Anwendung aus atDCS und kognitiven (OLM) Training auf die Lesitung des visuell-räumlichen Gedächtnisses bei Gesunden und Personen mit MCI erfasst anhand von neuronalen (Studie 1) Korrelaten und Verhaltenseffekten (Studie 1-3).
Methode: Durchführung eines computerbasierten OLM-Trainings an drei aufeinanderfolgenden Tagen zeitgleich mit atDCS (20min) oder einer Scheinstimulation (30s). Das OLM-Training bestand aus dem Erlernen korrekter Zuordnungen von Objekten (Gebäuden) zu Lokalisierungen (Positionen) auf einem fiktiven Stadtplan. Studie 1: In einem Zwischensubjektdesign wurde die Abrufleistung bei trainierten und nicht-trainierten Gedächtnisaufgaben unmittelbar nach dem Training, einen Tag und einen Monat später bei 20 gesunden jungen (HY) und 20 gesunden älteren (HE) Probanden gestestet. Die Ruheaktivität des Gehirns wurde mittels funktioneller Magnetresonanztomographie vor und nach dem mehrtägigen Training aufgenommen und der Einfluss der Intervention auf die funktionelle Kopplung (Konnektivität) im Ruhezustandsnetzwerk (engl. Default Mode Network) analysiert. Studie 2 und 3: In einem Meßwiederholungsansatz (cross-over) wurde die Auswirkung von OLM-Training und atDCS auf den Trainingserfolg (Leistung unmittelbar nach dem Training) und die verzögerte Abrufleistung (nach einem Monat) bei 32 HE (Studie 2) gemessen und mit der Leistung von 16 MCI-Patienten (Studie 3) verglichen.

Ergebnisse: Studie 1: Das Leistungsniveau in HY war höher verglichen zu HE, aber beide Gruppen profitierten von atDCS bezogen auf die Gedächtnisleistung. Eine erhöhte funktionale Konnektivität neuronaler Netzwerke ging mit einer verbesserten Gedächtnisleistung einen Tag nach dem Training einher. atDCS hatte einen postiven Einfluss auf trainierte und nicht-trainierte Aufgaben nach einem Monat. Studie 2: In HE hatte atDCS in Kombination mit OLM-Training keinen positiven Einfluss auf den Trainingserfolg, den verzögerten Abruf und trainierte und nicht-trainierte Transferaufgaben. Studie 3: Gemessen am Trainingserfolg profitierten MCI Patienten von atDCS, jedoch nicht in Bezug auf das verzögerte Gedächtnis. In explorativen Analysen deuten auf einen Vorteil von tDCS auf die online, nicht aber die offline-Leistung bei MCI hin.

Schlussfolgerung: Verhalten scheint durch atDCS modulierbar wodurch sich potentielle Interventionsstrategieansätze zur Gedächtnisverbesserung ergeben. Die hohe individuelle Variabilität und Heterogenität zwischen den Studien scheint jedoch die Effekte erheblich einzuschränken und macht die Entwicklung von individualisierten Protokollen erforderlich.

1. Introduction

Object-location memory (OLM) is known as the ability to remember where things are, along with the binding characteristics within the visuospatial context. This represents a special type of episodic memory that plays an essential role in orientation in the environment and adapting to changing surroundings [1]. Despite its critical importance in daily life functioning, memory suffers progressive deterioration over time, and among other cognitive domains, episodic memory represents the largest degree of age-related decline [2]. As the older population continues to grow worldwide, finding strategies for maintaining functional independence and mitigating the negative impact of aging on quality of life is of utmost importance [3,4].

Age-related cognitive decline is accelerated in conditions like amnestic Mild Cognitive Impairment (MCI) and Alzheimer's disease [5]. Evidence relating neural circuits of spatial memory to evolution of brain degenerative processes showed spatial disorders as potential cognitive markers of risk of conversion to Alzheimer's disease [6]. In this sense, early therapeutic approaches that counteract or delay the onset of cognitive decline become increasingly important. Given the scarcity of effectiveness from pharmacological treatments, studies have been encouraged to conduct non-pharmacological approaches, including nutritional supplementation, physical activity, cognitive training (CT), and non-invasive brain stimulation techniques like transcranial direct current stimulation (tDCS) [7].

