Association of cognitive performance with hippocampal network integrity of healthy adults and its modulation through non-invasive brain stimulation

I n a u g u r a l d i s s e r t a t i o n

zur

Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften (Dr. rer. nat.)

der

Mathematisch-Naturwissenschaftlichen Fakultät

der

Universität Greifswald

vorgelegt von

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Geboren am 10.01.1990

Greifswald, den 27.03.2019
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Tag der Promotion: 14.08.2019
Abstract

Brain aging even in healthy older adults is characterized by a decline in cognitive functions including memory, learning and attention. Among others, memory is one of the major cognitive functions affected by aging. Understanding the mechanisms underlying age-related memory decline may help pave the road for novel treatment strategies. Here, we tried to elucidate the neural correlates associated with memory decline using structural and functional neuroimaging and neuromodulation with transcranial direct current stimulation (tDCS).

Over the course of three studies, we investigated 1) the influence of white matter integrity and grey matter volume on memory performance in healthy older adults, 2) the role of functional coupling within the memory network in predicting memory performance and the impact of tDCS in modulating retrieval performance in healthy older adults, 3) the effect of tDCS over the sensorimotor cortex on cognitive performance in young adults.

MRI was used to study associations of cognitive performance with white matter integrity and grey matter volume, and examine their causal relationship in the course of aging. White matter integrity was assessed by acquiring diffusion tensor imaging (DTI) and performing deterministic tractography based on constrained spherical deconvolution. Grey matter volume was estimated using fully automated segmentation. Both white matter integrity and grey matter volume were correlated with behavioral data of a verbal episodic memory task. Percentage of correct answers at retrieval was used to measure memory performance (Manuscript 1). In addition, anodal tDCS (atDCS) (1 mA, 20 min) was applied over CP5 (left temporoparietal cortex) to modulate memory formation in healthy older adults. Participants underwent resting-state fMRI before the stimulation. Functional connectivity analysis was performed to determine whether functional coupling within the memory network predicted initial memory performance, and to examine its association to tDCS-induced enhancement effect (Manuscript 2). Finally, atDCS (1 mA, 20 min) was applied over C3 (left sensorimotor cortex) to explore the effect of tDCS over the sensorimotor cortex on cognitive performance in young adults. During the stimulation, participants performed three tasks; gestural task, attentional load task and simple reaction time task (Manuscript 3).
Results showed that volumes of the left dentate gyrus (DG) and tractography-based fractional anisotropy (FA) of individual fornix pathways were positively related to memory retrieval in older adults. Brain-behavior associations were observed for correct rejections rather than hits of memory performance, indicating specificity of memory network functioning for detecting false associations. Thus, the data suggested a particular role of neural integrity that promotes successful memory retrieval in older adults. Subsequent mediation analysis showed that left DG volume mediated the effect of fornix FA on memory performance (48%), corrected for age, revealing a crucial role of hippocampal pathway microstructure in modulating memory performance in older adults (Manuscript 1). tDCS results showed that atDCS led to better retrieval performance and increasing learning curves, indicating that brain stimulation can induce plasticity of episodic memory processes in older adults. Combining tDCS and fMRI, hippocampo-temporoparietal functional connectivity was positively associated with initial memory performance in healthy older adults and was positively correlated with the magnitude of individual tDCS-induced enhancement, suggesting that individual tDCS responsiveness may be determined by intrinsic network coupling (Manuscript 2). Finally, our findings suggested that atDCS over left sensorimotor cortex reduced reaction times in the gestural-verbal integration task, specifically for incongruent pairs of gestures and verbal expressions, indicating the role of sensorimotor cortex in gestural-verbal integration in young adults (Manuscript 3).

The results of all three studies may help to elucidate age-related structural deterioration and functional coupling network underlying cognitive processes in healthy adults. Furthermore, these studies emphasized the importance of interventions like tDCS in modulating cognitive performance, specifically episodic verbal memory and gestural-verbal integration. By unveiling the specific role of brain structures and functional network coupling as well as the role of tDCS in modulating cognitive performance, our results contribute to a better understanding of brain-behavior associations, and may help to develop clinical interventional approaches, tailored for specific cognitive functions in aging.
1 Introduction

Brain aging even in healthy older adults is characterized by a decline in cognitive functions including memory, learning, attention and word comprehension (Tran et al. 2016, van de Vijver, Cohen, and Ridderinkhof 2014, Oren et al. 2018). Among others, memory is one of the major cognitive domains affected by aging. Memory is divided into two major classifications: Short-term memory and long-term memory. Short-term memory is the information processed in a short period of time, where working memory is responsible for this processing. Long-term memory refers to the storage of information for long periods of time, and the retrieval of this information may be conscious (declarative memory) or unconscious (non-declarative memory) (Camina and Guell 2017) (Figure 1). In our studies, we were interested in declarative memory, more specifically episodic memory which has exhibited the most crucial age-related impairment (Nyberg 2017). Episodic memory stores personal information that includes the time and place of an event (Dickerson and Eichenbaum 2010). The hippocampus and the neuronal networks that connect it to its neighboring brain areas have been shown to be responsible for episodic memory formation (for review (Dickerson and Eichenbaum 2010, Eichenbaum, Yonelinas, and Ranganath 2007)).

![Figure 1: Memory classification. Diagram showing the classification of short-term and long-term memory functions; Short-term memory includes working memory, long-term memory includes non-declarative and non-declarative memory. Episodic and semantic memory constitute the declarative memory (adapted from (Camina and Guell 2017)).](image-url)
In addition, studies have suggested that age-related deficits in working memory explain language comprehension difficulties of older adults (DeDe et al. 2004, Bopp and Verhaeghen 2005). The engagement of the sensorimotor system in language comprehension has been an intriguing question in brain research (Hauk, Johnsrude, and Pulvermuller 2004, Tettamanti et al. 2005, Pulvermuller and Fadiga 2010, Vukovic et al. 2017). Moreover, gestures were found to improve language comprehension of listeners (Hostetter 2011), possibly via embodiment. Parzuchowski et al. (2014) used embodied cognition to show that hand gestures enhance language comprehension. According to Hostetter and Alibali (2008), the embodiment approach suggests that language understanding is based on perceptual experience, that is, words start to have meaning when linked to real world perception.

As such, elucidating neural mechanisms underpinning age-related cognitive decline may help to develop tailored interventions. In the human brain, this is implemented through neuroimaging and neuromodulation techniques.

1.1 Magnetic Resonance Imaging: structural and functional analysis

Cognition arises from the interplay between brain structures, including grey and white matter, and brain function. Given the importance of these features in cognition, extensive research has been conducted to develop techniques that facilitate their assessment. Magnetic resonance imaging (MRI) is one commonly used technique to determine volumes of grey matter, integrity of white matter and functional connectivity within brain networks. This technique was characterized by non-invasiveness and high resolution imaging ((Lim and Pfefferbaum 1989), for review (Helms 2016)).