CT refers to guided drill-and-practice on standardized tasks directed to specific cognitive processes, without explicit instructions regarding problem-solving strategies. This approach usually involves strengthening of neural networks through repeated co-activation of specific neurocognitive circuits active during task performance [8,9]. The literature indicates that CT is beneficial for older adults' memory, but it shows a small effect if conducted alone [10]. tDCS, in turn, is a non-invasive brain stimulation tool which induces plasticity by delivering a weak direct electrical current through the scalp. Primary effect is a polarization of neuronal membranes, which depends on electrical field orientation, and results in enhanced (anodal current) or reduced (cathodal current) neuronal excitability. Anodal tDCS (atDCS) generates immediate effects via subthreshold alteration of resting membrane potential or induces long-lasting changes at synaptic level through balance of long-term potentiation (strengthening of neuronal

connections) and long-term depression (weakening of neuronal connections) processes [11], which are considered physiological basis of learning and memory [12]. Although each approach has a potential for effectiveness, larger or more sustained improvements may be induced by the combination of different techniques, as well as by their application over several sessions [10].

Several studies demonstrated efficacy of atDCS on episodic memory performance in healthy young individuals, and against age-associated cognitive decline both in healthy persons and in patients with Alzheimer's disease. However, potential stimulation effects had been little explored in MCI, a stage that may be more promising for early interventions than Alzheimer's disease, where major brain pathology is already present [13]. Moreover, most atDCS studies evaluated working memory [14–19], little is known about a putative synergistic effect of combined intervention on episodic memory [10,20,21], and most atDCS studies investigating episodic memory focused on its verbal component. In addition, heterogeneity between stimulation protocols regarding the number of sessions, interval between sessions, duration of each session, intensity of stimulation parameters and timing of the application (online or offline) makes it harder to associate improvements from atDCS to a specific methodology [10].

Therefore, taking all these aspects into account, the three studies described in this thesis were guided by the same overall research question: which are the effects of a 3-day OLM-training (CT) combined with atDCS over the right temporoparietal cortex on immediate learning and on long-term episodic memory? We explored three distinct samples using the same stimulation protocol: healthy young (HY), healthy elderly (HE) and patients with MCI.

2. Objectives

The overall goal of this thesis was to study effects of a combined multi-day intervention comprising visuospatial CT in addition to atDCS effects on visuospatial episodic memory regarding trained and untrained functions.

Study 1 [22] focused on neuronal correlates. As default mode network (DMN) represents the most prominent large-scale brain network–mediating episodic memory processes, the effects on DMN functional connectivity were investigated and correlated to visuospatial memory performance. The DMN is an anatomically defined brain system

that preferentially activates during the awake resting state, showing lower levels of activity during tasks which require attention [23]. Declines in DMN connectivity predict age-related decrease in episodic memory performance, affecting areas like medial temporal lobes, and may thus represent a sensitive biomarker for memory deficits [22,23]. As brain-behavior functions changes with age, with stronger relationships in later life for most cognitive domains [24–27], effects on neuronal correlates were investigated in HY and HE adults in order to detect possible different behavioral responses between groups.

Study 2 [10] investigated atDCS effects on training success immediately after the end of training as well as long-term changes after one month in HE adults. Further differentiated effects on aspects of learning and memory (within and between-session changes, behavioral measures of on- and offline effects) were considered, since tDCS response can be driven by distinct consolidation mechanisms depending on time point assessed, with a minimal role of online stimulation and more pronounced differences over the course of training. Therefore, atDCS may contribute to offline effects more than to online effects [28–30].

Study 3 [31] included MCI patients and compared their perfomance to HE. Given that atDCS is a weak form of modulation, it may promote better results in individuals with lower performance levels [32]. In this sense, MCI patients may be particularly susceptible to the beneficial effects of atDCS. Meinzer et al. [13] assessed impact of a single session atDCS on cognition and brain functions in MCI and found improvements to the level of controls, which provided a strong rationale to explore whether repeated stimulation sessions would be able to induce long-lasting beneficial effects on cognition in this population.