With the emergence of MRI, the development of structural image processing and segmentation became fundamental for the assessment of grey matter tissues. T1-weighted sequences are one of the most commonly used sequences to acquire high-resolution structural anatomical images of brain regions in order to generate images with a maximum contrast difference between grey matter, white matter, and cerebrospinal fluid (CSF) (for review (Lockhart and DeCarli 2014). Advances in the segmentation algorithms of the two-dimensional structural image (T1-weighted image) yielded to the extraction of brain metrics, for instance volumes of various cortical and subcortical structures (Makris et al. 2006,
Fischl 2012). Similarly, DTI allows the depiction of diffusion processes in white matter microstructures. Diffusion properties in the brain provide information about tissue properties like membrane permeability and integrity, axonal diameter, axonal myelination and axonal density (Goveas et al. 2015, Beaulieu 2002). Fractional anisotropy (FA) is one DTI variable that measures the degree of directionality of the diffusion in a specific white matter structure (Basser and Pierpaoli 1996), and has been shown to decrease with age (for review (Assaf and Pasternak 2008). In diffusion MRI, tractography technique was used to explore diffusion anisotropy in order to assess the orientation of white matter tracts in each voxel, and use these orientation estimates to reconstruct continuous trajectories (for review (Mori and van Zijl 2002)). Even with technical advances in diffusion MRI, it is still complicated to evaluate microstructure in areas with crossing fibers, i.e when voxels contain complex fiber architecture (Jones, Knosche, and Turner 2013). As such, constrained spherical deconvolution (CSD) was recently introduced to overcome limitations of the widely used DTI techniques that cannot resolve these complex white matter configurations. CSD technique applies fiber tracking and estimates the orientation of multiple intravoxel fiber populations in regions of white matter structures with crossing fibers like the fornix (Jeurissen et al. 2011).

Unveiling the functional relationship between brain structures has become indispensable for understanding cognition. “Resting-state” functional MRI (fMRI) is used to assess changes of neural activity while the subject is resting without performing any task (Smitha et al. 2017). The concept of fMRI is based on blood oxygenation level dependent (BOLD) alterations in brain tissue, manifested when levels of oxygen intake change due to metabolic activity (Ogawa et al. 1992). As such, MRI sequences are acquired to reveal this BOLD contrast, and therefore unveil the underlying neural activity at rest. Generally, brain connectivity techniques include the assessment of the interaction between two different brain regions. Functional connectivity (FC) is implemented to establish the interaction between two regions of interest using temporal correlation. This type of analysis allows the estimation of the causal connections between functionally connected brain regions (Friston 2011, Smith 2012).

In sum, MRI analysis emerged as an efficient tool to examine white matter integrity, grey matter volume, and neural activity, and therefore explore their associations with cognitive functions.
1.2 The involvement of brain structures in memory formation

Brain tissue includes white and grey matter. White matter contains a wide range of axons that assure neural communication and grey matter contains local networks of neurons that are connected by dendrites and mainly nonmyelinated axons (Wen and Chklovskii 2005). Variability of white matter microstructure integrity and grey matter volumes have been shown to be efficient predictors of performance level in many cognitive functions, such as memory performance (Metoki et al. 2017, Lett et al. 2016, Moore et al. 2017, Xing et al. 2016). In healthy aging, studies have shown that white matter integrity and grey matter volumes decrease with age, causing impairment in memory functions (Salat 2011, Minkova et al. 2017).

1.2.1 Effect of fornix integrity on memory performance

Fornix is a C-shaped tract that carries a major efferent pathway of the hippocampus and diverges into two entities that pass through the hypothalamus and reach the mamillary bodies (Lovblad, Schaller, and Vargas 2014). Fornix is the most commonly studied white matter structure in the context of aging and memory (Chen et al. 2015, Gunbey et al. 2014, Ren et al. 2018, Antonenko et al. 2016). In normal aging, fornix integrity has been shown to decrease (Jang, Cho, and Chang 2011, Bennett et al. 2017). This decrease in integrity of fornical pathways has been associated with memory decline in healthy older adults (Fletcher et al. 2013, Henson et al. 2016), specifically of episodic memory functions (Metzler-Baddeley et al. 2011, Lockhart et al. 2012, Douet and Chang 2014). For instance, a recent study suggested that changes in fornix microstructure of middle-to-late aged adults was associated to face recognition memory and partly explained the preserved functional connectivity within hippocampal networks (Ly et al. 2016). Moreover, Metzler-Baddeley and colleagues conducted individual fiber tracking technique and found that specifically age-related degradation of fornix microstructure was related to episodic memory recall performance in strategic and visual memory tasks in older adults (Metzler-Baddeley et al. 2011). Age-related fornix degeneration was not only associated with memory performance, but also was among the earliest impairments in healthy older adults at risk of Alzheimer’s disease (AD) (Douaud et al. 2013). Since the fornix is the major efferent pathway of the hippocampus, understanding the role of hippocampus in memory formation is therefore indispensable.
1.2.2  Effect of hippocampus volume on memory performance

The hippocampus is an elongated structure that resides in the medial temporal lobe (MTL). The entorhinal cortex is the structure that provides input for the hippocampus. Within the hippocampus, information circulates from the dentate gyrus (DG), through the cornu ammonis (CA) fields (1-4) and reaches the subiculum (van Strien, Cappaert, and Witter 2009, Sloviter and Lomo 2012). This information flow is known as the “trisynaptic loop” (Knierim 2015). From the CA1 and subiculum, information flows back into the entorhinal cortex and via hippocampal efferent pathways through the fornix (Kerr et al. 2007) (Figure 2). Degeneration of the hippocampus has been shown to be implicated in age-related memory deficits (Hong et al. 2015, Irish et al. 2014, Catheline et al. 2015, Mack, Love, and Preston 2017). Studies have shown that the hippocampus is affected by advanced age which led to smaller volumes of its subfields, such as CA1-4, fimbria, subiculum and dentate gyrus (DG) (Pereira et al. 2014, Raz et al. 2015). The DG was observed to have a stronger age-associated cerebral blood volume decrease compared to other hippocampal subfields (Brickman et al. 2014). Due to its particular vulnerability to age-related processes (Wilson et al. 2006, Small et al. 2004) and activity during memory discrimination tasks, changes in DG has been suggested to drive age-related cognitive decline (Yassa, Lacy, et al. 2011, Yassa, Mattfeld, et al. 2011, Doxey and Kirwan 2015). It has been shown that the discrimination ability is a critical feature of episodic memory (Norman and O’Reilly 2003, Norman 2010). Previous studies have found an association between age-related decrease in DG volume and memory decline in healthy older adults (Small et al. 2002, Toner et al. 2009, Stark and Stark 2017, Bennett, Stark, and Stark 2018).
1.3 Non-invasive brain stimulation

Despite the use of MRI to investigate the relationship between brain structures and functions, non-invasive brain stimulation emerged as a promising approach to elucidate the involvement of the stimulated cortex in the respective function (Jacobson, Koslowsky, and Lavidor 2012, Tremblay et al. 2016, Parkin, Ekhtiari, and Walsh 2015, Polania, Nitsche, and Ruff 2018). To do that, stimulation of a specific cortical region may modulate performance in the task under study. As such, the function of this region can be revealed depending on the cognitive domains recruited to perform the task. This has led to major advances in understanding cognition. Non-invasive brain stimulation (NIBS) includes magnetic and electric stimulation. Transcranial magnetic stimulation (TMS) delivers a magnetic pulse that stimulates nerve cells in the underlying brain region. TMS has been associated with several cognitive domains, for instance the perception of movement (Tadin et al. 2011, Wokke, Scholte, and Lamme 2014), language functions (Sliwinska, James, and Devlin 2015, Papeo et al. 2015) and production of actions (Duque, Olivier, and Rushworth 2013, Buch et al. 2010).