3. Methods

3.1. Participants

After recruitment of healthy individuals via advertisements in Berlin, Germany, and of MCI patients from the memory clinic of the Department of Neurology, Charité – Universitätsmedizin Berlin, Germany, subjects were pre-screened by a structured phone interview, underwent a medical and neuropsychological tests battery. A structural magnetic resonance imaging (MRI) was conducted in order to exclude brain

pathologies like stroke or tumor. After following inclusion and exclusion criteria, a total of 68 fluent German language speakers were selected to participate in the three studies. In study 1, the sample comprised 20 HY (12 women; mean age (SD) [range] in years: 25 (4) [19–34]) and 20 HE (14 women; mean age (SD) [range] in years: 70 (6) [60–78]). In study 2, 32 HE (22 females; mean age (SD) [range] in years: 68 (7), [53–79]) were included (sample used in study 1 was composed by 20 HE taken from this larger sample of 32 HE). In both studies, participants without history of neurological or psychiatric disorders and within age-related norms in standardized tests (Consortium to Establish a Registry for Alzheimer's Disease, CERAD-Plus Test Battery, https://www.memoryclinic.ch) were selected. In study 3, data of 16 patients with amnestic MCI (5 women; mean age (SD) [range] in years: 70 (6) [62–82]) were compared with previously reported data of 32 HE. Subjects were included if they fulfilled the Peterson criteria for MCI [33–35] and comprised subjective cognitive complaints, objective memory impairments in standardized tests (at least 1 SD below age and education adjusted norms in relevant memory-related subtests of CERAD, to include both, early and late MCI [36]), preserved general cognition, no constraints in activities of daily living (Mini-Mental State Examination (MMSE) [37] above the normality cut-off: MMSE> 24), and no evidence of manifest dementia. The studies were approved by the Ethics Committee of the Charité – Universitätsmedizin Berlin, Germany, and were conducted according declaration of Helsinki. All participants signed informed written consent before study-specific procedures and received a reimbursement for participation.

3.2. Object-Location Memory Training

A visuospatial memory training was administered using the OLM-paradigm [29,38], in which 30 pictures of real-life buildings were associated to 30 specific positions on a schematic bidimensional street map. On each training day, in a total of 600 trials for older individuals and 360 trials for younger (in order to avoid ceiling effects), distributed respectively over 5 learning blocks or over 3 learning blocks (each block with 120 trials), subjects had to indicate by button press on a response pad whether the building was associated to its correct or incorrect position. Each correct association appeared twice per block, 10 times per day (100% of daily success training was equivalent to 300 hits and 300 correct rejections in older, 180 hits and 180 correct rejections in younger). Each building was presented for 3000 ms with an inter-stimulus interval of 1000 ms.

Performance was assessed by cued recall using two different test formats, namely item recognition (IR) and 3-alternative forced choice (3-AFC) test. For IR, 15 correct object-location associations were intermixed with 15 new incorrect pairings and subjects had to indicate by button press whether each association was correct or not. In the 3-AFC test, subjects had to choose among three possible locations the correct location for a building presented above the street map. In study 1, only 3-AFC format was used as cued recall, whilst in study 2 and 3 both recall tests were used.

3.3 Testing

In all three studies, questionnaires and standardized neuropsychological tests were carried out in order to characterize the sample. In study 1, Digit span [39], Phonemic and semantic word fluency test [40] and Mini Mental State Examination (MMSE) [37] were applied to determine cognitive status of participants. Mood was assessed by using Beck's Depression Inventory (BDI) [41] and handedness were controlled by the laterality quotient (Oldfield) [42]. At pre-training (baseline) as well as one day and one month later, positive and negative affective states were self-rated by means of Positive and Negative Affect Schedule (PANAS) [43], and transfer tasks to measure transfer to a similar (short form of LOCATO learning and retrieval) and less similar memory tasks (Auditory Verbal Learning Test (AVLT) [44]) were applied. At each training session, affective states were rated by means of the German questionnaire "Befindlichkeitsskalierung anhand von Kategorien und Eigenschaftswörtern" (BSKE) [45], and subjective perception of sleep quality and sleep duration of prior night were determined by the questions "How did you sleep last night?" and "How many hours did you sleep last night?". At re- and post-training, trained and untrained memory functions were measured from shorter version of OLM-paradigm (LOCATO-15), Rey–Osterrieth Complex Figure Test (ROCF)[46,47] and AVLT [44].