Transcranial direct current stimulation (tDCS) consists of the application of two saline-soaked sponge electrodes divided into anode and cathode. A weak non-invasive constant current (1-2 mA) is generated for several minutes leading to either facilitation (anodal tDCS) or inhibition (cathodal tDCS) of neuronal activity within the stimulated brain region (for review (Antonenko and Floel 2016)). tDCS has been used...
to elucidate the function of the stimulated area and to enhance cognitive functions. tDCS has been implemented to modulate several cognitive functions and underlying neuronal activity and connectivity (Parkin, Ekhtiar, and Walsh 2015, Yavari et al. 2017, Meinzer et al. 2012, Lavido 2016, Strobach and Antenenko 2017), for instance memory (Antenenko et al. 2018), language (Meinzer, Lindenberg, et al. 2014) and gesture comprehension (Cohen-Maximov et al. 2015). The effect of tDCS on cognitive performance has been investigated from different perspectives. Some studies have explored the anatomical and functional specificity of tDCS; where the anatomical specificity relies on targeting specific brain regions by guiding the electrical current and the functional specificity is based on the selectivity of tDCS for neuronal networks (Bikson, Name, and Rahman 2013). Other studies have investigated the functional connectivity changes that result from tDCS and their effect on cognitive functions (Meinzer et al. 2013).

1.3.1 The effect of tDCS on cognitive functions

Recent studies have investigated the involvement of tDCS in modulating cognitive functions. More specifically, atDCS has been applied to enhance memory performance in young adults (Zaehle et al. 2011, Martin et al. 2013, Ruf, Fallgatter, and Plewnia 2017, Habich et al. 2017). For instance, Ruf et al. (2017) applied anodal (atDCS) or sham tDCS (stDCS) (1 mA) to the right or left dorsolateral prefrontal cortex on healthy young participants during three working memory training sessions. Results showed that learning curve increased under atDCS compared to stDCS and that these effects lasted for up to nine months. In the aging brain, tDCS has been an efficient tool to enhance memory performance (Sandrini et al. 2014, Sandrini et al. 2016). In a recent study, researchers applied atDCS or stDCS over the left lateral prefrontal cortex of healthy older adults while learning lists of words. Memory recall was examined 48 hours and one month later. Results showed an enhanced delayed recall (after 48 hours) under atDCS compared to stDCS (Sandrini et al. 2016). Taken together, these findings indicate that atDCS in the learning phase of a memory task has an efficient long lasting role in enhancing recall performance in both young and older adults.

Similarly, studies have used tDCS to unveil the role of M1 in language comprehension, especially for action-related words (Branscheidt et al. 2018, Meinzer et al. 2016). These results suggested an
association between motor cortex activity and language processing. A more recent study found that for healthy individuals, anodal tDCS (atDCS) over the motor cortex improved semantic word retrieval performance (Martin et al. 2017). In sum, previous studies provided evidence for the involvement of the sensorimotor cortex in language processing.

1.4 Applications of tDCS and fMRI

The relationship between brain structure and function has been traditionally simplified, where distinct brain regions possess different specialized functions. Novel insights into brain function have been developed; in which complex behavioral tasks rely on interactions between brain areas. These insights were derived from studies that used brain imaging modalities with NIBS. Combination of MRI with tDCS allows for more sophisticated studies of functional connectivity networks and their associations with cognitive functions (Dayan et al. 2013). In the aging brain, tDCS has been used to investigate the causal relationship between functional activity of brain region and memory formation (Antonenko et al. 2018, Martin et al. 2017). For instance, Antonenko et al. (2018) conducted fMRI on young and older adults. Following fMRI, atDCS or stDCS was applied during an object-location-memory task on three consecutive days. Recall performance was examined immediately after training, a day and 1 month later. Results showed that atDCS enhanced recall performance after training, and that this was associated with an increase in default mode network strength. Besides the variability in functional brain networks, inter-individual differences in tDCS-induced effect might as well be explained by differences in individual baseline functional connectivity.

Studies have used fMRI to show that regions of the MTL are involved in both the formation and the retrieval of episodic memory (Zeineh et al. 2003, Maass et al. 2014). Several regions in the brain exhibited higher functional activity associated with a specific memory feature. For instance, prefrontal cortex has been related to strategic encoding and retrieval as well as to working memory ((Badre and Wagner 2007), for review (Blumenfeld and Ranganath 2007)), the hippocampus has been associated with recognition memory (for review, (Eichenbaum, Yonelinas, and Ranganath 2007)), posterior parietal cortex has been associated with retrieving details of memory features (Bonnici et al. 2016, Kuhl and Chun 2014), and default mode network has been involved in working memory (Vatansever et al. 2017,
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Piccoli et al. 2015). The architecture of brain regions and their interconnected aspect widened the fMRI usage by not only measuring the metabolic activity in one specific region, but also the functional connectivity between different regions. This unveiled the relationship between brain structure and function. For instance, a recent study conducted resting state functional MRI in either a stressful or a neutral context on healthy participants after performing an encoding task. Results showed that amygdala-hippocampus functional connectivity was related to enhanced memory performance in the stressful context (de Voogd et al. 2017). As such, memory formation and retrieval reside in both the structural brain metrics and their underlying functions. In addition, fMRI analysis on distinct brain regions helps unveiling the relationship between these regions and their functional network coupling effect.

1.5 Aims


Although previous studies showed that fornix white matter fiber pathway degradation and DG atrophy have been associated with age-related memory decline, the specific association between fornix microstructure and DG volume affecting memory performance is still scarce. Previous studies suggested that DG and fornix possess a unique connection and that DG itself has features which discern it from neighboring subfields (Gondard et al. 2015, Hao et al. 2015). As such, DG is vulnerable to impairments of its neighboring synaptic contacts, mainly the fornix. In our study, we aimed to investigate the relationship between fornix integrity, DG volume and episodic memory performance in the aging brain.


Previous studies have discussed the neural and behavioral effects of tDCS in the aging brain; some have found an age-dependent beneficial effect of tDCS (Fiori et al. 2017, Martin et al. 2017), others did not find any positive effects of tDCS in older adults (Kulzow et al. 2017, Leach et al. 2018). This interindividual variability in tDCS responsiveness still present heterogeneous explanations and the understanding of tDCS effects on the aging brain is still unclear. In addition, episodic memory is one of
the cognitive domains the most vulnerable to aging (Reuter-Lorenz and Park 2010). In our study, we aimed to explore the effect of tDCS on episodic memory performance, and whether individual memory network coupling interacts with baseline memory performance and with tDCS enhancement effect.


After understanding the neural correlates of cognitive functions, we were interested in the effect of tDCS over the sensorimotor cortex (M1) on cognitive performance in young adults. tDCS has been applied over the inferior frontal gyrus (IFG) to examine the role of this region in processing gestural-verbal stimuli (Cohen-Maximov et al. 2015, Schulke and Straube 2017, 2018). Cohen-Maximov et al. (2015) implemented gesture prime clips for word targets. Subjects were instructed to make a semantic decision whether the gesture prime describes the word target or not. They showed that subjects responded faster under atDCS of the right IFG compared to sham tDCS (stDCS). The study suggested that inferior frontal atDCS may enhance gestural-verbal integration, which in turn enhances gesture comprehension. We believe that modulation of M1 may be either mediated by frontal functions or by an interaction between frontal and motor areas. However, no previous study, to our knowledge, used tDCS to investigate the role of left M1 for gestural-verbal integration. As such, given the role of left M1 in language processing, our study aimed to explore tDCS-induced effect of M1 on the gestural-verbal integration performance.

2 Methods

Manuscript 1: Thirty healthy older subjects between the age of 50 and 79 years were recruited in this study (14 f; mean/SD age: 62/6 years). They performed an episodic memory paradigm outside of the scanner that required learning and subsequent retrieval of picture-pseudoword associations (for detailed description of the paradigm, see manuscript 1). Main outcome measure for memory performance was percentage of correct responses, and the two response categories (hits and correct rejections) during the immediate retrieval block. To assess fornix integrity, 3T MRI was conducted prior to stimulation to acquire DTI and perform a deterministic tractography based on constrained spherical deconvolution. To
extract hippocampal subfields volumes, high-resolution T1-weighted images were acquired. A fully automated cortical and subcortical reconstructions and volumetric segmentations, including the hippocampus were performed (Figure 3A-B). Individual DG volumes were adjusted for intracranial volume. Partial correlation coefficients were computed to correlate brain structural variables and behavioral performance, corrected for age. Subsequently, we implemented a simple mediation analysis in order to test whether the association between a predictor (fornix FA) and an outcome (percentage of correct rejections) is mediated by a mediator (volume of left DG), with age added as a covariate (Baron and Kenny 1986).

**Manuscript 2:** Thirty-four healthy older adults participated in the study (16 f, mean/SD age: 63.1/7.7 years). We applied atDCS (1 mA, 20 min) over CP5 according to the 10–10 EEG system (left temporoparietal cortex) on older participants, while performing an episodic memory paradigm. Memory performance was assessed during immediate retrieval. Healthy older adults participated in three experimental sessions. Baseline session included fMRI and subsequent memory task. Second and third sessions included either atDCS or stDCS during the learning phase of the task. Percentage of correct responses and mean reaction time of each block were assessed. Main outcome measure for memory performance was percentage of correct responses during the immediate retrieval block. Functional connectivity analysis was performed to examine the association of hippocampal connectivity with baseline memory performance. Then, connectivity coefficients of hippocampal-temporoparietal coupling were extracted between the left hippocampus and the cluster derived from the functional analysis (angular gyrus). Linear mixed models were computed for dependent variables with the factor stimulation condition (anodal, sham). Models were adjusted for age and experimental session. Linear regression analysis was conducted for association between functional connectivity and individual responsiveness to tDCS (defined by performance in anodal minus performance in sham stimulation condition), adjusted for age, order of experimental sessions and sham stimulation performance.

**Manuscript 3:** Twenty-two healthy young adults participated in two experimental sessions where either atDCS (1 mA, 20 min) or stDCS was applied over C3 according to the 10–10 EEG system (left sensorimotor cortex) (Figure 5A). During the stimulation, subjects performed three different tasks in a
counterbalanced order: gestural task, attentional load task and simple reaction task. The main task was the gestural task where subjects had to make a semantic decision of the prime-target (gesture-word) congruency. The two control tasks (attentional load task and simple reaction time task) were included to exclude that the effect on the gestural-verbal task would be based on improved attentional and motor processes. Both percentage of correct responses and RT were analyzed separately as dependent variables for each task. In order to test for differences between atDCS and stDCS, repeated-measures ANOVAs were performed, separately for all dependent variables. For the gestural task, congruency was added as within-subject factor.

3 Results

**Manuscript 1:** Fornix FA and left DG volume did not correlate with percentage of hits (Fornix FA: partial r = -0.001, p = 0.995; left DG volume: partial r = -0.133, p = 0.492) but showed a significant positive correlation with percentage of correct rejections (Fornix FA: partial r = 0.403, p = 0.030; left DG volume: partial r = 0.501, p = 0.006). Single mediation analysis (model A) showed a significant indirect effect of fornix FA on percentage of correct rejections, mediated by left DG volume, corrected for age (β = 1.50, 95% CI: 0.03, 4.57, Table 1, Figure 3C-D). The mediation effect (path ab) constituted 48% of the total effect of fornix FA on percentage of correct rejections (path c). Reverse mediation analysis (model B) showed that fornix FA did not mediate the effect of DG volume on percentage of correct rejections, corrected for age (β = 0.0007, 95% CI: -0.0005, 0.0019, Table 1). These findings support the mediation effects of DG volume on the relationship between fornix FA and percentage of correct rejections.
Table 1. Mediation of fornix FA and percentage of correct rejections by left DG volume (n = 30).

<table>
<thead>
<tr>
<th>Effect</th>
<th>Coefficient ± SE (% Mediation)</th>
<th>t</th>
<th>p</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total effect c (fornix FA on % correct rejections)</td>
<td>3.13 ± 1.37</td>
<td>2.28</td>
<td>0.030*</td>
<td></td>
</tr>
<tr>
<td>a (fornix FA on left DG volume)</td>
<td>545.31 ± 189.80</td>
<td>2.87</td>
<td>0.008**</td>
<td></td>
</tr>
<tr>
<td>b (left DG volume on % correct rejections)</td>
<td>0.0028 ± 0.0013</td>
<td>2.10</td>
<td>0.045*</td>
<td></td>
</tr>
<tr>
<td>Mediation effect ab (fornix FA on % correct rejections via left DG volume)</td>
<td>1.50 ± 1.03 (48)</td>
<td>–</td>
<td>–</td>
<td>0.03, 4.57</td>
</tr>
<tr>
<td>Direct effect c' (fornix FA on % correct rejections)</td>
<td>1.63 ± 1.47</td>
<td>1.10</td>
<td>0.279</td>
<td></td>
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<tr>
<td><strong>Model B</strong></td>
<td></td>
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<tr>
<td>Total effect c (left DG volume on % correct rejections)</td>
<td>0.0035 ± 0.0012</td>
<td>3.0</td>
<td>0.006**</td>
<td></td>
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<tr>
<td>a (left DG volume on fornix FA)</td>
<td>0.0004 ± 0.0001</td>
<td>2.87</td>
<td>0.008**</td>
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<tr>
<td>b (Fornix FA on % correct rejections)</td>
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<td>0.279</td>
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<tr>
<td>Mediation effect ab (left DG volume on % correct rejections via Fornix FA)</td>
<td>0.0007 ± 0.0006</td>
<td>–</td>
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<td>-0.0005, 0.0019</td>
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<td>Direct effect c' (left DG volume on % correct rejections)</td>
<td>0.0028 ± 0.0013</td>
<td>2.10</td>
<td>0.045*</td>
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Abbreviations: FA, fractional anisotropy; DG, dentate gyrus; CI, confidence interval, * indicates significance with p<0.05 and ** indicates significance with p<0.01. Significant bias-corrected confidence intervals are shown in bold.
Figure 3: Overview of results from the simple mediation analysis, including fornix FA, memory performance and left DG volume (n = 30). (A) Figure showing hippocampal subfields segmentation performed using FreeSurfer v6.0 algorithm (http://surfer.nmr.mgh.harvard.edu/) introduced by (Iglesias et al. 2015). This segmentation resulted in 12 subfields; DG is shown in red and all remaining subfields are shown in colored outline. (B) Tractography using ROI waypoints (Leemans et al. 2009) (“SEED” ROI is shown in blue, “AND” ROI is shown in green and “NOT” ROIs are shown in red) for the fornix in the native space of one participant. (C) “c” indicates the total effect (direct and indirect) of fornix FA on % correct rejections. (D) “a” indicates the effect of fornix FA on left DG volume, “b” indicates the effect of left DG volume on % correct rejections, adjusted for fornix FA, i.e., direct effect of left DG volume on % correct rejections. “c’’ indicates the direct effect of fornix FA on % correct rejections.