In addition in study 2 and 3 WHO Quality of life (WHOQoL) [48], Stress coping strategies—habitual form (SVF120) [49], Pittsburgh Sleep Quality – habitual sleep score (PSQI)[50] and Need for Cognition (NFC) [51] were assessed to characterize cohorts, since these tests quantify motivation and difficulty to execute cognitive tasks, factors which may directly interfere on performance during training.

Baseline cognitive domains encompassed: MMSE scores [37]; CERAD (Memory Clinic Basel, www.memoryclinic.ch); Digit span [39]; Trail Making Test

(TMT) [52]; Regensburger Verbal Fluency Test [38]; computerized test battery to test attention (Test of attentional performance: TAP) [53]; and Multiple-Choice Vocabulary Intelligence Test (MWT) [54].

In addition, in all three studies blood samples were drawn at baseline to determine genotypes of learning relevant polymorphisms (ApoE 4, COMT Val158 Met, BDNF Val66Met).

3.4 Functional magnetic resonance imaging

For acquisition of imaging data in study 1, functional MRI (fMRI) was conducted 1 day before and 1 day after the multi-day training with a 3T Siemens Trio MRI system using a 12-channel head coil. A three-dimensional structural scanning protocol was applied using high-resolution T1-weighted magnetization prepared rapid gradient echo imaging. Resting state fMRI data were obtained using an echo-planar imaging sequence.

FMRIB Software Library (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) [55] was manipulated for extraction of information from image. T1-weighted images were used at baseline and individual brain tissue volume was estimated, normalized for head size using SIENAX [56], in order to measure brain atrophy level. After determining the means and standard deviations (SD) for participants, normalizing brain volumes, preprocessing of individual 4D data sets included motion (using MCFLIRT) and slice time correction, brain extraction, spatial smoothing using a gaussian kernel of full-width half maximum of 5 mm, and high-pass temporal filtering of 100 seconds (0.01 Hz). Functional images were aligned to individual structural T1-weighted images using boundary-based registration [57] and then to standard space (Montreal Neurological Institute 2 mm) using the nonlinear registration tool (12 degrees of freedom; warp resolution: 10 mm; resampling resolution: 2 mm). Independent component analyses were carried out using FMRIB Software Library's MELODIC tool 3.14[58]. A single 4D data set was generated from individual data which were temporally coupled across participants and time points. This data set was then decomposed into 20 independent components, and dual-regression approach was used to identify separate maps for each participant and time point, in order to determine strength of functional connectivity within a network. For seed-based analysis, we used a probabilistic mask from the Harvard-Oxford cortical atlas (right middle temporal gyrus, temporo-occipital part, thresholded at 50%) [59] to find the

stimulation target brain area [60,61]. The time course of the blood oxygenation level dependent signal in this region-of-interest was extracted from each participant's preprocessed resting-state 4D data set, and resulting time courses were correlated with the time course extracted from the DMN to obtain individual functional correlations between the stimulation target and the DMN for each time point. The resulting r values were transformed into z scores using Fisher r-to-z transformation.

3.5 Transcranial Direct Current Stimulation

On each training day, during the beginning of OLM-paradigm, a non-invasive brain stimulation was delivered by a direct current stimulator (NeuroConn GmbH, Ilmenau, Germany) through two saline soaked- surface sponge electrodes: an active electrode (size:7x5 cm^2) centered over the right temporoparietal cortex, and a reference electrode (size:10x10 cm^2) over the contralateral supraorbital cortex, both attached to the scalp using rubber bands. Electrode placement was defined according to the international EEG 10-20 System. A current of 1mA was applied for 20 min (atDCS) or 30 s (sham) in a ramp-like fashion of 10 s. Given that right temporoparietal region is implicated in the acquisition of OLM [1] and atDCS over this area showed benefits in previous studies using similar version of the same OLM task [29,62], this site was selected for receiving simulation.