Manuscript 2: Performance after anodal stimulation was on average 2.8% better compared to sham in the 1st retrieval block ($\beta = 2.8$, 95%-CI: [0.3, 5.4], $F(1,89) = 4.815$, $p = 0.031$; linear mixed model post-hoc, N = 34, 127 data points, Figure 4A). The effect of stimulation condition itself was not significant (main effect: $\beta = 0.4$, 95%CI: [-0.8, 1.6] $F(1,287) = 0.52$, $p = 0.473$; linear mixed model, N = 34, 320 data points), but the interaction of stimulation condition and learning block indicated steeper learning curves in the anodal compared to the sham stimulation condition ($\beta = 1.0$, 95%-CI: [0.2, 1.8], $F(1,281) = 6.16$, $p = 0.014$, Figure 4A). fMRI analysis for the dependent variable baseline memory performance revealed an association of task performance with left-hemisphere hippocampo-temporoparietal coupling (significant cluster in left angular gyrus, peak coordinates: $x = -40$, $y = -52$, $z = 18$, $T = 4.45$, $k = 232$ voxel, cluster-p-FDR = 0.040, cluster-p-unc < 0.001, corrected for age, Figure 4B-C). We then aimed to
investigate whether the individual left-hemisphere hippocampo-temporoparietal coupling at baseline was also associated with the magnitude of responsiveness to tDCS (“percentage of correct responses during immediate retrieval in atDCS” minus “percentage of correct responses during immediate retrieval in stDCS”). We found that individual responsiveness to tDCS was positively associated to functional connectivity (standardized $\beta = 0.31$, $p = 0.036$, overall model $R^2 = 0.58$, adjusted $R^2 = 0.52$).

Figure 4: (A) Percentage of correct responses in the five learning blocks (L1-L5) and the two retrieval blocks (R1, R2). Means and (one side of) two-sided 95%-CIs are shown. Adapted from (Antonenko et al. 2019) (B-C) Schematic of the functional connectivity between the left hippocampus and left temporoparietal cortex correlated with task performance at baseline (R, retrieval). The significant cluster that emerged from whole-brain seed-to-voxel analyses had peak coordinates of $x = -40$, $y = -52$, $z = 18$, $T = 4.45$. IHP: left hippocampus; IAG: left angular gyrus.

Manuscript 3: In the gestural task, a 2x2 ANOVA showed that there was no significant main effect of stimulation condition on RT ($F (1, 21) = 2.41$, $p = 0.135$). Interestingly, there was a significant interaction between stimulation condition and congruency, ($F (1, 21) = 5.21$, $p = 0.033$, partial eta squared = 0.20). Subsequent ANOVAs conducted per congruency, showed that, for congruent stimuli, there was no significant difference in RT between atDCS and stDCS ($F (1, 21) = 0.013$, $p = 0.911$). However, for incongruent stimuli, RT under atDCS was significantly faster compared to stDCS, ($F (1, 21) = 6.15$, $p=0.022$, partial eta squared = 0.23, Figure 5B). In both control tasks, there was no significant main
effect of stimulation condition on RT (for attentional load task: \((F(1, 21) = 1.33, p = 0.262)\); for simple reaction time task: \((F(1, 21) = 0.01, p = 0.926)\).

Figure 5: Figure showing tDCS montage and mean RT of the gestural task (A) Image of the two stimulation electrodes placed over the left sensorimotor cortex (C3, 5 x 7 cm²) and the right supraorbital area (10 x 10 cm²) (B) Gestural task for congruent and incongruent stimuli. Only for incongruent stimuli, RT was significantly faster under atDCS compared to stDCS. Plots were constructed using BoxPlotR ((Spitzer et al. 2014); http://shiny.chemgrid.org/boxplotr/). The white circles represent the individual data points, the red and blue lines represent the mean values across the group. Error bars represent 95% confidence interval. Violin plots show the distribution across subjects. *p < 0.05. Adapted from (Hayek, Floel, and Antonenko 2018).

4 Discussion

Manuscript 1: Results showed that tractography-based fractional anisotropy (FA) of individual fornix pathways and volumes of the left DG were positively related to memory retrieval. Brain-behavior associations were in particular observed for correct rejections rather than hits of memory performance. These results corroborate with previous studies (Ly et al. 2016, Metzler-Baddeley et al. 2011) and further extend the role of fornical fiber pathway integrity in enhancing the ability to detect incorrect associations in older adults. Similarly, the specific association of left DG and correct rejections unveil the function of DG in implementing retrieval strategies (for instance, novelty detection) to prevent memory distortions caused by increasing age (for review (Kesner, Lee, and Gilbert 2004)). As such, our finding assumed that participants may have implemented DG-related strategies to successfully detect new (i.e.,
incorrect) associations. Subsequent mediation analysis, corrected for age, showed that the effect of fornix FA on memory performance was mediated (48%) by the left DG volume. Our findings corroborate with two recent studies that supported the hypothesis of age-related fornix white matter damage leading to hippocampal gray matter decline, but not vice versa (Gazes et al. 2018, Metzler-Baddeley et al. 2019). Gazes et al. (2018) conducted DTI and T1-weighted imaging for both young and older adults who subsequently performed an episodic memory task. The authors examined whether the association of hippocampal volume with fornix microstructure differed between age groups, and how this relationship was linked to recall performance of a word list. The association between structural integrity of fornix and volume of hippocampus was only evident in older adults, suggesting that the two are interdependent once neuronal atrophy is present. Interestingly, they also suggested that, in the presence of age-related neuronal atrophy, fornix integrity is a more sensitive predictive factor for episodic memory than hippocampal volume. More recently, Metzler-Badeley et al. (2019) reconstructed the fornix using deterministic tractography and segmented hippocampal volumes using Freesurfer. The authors assessed the effect directionality of age-related differences in fornix and hippocampus. Using fornix and hippocampus metrics, they performed two mediation model analyses; in one model hippocampal variables mediate the effect of age on fornix microstructure metrics, while in the other model, fornix metrics mediate the effect of age on hippocampal variables. They found that only the first model was significant, indicating that fornix white matter damage may cause hippocampal gray matter deterioration with increasing age. The current study complements previous evidence by demonstrating that fornix microstructure and DG subfield volume interact to specifically mediate memory for false associations. Our mediation model offered a neurobehavioral model in which preserved fornix white matter microstructure in older age predicts successful memory formation through its protective effect on grey matter volume in the DG. Thus, demyelination, even in healthy aging, may induce grey matter loss reflecting altered intracellular metabolism or neural death in connected structures, and leading to constrained memory performance (Metzler-Baddeley et al. 2019, Bartzokis 2004).

**Manuscript 2:** Results showed better retrieval performance with augmented learning curves under atDCS compared to stDCS. Our results extend previous studies conducted on young adults and
showing that atDCS over left temporoparietal cortex enhance learning and memory performance (Meinzer, Jahnigen, et al. 2014, Floel et al. 2008). Here, same effects were found in older adults where subjects showed faster learning and enhanced retrieval when learning was performed under atDCS. Given that age-related memory impairment is a process that involves brain regions and their underlying function, we found that hippocampo-temporoparietal functional connectivity was positively associated with both baseline memory performance and the individual tDCS-induced enhancement effect. The interaction of hippocampo-temporoparietal functional connectivity and baseline performance may be explained by previous studies suggesting an inter-regional interaction within a neuronal network that further mediate hippocampus-dependent memory processes in the aging brain (Rugg and Vilberg 2013, Simons and Spiers 2003). Results showed better retrieval performance with augmented learning curves under atDCS compared to stDCS. Our results extend previous studies conducted on young adults and showing that atDCS over left temporoparietal cortex enhance learning and memory performance (Meinzer, Jahnigen, et al. 2014, Floel et al. 2008). Here, same effects were found in older adults where subjects showed faster learning and enhanced retrieval when learning was performed under atDCS. Moreover, our finding that functional connectivity was associated with tDCS-induced enhancement effect support the hypothesis that functional connectivity can be an efficient predictor of individual tDCS responsiveness. The current findings give insights on the tDCS-induced plasticity of episodic memory in healthy older adults. We suggest that the inter-individual variability in tDCS responsiveness might be due intrinsic network coupling.

**Manuscript 3:** Furthermore, the detecting of incorrect associations are not only restricted to DG strategies but also to the implementations of a cognitive control mechanism that has been previously involved in decreasing interference effect (Ouellet et al. 2015, Zmigrod, Zmigrod, and Hommel 2016). Results showed that anodal compared to sham tDCS reduced reaction times in the gestural-verbal integration task and that this decrease was specific for incongruent pairs of gestures and verbal expressions. tDCS had no effect on control tasks performance. These findings corroborate with previous studies that showed a tDCS-induced enhancement of both gestural-verbal integration and language comprehension, when tDCS is applied over frontal and parietal cortices (Cohen-Maximov et al. 2015,
Schulke and Straube 2017, Bianchi et al. 2015). Recent studies also investigated the role of motor cortex in language processing using tDCS (Branscheidt et al. 2018, Meinzer et al. 2016). Our results are specific in that atDCS decreased reaction times for incongruent gestural-verbal associations. This indicates the involvement of motor areas in the processing of gesture and language when the information conveyed by the gesture does not describe the information conveyed by language. These findings fit with previous studies suggesting that left IFG and its adjacent motor areas are activated during the processing of incongruent speech-gesture associations (Green et al. 2009, Kircher et al. 2009, Willems, Ozyurek, and Hagoort 2007). Cognitive control mechanism has been shown to be related to a cascade of distinct control types performed by different brain regions. Dorsolateral prefrontal cortex (DLPFC) was shown to implement its control through the top-down modulation of task-dependent information processing in M1, also referred to as sensory control (Koechlin, Ody, and Kouneiher 2003).

As such, we assumed that modulation of brain circuitry underlying sensory control in M1 area led to an increase in cognitive control mechanisms, and therefore improved the subjects’ ability to detect incongruent gestural-verbal associations.

5 Strengths and limitations

Strengths of our studies lie in the robust tractography method, since CSD-based tractography shows favorable outcome compared to the usual DTI-based approaches, especially in regions with complex crossing fibers (Tournier et al. 2008). In addition, our studies include the multimodal imaging approach of combined grey matter volumetric analysis and white matter fiber tractography to assess the impact of structural integrity that promotes successful cognitive function. Finally, combining brain stimulation and neuroimaging allowed for the investigation of differences in tDCS response at the functional network level, unveiling the neural changes induced by tDCS (Bergmann et al. 2016).

Several technical limitations should be taken into consideration. First, it was shown that potential partial volume effects may affect DTI-based indices (Alexander et al. 2001, Szczepankiewicz et al. 2013) and hippocampal subfields segmentation (Duche et al. 2017). However, we set the FA threshold to 0.2, as a mean to eliminate all the possible underestimated FA values. Second, conventional dual-electrode tDCS set-up stimulates networks rather than brain regions (Nasseri, Nitsche, and Ekhtiari 2015, Polania,
Nitsche, and Ruff 2018). Although other brain regions might as well be stimulated, it is conceivable that the interaction of brain stimulation with a task induces more focused effects (Holland et al. 2011, Meinzer et al. 2012). To better infer specificity of tDCS, a control tDCS site would have to be included. Nevertheless, specificity of left M1 atDCS for the gestural task was demonstrated by the absence of effects on the control tasks.

6 Summary and future directions

Age-related cognitive impairments have been linked to both structural and functional neural determinants. Recent advances in neuroimaging and brain stimulation techniques have given novel perspectives of the brain-behavior relationship. In this work, we examined the impact of brain structural and functional factors on cognitive performance in healthy adults, and applied tDCS to modulate performance.

First, we showed that fornix FA and DG volume were independently associated with memory performance. Interestingly, the effect of fornix FA on memory retrieval was mediated by DG volume in the healthy aging brain. This interdependent association was observed specifically for successful retrieval of incorrect associations. Our results supported the relationship between fornix integrity and DG volume in the course of healthy aging, and highlight the dependency of fornix integrity on DG volume in affecting episodic memory performance. Preserved fornix microstructure may thus positively impact on memory for false associations through a protective effect on DG subfield volume. More generally, the results lend further support to the hypothesis that structural disconnection plays a crucial role in mediating deficits in the course of aging. Our study provides a neurobehavioral model for the linkage between structural memory network properties to explain inter-individual variability behavioral outcomes in older adults.

Second, we applied tDCS over brain areas implicated in memory formation and semantic processing, to further understand neural mechanisms underlying cognitive performance. We showed that hippocampo-temporoparietal functional connectivity was associated with initial memory performance and with tDCS-induced enhancement effect, indicating that hippocampo-temporoparietal functional connectivity predicts memory performance and may explain inter-individual differences in tDCS responsiveness.
Taken together, these findings support the hypothesis that hippocampus-dependent memory processes are mediated by the inter-regional interaction within a distributed network in older adults (Rugg and Vilberg 2013, Simons and Spiers 2003), and can be modulated through lateral frontal and parietal brain stimulation (Medvedeva et al. 2019, Sandrini et al. 2014).

Third, we found that excitatory tDCS over left M1 improves semantic processing by enhancing performance of incongruent stimuli, possibly mediated by facilitation of action perception sensitivity of the MNS. In addition, up-regulation of M1 might have increased its cognitive control potential, leading to lower interference. Our findings suggested that atDCS over left M1 might have affected cognitive control mechanisms, leading to better performance in the detection of incongruent associations. This supports previous findings suggesting that left IFG and its adjacent motor areas are activated during the preprocessing of speech-gesture associations, when the information conveyed by speech is incongruent with the information conveyed by the gesture (Green et al. 2009, Kircher et al. 2009, Willems, Ozyurek, and Hagoort 2007). This effect might be explained by the implementation of a sensory cognitive control mechanism through the top-down modulation of information processing in M1 (Koechlin, Ody, and Kouneiher 2003).

Our studies pave the road for novel treatment strategies, especially in the aging brain. First, we provide novel insights into the neurophysiological mechanism underpinning age-related deficits of associative episodic memory. Understanding this relationship is an important prerequisite for the development of interventions to counteract cognitive decline such as aerobic exercise, cognitive training or dietary interventions (Antonenko et al. 2016, Brickman et al. 2014, Kobe et al. 2016). Due to the cross-sectional design of our study that still limits conclusions about causality, future longitudinal studies are needed to support this hypothesis. Moreover, future studies with larger sample sizes should examine volumes and their link to age-related memory decline using high resolutions 7-Tesla MRI scans to allow for a precise separation between DG and its neighboring structures, like CA3 and CA4 (Boretius et al. 2009).

Second, by unveiling the hippocampo-temporoparietal connectivity and its association with memory performance, we would then allow to modulate hippocampus-dependent processes by targeting connected cortical sites (Nilakantan et al. 2017, Tambini, Nee, and D'Esposito 2018). In our study, we
investigated neural factors predicting cognitive functions. As such, future studies using task-related fMRI may be relevant to better understand neural factors of the task-related cognitive demands.

Third, our results of tDCS effect over left M1 were revealed on cognitive performance of young adults. Previous studies showed that tDCS over M1 also affect cognitive functions in older adults (Meinzer et al. 2016, Martin et al. 2017). As such, future studies are needed to extrapolate our results to older adults. In the clinical context, tDCS effects on speech and gesture processing may be relevant for patients with schizophrenia who suffer from severe deficits in speech and gesture processing (Schulke and Straube 2018).
7 References


https://www.nature.com/articles/nn.3850#supplementary-information.