3.6 Experimental Design

The three studies [10,22,31] were randomized subject-blind placebo-controlled trials. Study 2 [10] and study 3 [31] were conducted in a cross-over design (within-subject) involving two blocks of testing for each participant. In study 1 [22], a cross-sectional analysis was carried out using data of a subgroup composed by 20 HE and including 20 HY for comparison purposes. All procedures were performed over three phases: baseline testing (pre-training: MRI, neuropsychological tests involving cognitive and non-cognitive domains, performance on trained function and transfer tasks); a 3-day visuospatial training combined with atDCS; and follow-up sessions, after 1-day and after 1-month (post-training: retrieval performance on trained paradigm, trained function and transfer tasks). With regard to retrieval, in study 1 recall tests data at one time point were used, whilst study and study 3 used data of both recall and recognition tests.

4. Statistical analysis

Statistical analysis was conducted using the *Statistical Package for the Social Sciences* (SPSS), version 24.0 (www.ibm.com/software/de/analytics/spss/), and R software, version 3.3.2 (https://www.rproject.org).

Study 1: Repeated measurements analyses of variance (ANOVAs) were conducted using TIME (baseline, 1-day after training), INTERVENTION (anodal, sham) and GROUP (HY or HE) as factors. Resting-state functional connectivity and behavioral performance were the main outcome variables. Two-factorial univariate ANOVAs were conducted for comparison of behavioral performance between conditions (anodal, sham) and groups (HY, HE) as well as their interaction. Pearson's correlation coefficients were computed for linear associations between variables. Additional covariates were included, where appropriate. All statistical tests were considered significant at a level of $p<0.05$.

Study 2 and 3: Linear Mixed Models (LMM) [63] were used to test the effects of atDCS concomitant to training. For each outcome variable (training success; delayed memory after 1-month), 2 repeated measures regarding to the factor INTERVENTION (atDCS or sham) were entered, totalizing 64 points for HE and 31 points for MCI patients. Exploratory analyses using the factor DAY were added in order to investigate online and offline effects, as well as changes in emotional state and sleep characteristics, totalizing 192 points for HE and 93 points for MCI patients. Pre and post-training mood and changes in trained and untrained transfer tasks as a function of intervention were analyzed by separate LMM with three factors INTERVENTION (atDCS, sham), TIME (Baseline, FU1, FU2), and SEQUENCE (study block 1, study block 2). All analyses were repeated with covariates, in order to exclude the influence of other variables on the results. In study 2 [10], impact of intervention was measured by regression coefficients ß, using d as a coefficient to measure effect size, in a 95% CI. In study 3 [31], model-based post-hoc pairwise comparisons of the fixed effects were calculated and presented as mean differences in % (atDCS – sham or MCI – HE), and semi-partial R^2 as implemented in the r package r^2glmm were computed as measures of effect size for fixed effects in a 95% CI of these differences. All statistical tests were considered significant at a level of $p<0.05$.

5. Results

Study 1: For all individuals, mean learning performance during training did not differ between stimulation conditions, but mean recall on each training day revealed significant differences between conditions ($F(1,36)=4.72$, $p=0.037$. With regard to delayed recall performance, a main effect of condition was found for atDCS after 1 day ($F(1,26) = 6.62$, $p = 0.016$), which did not persist after 1 month. atDCS also generated an increased DMN strength (time x condition interaction: $F(1,34) = 4.86$, $p = 0.034$), as well as an increased functional connectivity between the stimulation target and DMN (time x condition interaction: $F(1,34)=5.38$, $p=0.026$) compared with sham stimulation. The increase in DMN strength was positively associated with enhanced memory performance ($r=0.36$, $p=0.0319$), mainly of HE. atDCS combined with training promoted also benefits on trained function (OLM-training) and transfer task performance (AVLT) assessed 1 month after episodic memory training. For all variables, there was a main effect of GROUP, with HY performing better than HE. Mood ratings were not affected by stimulation.

Study 2: No significant differences were found between atDCS and sham condition for the main outcome variables training success and delayed memory, which did not confirm stimulation efficacy on OLM in HE. There were also no significant differences for online effects, trained and untrained transfer tasks. In contrast, for between-session (offline) measurements significant less forgetting overnight after atDCS compared to sham was found after first night in IR test (4.2 [0.3, 8.0], $d = 0.5$).