Appendix A: Publications
(Peer-reviewed journal articles)

Manuscript 1


Manuscript 2


Manuscript 3

Manuscript 1

Dentate gyrus volume mediates the effect of fornix microstructure on memory formation in older adults

Dayana Hayek, Friederike Thams, Agnes Flöel, Daria Antonenko

(Submitted)

Authors contributions:
D.H, D.A. and A.F. designed research. D.H., F.T., and D.A. analyzed the data. D.H. performed tractography and volumetric analysis D.H. prepared all figures. D.H. and D.A. wrote the manuscript. All authors reviewed and revised the manuscript.
Dentate gyrus volume mediates the effect of fornix microstructure on memory formation in older adults

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Competing interests: The authors declare no competing interests.

Keywords: Aging, brain plasticity, white matter integrity, hippocampal subfields, tractography
Abstract

Age-related deterioration in white and grey matter is linked to cognitive deficits. Reduced microstructure of the fornix, the major efferent pathway of the hippocampus, and volume of the dentate gyrus (DG), may cause age-associated memory decline. However, the linkage between these anatomical determinants and memory formation in healthy aging are poorly understood. In 30 older adults, we acquired diffusion tensor and T1-weighted images for individual deterministic tractography and volume estimation. A memory task, administered outside of the scanner to assess memory formation, required discrimination of previously acquired picture-word pairs. The results showed that fornix fractional anisotropy (FA) and left DG volumes were related to successful retrieval. These brain-behavior associations were observed for correct rejections, but not hits, indicating specificity of memory network functioning for detecting false associations. Mediation analyses showed that DG volume mediated the effect of fornix FA on memory (48%), but not vice versa. These findings suggest that reduced microstructure induces volume loss and thus negatively affects memory formation, complementing evidence of a pivotal role of the fornix in healthy aging. Our study offers a neurobehavioral model to explain variability in memory formation in older adults, an important prerequisite for the development of interventions to counteract cognitive decline.
Introduction

Age-related deterioration of white and grey matter in the human brain contributes to cognitive impairment in the course of aging (Grady 2012, Marstaller, Williams et al. 2015). Microstructure in white matter tracts as assessed by diffusion tensor imaging (DTI) and grey matter volume estimated from high-resolution structural magnetic resonance images predict behavioral task performance in healthy older adults (Antonenko, Meinzer et al. 2012, Sasson, Doniger et al. 2013). Episodic memory, as one of the most vulnerable cognitive domains in aging (Nyberg, Lövdén et al. 2012), is mediated by hippocampal networks, including the hippocampus itself and the fornix as its major efferent white matter pathway (Metzler-Baddeley, Jones et al. 2011, Lovblad, Schaller et al. 2014, Antonenko, Kulzow et al. 2016, Gorbach, Pudas et al. 2017, Anblagan, Valdes Hernandez et al. 2018).

Reduced fornix white matter microstructure has been observed in the course of healthy, but also pathological aging processes (Metzler-Baddeley, Hunt et al. 2012, Pelletier, Periot et al. 2013, Zhuang, Sachdev et al. 2013). Deteriorated fornix pathways were suggested to be one of the earliest abnormalities in older individuals with mild cognitive impairment (MCI) who progress to Alzheimer’s dementia (Douaud, Menke et al. 2013, Kantarci 2014). Inter-individual variability in fornix macro- and microstructure has been associated with older adults’ variability in performance on various episodic memory tasks that require verbal or visual recall or recognition of items and associations (Metzler-Baddeley, Jones et al. 2011, Lockhart, Mayda et al. 2012, Douet and Chang 2014, Henson, Campbell et al. 2016, Gazes, Li et al. 2018). Likewise, the hippocampus is susceptible to healthy and pathological aging, involved in altered episodic memory function (Frisoni, Ganzola et al. 2008, Walhovd, Westlye et al. 2011, den Heijer, der Lijn et al. 2012). Whether or not volumetric atrophy of the whole hippocampus contributes to age-related memory decline is unclear (Head, Snyder et al. 2005, Fjell, Walhovd et al. 2010, Pereira, Valls-Pedret et al. 2014), probably due to its composition in separate anatomic subregions being differentially vulnerable to aging (Small, Tsai et al. 2002, Small, Schobel et al. 2011). Within the hippocampal formation, advanced age has been associated mainly with reduced volumes in its subfields cornu ammonis 1-4 and dentate gyrus (DG) (Mueller and
Weiner 2009, Pereira, Valls-Pedret et al. 2014). The DG was observed to have a stronger age-associated cerebral blood volume decrease compared to other hippocampal subfields (Brickman, Khan et al. 2014). Due to its particular vulnerability to age-related processes (Small, Chawla et al. 2004, Wilson, Gallagher et al. 2006) and activity during memory discrimination tasks, changes in DG has been suggested to drive age-related cognitive decline (Yassa, Lacy et al. 2011, Yassa, Mattfeld et al. 2011, Doxey and Kirwan 2015).

Of particular importance in the context of healthy aging and AD is the question of mutual dependency of grey and white matter damage, i.e., their directional relationship and interaction to predict cognitive decline (cf. Bartzokis 2011, Dansokho and Heneka 2018, Metzler-Baddeley, Mole et al. 2019). Within structural hippocampal networks, correlational studies have found positive associations between hippocampal atrophy and loss of fornix connections in older adults, with findings pointing towards a pivotal role of the latter in older age (Fletcher, Raman et al. 2013, Pelletier, Periot et al. 2013, Zhuang, Sachdev et al. 2013, Gazes, Li et al. 2018). Using mediation analyses, a recent study applied individual white matter fiber tractography and hippocampal segmentation to investigate the linkage between increased age, reduced fornix microstructure and hippocampal atrophy in a directional approach (Metzler-Baddeley, Mole et al. 2019). The results revealed that white matter changes predicted grey matter deterioration, but not vice versa, concordant with the idea of age-related myelin damage causing abnormal intracellular metabolism and neuronal death (Bartzokis 2004, Metzler-Baddeley, Mole et al. 2019). Moreover, animal studies have observed a unique connection between the fornix and the DG subfield (Gondard, Chau et al. 2015, Hao, Tang et al. 2015). The authors showed that deep brain stimulation of the fornix activated the DG by modulating the expression of neurotrophic factors and markers of synaptic plasticity known to be crucial for memory processing. Taken together, these studies indicate that disconnection in forniceal white matter pathways might induce atrophy in the hippocampus, and more specifically in its DG subfield, which in turn may affect age-related decline in memory function.
The interactive effect of both structures on the ability to form novel memories in older adults has not been elucidated yet.

In the present study, we aimed to investigate this linkage between structural hippocampal networks and episodic memory formation in healthy older adults. We administered a task that required learning of new picture-word associations and subsequent discrimination of correct and incorrect pairings during retrieval in order to assess hippocampus-dependent memory performance (Antonenko, Faxel et al. 2016, Antonenko, Hayek et al. 2019). Individual fornical pathways were reconstructed on diffusion-weighted images using deterministic tractography based on the constrained spherical deconvolution (CSD) technique. Individual volumes of the DG were estimated on T1 images using automated subcortical segmentation (Iglesias, Augustinack et al. 2015). We aimed to explore correlational relationships between memory retrieval performance in different response categories reflecting the detection of correct and incorrect associations, fractional anisotropy in the fornix and volume in the left DG. Subsequent mediation models were conducted to evaluate the linkage between structural properties and memory performance.

Materials and methods

Participants and study design

Thirty healthy older subjects between the age of 50 and 79 years were recruited in this study (14 f; mean/SD age: 62/6 years). They were all right-handed, German native speakers, with no history of neurological diseases. Neuropsychological testing was performed for all participants to assure normal cognitive functioning within age- and education-related norms (CERAD-Plus, https://www.memoryclinic.ch/de/) (Table 1). The study was approved by the ethics committee of the Charité University Medicine and conducted in accordance with the Helsinki Declaration. Written informed consent was obtained from all participants prior to participation.
Table 1. Characteristics of participants.