Study 3: atDCS showed an impact on training success in both groups (HE and MCI), although not statistically significant GROUP x INTERVENTION interaction effect was found. With regard to INTERVENTION, MCI patients benefited from training under atDCS in comparison to sham, a result even more pronounced after analysis with covariates (13.8 [1.0, 26.7], $p = 0.04$), while no differences between atDCS and sham were obtained for HE. Furthermore, relative gain (calculated as difference in training success between atDCS and sham condition: atDCS – sham) was similar in MCI and HE under atDCS, but lower in MCI than in HE under sham. No significant differences were found for delayed memory. Regarding explorative analysis, for within-session performance, a significant training effect across days was evident in both groups, and although not statistically significant, an enhancement by atDCS was found as online

effects. For between-session performance, an adverse effect of atDCS was obtained in MCI patients after the third night in both cued recall tests (IR: –11.0 [–18.3, –3.7], 3-AFC: –14.6 [–24.6, –4.5]), even after adjustment for covariates, without differences in HE.

6. Discussion

Overall, the three studies investigated the effects of a 3-day OLM-training combined with atDCS over the right temporoparietal cortex on immediate learning and on long-term episodic memory.

Study 1: Study 1 [22] focused on neuronal and behavioral effects of intervention in HY and HE. With regard to behavioral variables, atDCS over multiple days did not demonstrate effectiveness on performance during CT and did not affect the learning curve, corroborating single-session results found by Flöel et al. 2012 [29] when used the same OLM-paradigm. However, despite no online effects, they found after-effects of atDCS in a recall test one week later [29]. Similarly, study 1 obtained positive after-effects of atDCS in a recall test (although at another time point: one day later), which shows a better offline responsiveness to stimulation, in line with other studies that compared HY and HE over multiple sessions and found benefits in the retrieval phase [64–68]. The main findings of study 1 corroborated previous results of studies associating atDCS and CT in HY [30,69,70,71], besides adding multi-session effectiveness to the existing knowledge about tDCS-benefits on visuospatial memory after single-sessions or working memory training in HE [29,66]. Although this effect did not persist in recall performance 1 month later, benefits have been shown regarding trained function and transfer tasks at this same time point, suggesting that long-term effects may not necessarily translate into improved performance in the trained task, but they may be evidenced by learning of new associations [22]. In contrast, Sandrini et al. 2016 tested atDCS effects on recall performance at one month and found enhancement in the atDCS group relative to the sham group [66]. However, it is important to note that they applied stimulation on the prefrontal cortex (PFC) and investigated its effects on verbal episodic memory, whilst our focus was on right temporoparietal cortex and visuospatial episodic memory. Sandrini et al. 2019 obtained positive recall effects by applying offline atDCS in HE [67], in contrast to our study, which applied atDCS during encoding phase of task. Therefore, methodological aspects including type of cognitive domain, target area,

electrode montage, electrode size, session duration, between-session intervals, stimulation parameters and stimulation application in combination with simultaneous task may considerably affect the cognitive outcome [10,22].

Another aspect that may interfere with the results concerns age. Comparison between age groups in study 1 showed that HY outperformed HE in learning and recall scores at all test sessions, suggesting age-related limits of cognitive reserve plasticity [72]. In line with this theory, results of Leach et al. 2019 showed improved face-name associative memory performance for both recall and recognition measures only for younger adults [73]. In contrast, systematic review conducted by Galli et al. 2018 showed stronger stimulation effects on memory accuracy of HE in comparison to HY [20]. This advantage may be explained by the fact that a worsened function at baseline allows larger changes resulting from interventions. Therefore, there is not yet consensus in the literature and each trend related to atDCS age-related differences warrants further investigation. Moreover, intraindividual variability may have influence on results, and not solely age differences.

Despite heterogeneity of results due to differences between subjects and studies, with both positive and null effects, atDCS concurrent to CT is considered an effective method to improve function and induce brain plasticity. With regard to neuronal effects, the increased strength on DMN was positively correlated with improved recall performance after 1 day and this association was mainly driven by older participants in the stimulation group, that is, individuals with cognitive disadvantage may benefit more from stimulation. Therefore, since these neural networks used to be impaired in conditions like MCI or Alzheimer's disease, atDCS combined with CT may modulate these regions and reduce or even prevent the impact of cognitive decline [22].

Study 2: In study 2, atDCS over multiple days did not generate any improvements related to learning or retrieval in HE, but both conditions (with or without atDCS) showed better memory performance at the end of training and after 1 month. One possible explanation would concern the study protocol, which involved not solely stimulation, but also CT. Thus, strong effects of practice arising from CT over multiple sessions would have masked the benefits of stimulation, generating carry-over effects and explaining the absence of improvements in this study in contrast to positive effects found by Flöel et al. 2012 after a single session [29]. Since atDCS is a weak form of modulation, stimulation intensity in our protocol may not have been powerful enough to overcome the effects of CT and reveal detectable differences in relation to sham [10]. On the other hand,

study 1 found atDCS positive effects even using the same protocol of CT. Despite this, both studies differed in parameters assumed to be relevant in atDCS/cognition modulating protocols such as primary cognitive outcomes, time point of assessment and selected statistical approach [74]. Furthermore, within-subject design of study 2 increases the chances of carry-over effects in comparison to the between-subject design of study 1. Although several studies have shown atDCS-induced beneficial effects on item memory performance in HE [5,64,65, 66,75,76], our results corroborate other studies that did not find stimulation benefits compared to sham [20,76]. The only parameter positively affected by atDCS effect was offline performance after the first night. After initial encoding, memory traces are unstable (vulnerable to interference or modification), but become stabilized and more resistant to disruption over time, a process referred to as consolidation [77]. In this sense, a relatively larger impact of atDCS may have occurred on more labile phases of consolidation [78,79], promoting immediate offline benefits, but these improvements have been not maintained probably due to CT strength, which could be confirmed if there would be atDCS-group without CT.

Study 3*:* Study 3 expanded the results obtained in study 2, since experimental protocol and research design were the same, with addition of MCI patients for comparing data. atDCS did not modulate any variables in HE, neither during learning nor in delayed memory, whilst MCI patients who received atDCS revealed significant differences regarding training success (a better learning performance compared to sham) and offline effects (a negative performance compared to sham) on the last training day. Such results show that atDCS as applied in our study protocol was able to promote immediate effects, but no long-term modifications (at least, no detectable differences between HE and MCI 1 month after our training protocol). Similarly, MCI studies conducted by Inagawa et al.2019 and Huo et al. 2020 did not find benefits regarding long-term memory [76,80]. In contrast, other researchers showed a positive impact of atDCS on delayed episodic memory in MCI patients. Fillecia et al. 2019 found that long-lasting atDCS over the left dorsolateral PFC improved overall cognition scores, immediate verbal memory (AVLT: immediate recall) and figure naming performance in MCI patients [81]. Manenti et al. 2020 found improvements on recognition memory after 1 month in MCI [82]. There is not yet a consensus in the literature, since there is a large methodological heterogeneity, and further investigations are needed to define the better parameters that generate long-term modifications on brain and behavior [8].

With regard to explorative analysis, negative offline performance after training was not expected in the atDCS group, since the hypothesis that there is a consolidation mechanism more susceptible to offline effects than online effects is well-stablished in the literature [25,26] . In our study, atDCS generated a positive impact on online performance (related to neuronal activity at subthreshold level of resting membrane potentials), whilst it promoted negative influence on offline performance (processes depending on NMDA receptors). Contrary to expected, MCI patients benefited more from online effects than offline effects. In this sense, effects of atDCS during and after training seem to be driven by distinct neuroplasticity mechanisms, although the basis for such differences remains uncertain. Stimulation could be causing offline impairments to MCI patients, but such argument seems unlikely, since the effects during training were positive (levels comparable to HE) and no long-term worsening was detected.

Sum:

In general, our study strengthens the body of evidence in favor of modulation of episodic memory by atDCS. We concluded that atDCS combined with CT is able to promote cognitive and neural changes, but this does not apply to all individuals, situations, cognitive tasks and contexts in the same way. In line with other reports in the literature and supported by our study, the assumptions that one protocol fits does not seem appropriate. The high variability in our outcomes despite the same intervention protocol reveals that not only type of cognitive domain and stimulation parameters per se are responsible for atDCS-effects, but also aspects regarding behavioral, structural and functional parameters of the individual participants. These parameters may interact and change intraindividually over time, which may also require adjustments in individualized protocols for multiple applications, constituting a challenge in developing appropriate criteria in the future studies.

7. References

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Statutory Declaration

"I, Angelica Vieira Cavalcanti de Sousa, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic "Influence of anodal transcranial direct current stimulation combined with cognitive training on visuospatial episodic memory in healthy young adults, healthy elderly adults and patients with Mild Cognitive Impairment" (Einfluss der anodalen transkraniellen Gleichstromstimulation in Kombination mit kognitivem Training auf das visuell-räumliche episodische Gedächtnis bei gesunden jungen Erwachsenen, gesunden älteren Erwachsenen und Patienten mit leichter kognitiver Beeinträchtigung) independently and without the support of third parties, and that I used no other sources and aids than those stated.

All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me."

……

Date

…….....

Signature of Angelica Vieira Cavalcanti de Sousa

Declaration of own contribution

Angelica Vieira Cavalcanti de Sousa contributed the following to the below listed publications:

Publication 1: Antonenko Daria*, Külzow Nadine*, **Sousa Angelica**, Prehn Kristin, Grittner Ulrike, Flöel Agnes. Neuronal and behavioral effects of multi-day brain stimulation and memory training. Neurobiology of Aging, 2018.
Contribution: Participants recruitment (via phone interview or e-mail); acquisition data (application of neuropsychological tests, stimulation and OLM-training; conversion of raw data into data table – Excel and SPSS), collaboration in writing (tables 2 and 3).

Publication 2: Nadine Külzow*, **Angelica Vieira Cavalcanti de Sousa***, Magda Cesarz, Julie-Marie Hank, Alida Günsberg, Solvejg Harder, Swantje Koblitz, Ulrike Grittner, Agnes Flöel. No effects of non-invasive brain stimulation on multiple sessions of object-location memory training in healthy older adults. Frontiers in Neuroscience, 2018.
Contribution: Participants recruitment (via phone interview or e-mail); data acquisition (application of neuropsychological tests, stimulation and OLM-training; conversion of raw data into data table); participation in statistical analysis and interpretation of data; participation in drafting, writing (co-creation of figures 1 and 2; co-creation of tables 1, 2 and 3) and revision of the paper.

Publication 3: Angelica Vieira Cavalcanti de Sousa, Ulrike Grittner, Dan Rujescu, Nadine Külzow, Agnes Flöel. Impact of 3-day combined anodal transcranial direct current stimulation-visuospatial training on object-location memory in healthy older adults and patients with mild cognitive impairment. Journal of Alzheimer`s Disease, 2020.
Contribution: Participants recruitment (via phone interview or e-mail); data acquisition (application of neuropsychological tests, stimulation and OLM-training; conversion of raw data into data table – Excel and SPSS); statistical analysis of data set using SPSS and R, supervised by Dr. Ulrike Grittner; writing (reference searching; drafting; creation of figures 1, 2, 3 and 4; creation of tables 1, 2 and 3; organization of ideas and each part of

text structure); revision (re-writing according corrections of Dr. Nadine Külzow and Prof. Dr. med. Agnes Flöel); submission of the paper (according journal's submission guideline); correction of the paper according to the reviewers' suggestions; writing a response letter to reviewers; re-submission of the paper.

*[*shared first authorship]*

.....
Signature, date and stamp of first supervising university professor

.....
Signature of doctoral candidate

Selected publications

Publication 1:

Antonenko Daria*, Külzow Nadine*, **Sousa Angelica**, Prehn Kristin, Grittner Ulrike, Flöel Agnes. Neuronal and behavioral effects of multi-day brain stimulation and memory training. Neurobiology of Aging, 2018.

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Impact factor: 3.566

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Publication 3:

Angelica Vieira Cavalcanti de Sousa, Ulrike Grittner, Dan Rujescu, Nadine Külzow, Agnes Flöel. Impact of 3-day combined anodal transcranial direct current stimulation-visuospatial training on object-location memory in healthy older adults and patients with mild cognitive impairment. Journal of Alzheimer's Disease, 2020.

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CURRICULUM VITAE

My curriculum vitae does not appear in the electronic version of my paper for reasons of data protection.

Complete Publication List

de Sousa AVC, Grittner U, Rujescu D, Külzow N, Flöel A. Impact of 3-day combined anodal transcranial direct current stimulation-visuospatial training on object-location memory in healthy older adults and patients with Mild Cognitive Impairment. J Alzheimers Dis. 2020; 75(1): 223–44.

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