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\(^a\) LQ, laterality quotient (Oldfield 1971). \(^b\) GDS, Geriatric Depression Scale (Yesavage, Brink et al. 1982). \(^c\) (Lehrl 2005)

3 Episodic memory task

The task was adapted from previous studies (Breitenstein and Knecht 2002, Breitenstein, Jansen et al. 2005, Floel, Rosser et al. 2008, Antonenko, Faxel et al. 2016), and presented using Presentation software (Neurobehavioral Systems, http://www.neurobs.com/, version 18.1). The paradigm consists of the presentation of a combination of pseudowords and pictures of daily life. For each subject, a group of 30 pseudowords and 30 pictures of daily life were randomly combined to 30 correct pairings resulting in different correct and incorrect associations. The paradigm consisted of two phases: a learning and a retrieval phase. The learning phase was divided into 5 blocks with 120 trials each (600 trials in total). In each block, one of the 30 pictures was presented with two different “incorrect”
pairings (10 different “incorrect” pairings in total). Incorrect pairings occurred only once and correct pairings occurred 10 times (twice in each block). Participants were blinded for the pairings presentation frequency and were instructed to answer as quickly as possible if the pairing was correct or not. As such, the learning basis of this phase lies in higher co-occurrences of “correct” pairings compared to “incorrect” pairings. The order of the trials was randomized. Half of the subjects had response buttons reversed. In each trial, the picture was presented for 200 ms after the onset of an auditory spoken pseudoword (normalized at the same loudness and length of 600 ms). Response window was set to 1500 ms. During the retrieval phase, learning success was assessed in a “transfer” block. Pictures were replaced with corresponding spoken German words that were presented together with the pseudowords, using the same stimuli number, trial timings, and frequency. Duration of the task was 35 min. The task generated four response categories: hits (i.e., correct classifications of newly acquired associations), correct rejections (i.e., correct detection of incorrect associations), false alarms, and misses. Percentage of total correct responses and percentage of correct responses in different response categories were examined in the retrieval phase.

MRI acquisition

MRI was performed using 3T Siemens Trio MR-System using 12-channel head coil at the Berlin Center for Advanced Neuroimaging. First, a 3D structural high-resolution T1-weighted magnetization prepared rapid gradient echo image was acquired with the subsequent characteristics; TR=1900 ms, TE=2.52 ms, 192 sagittal slices, voxel size = 1.0 x 1.0 x 1.0 mm³, flip angle = 9°. Second, a diffusion-weighted spin-echo echo-planar imaging image was acquired with the subsequent characteristics; TR = 7500 ms, TE = 86 ms, 61 axial slices, voxel size = 2.3 x 2.3 x 2.3 mm³; 64 directions, b-value of 1000 s/mm², 1 b0.

MRI data analysis

Tractography and tract variables
Tractography was performed using ExploreDTI (Leemans, Jeurissen et al. 2009). Diffusion MRI images were corrected for eddy currents and distortions caused by head motion. Metzler-Baddeley (2011) previously described that diffusion tensor model tractography (Basser, Pajevic et al. 2000) was not found to be the most appropriate technique to reconstruct the fornix because it is positioned next to other white matter tracts. Thus, we used deterministic tracking based on constrained spherical deconvolution which was shown to resolve crossing fibers problem (Tournier, Calamante et al. 2004, Tournier, Calamante et al. 2007, Tournier, Yeh et al. 2008). Deterministic tracking algorithm was applied as following: The diffusion orientation was estimated at each seed point and then moved further in 0.5 mm steps along the direction. Next, the fiber orientation was again estimated at the subsequent seed point and propagated in another 0.5 mm step along the direction that traversed the smallest seed point and propagated in another 0.5 mm step along the direction that traversed the angle >60° or a drop of FA value below 0.2 occurred (Carballedo, Amico et al. 2012, Antonenko, Kulzow et al. 2016).

Whole brain tractography was performed using every voxel as seed point. In order to extract three-dimensional reconstructions of the fornix, multiple way-point regions of interest (ROIs) masks was implemented. This technique uses Boolean logical operations to delineate ROIs specific masks, for instance, one can choose to reconstruct a tract that passes through ROI-1 but NOT ROI-2. All ROIs were manually drawn in native space using color-coded fiber orientation maps for individual subjects while blinded to age, neuropsychological data and memory performance (for individual tract, see Supplementary Figure S1). The main outcome measure was mean fractional anisotropy (FA).

Landmark techniques that were used to draw tract specific ROIs were defined according to previously published methods (Catani, Howard et al. 2002, Metzler-Baddeley, Jones et al. 2011, Carballedo, Amico et al. 2012), see Figure 1.

In order to delineate the individual tracts, first, medial level of a coronal section where the anterior pillars enter into the body of the fornix was located. “SEED” point ROI was drawn around the body of the fornix bundle. Second, at the inferior part of the splenium of the corpus callosum, “AND” ROI
around the crus fornici of each hemisphere was drawn. Finally, to eliminate unwanted tracts, three “NOT” ROIs were drawn; one is rostral to the fornix pillars; one is caudal to the crus fornici, and a third one on an axial slice through the upper pons and the corpus callosum. Every individual tract was visually inspected and inadequate outlier tracts were removed using additional “NOT” ROIs.

**Dentate gyrus volume**

Individual volumes of the DG were segmented using FreeSurfer (version 6.0) (http://surfer.nmr.mgh.harvard.edu/) algorithm, introduced by Iglesias et al. (2015). Fully automated cortical and subcortical reconstructions and volumetric segmentations, including the hippocampus were performed (for individual volumetric segmentation of all hippocampal subfields, see Supplementary Figure S2). T1-weighted images were preprocessed for motion correction, intensity normalization, skull stripping automated topology correction using a watershed algorithm (Fischl, Salat et al. 2002). Individual DG volumes were adjusted for intracranial volume (ICV) (den Heijer, der Lijn et al. 2012, Kerti, Witte et al. 2013, Kobe, Witte et al. 2017) with the following formula:

\[
\text{Adjusted Volume} = \text{raw volume} - b \times (\text{ICV} - \text{mean ICV}).
\]

The coefficient \(b\) indicates the regression slope of the region to be adjusted on the ICV. Individual hippocampal subfields were superimposed on anatomical images and segmentation quality was visually inspected.

**Statistical analysis**

We used SPSS 25.0 (http://www-01.ibm.com/software/uk/analytics/spss/) to perform all statistical analyses. Partial correlation coefficients were computed for correlation analyses of brain structural variables and behavioral performance, corrected for age. We implemented a simple mediation analysis using PROCESS (Hayes and Preacher 2014, Zamroziewicz, Paul et al. 2017, Zhang, Beyer et al. 2018) in order to test whether the relation between a predictor (fornix FA) and an outcome (percentage of correct rejections) is mediated - in total or in part - by a mediator variable (volume of left DG) (Baron and Kenny 1986). We assessed the indirect effect of the independent variable (IV)
and the dependent variable (DV) through a mediator variable (MV) with age added as covariate. To
test this hypothesis, a bootstrapping resampling strategy was implemented while taking 5000
bootstrap samples. Here, path $a$ describes the direct effect of the IV (fornix FA) on the MV (left DG
volume), path $b$ represents the direct effect of the MV (left DG volume) on the DV (percentage of
correct rejections), and path $c$ indicates the total effect of IV and MV on the DV. Finally, path $c'$
reveals the direct effect of the IV (minus MV) on the DV. Bias-corrected 95% confidence interval (CI)
was computed to evaluate the contribution of the MV (indirect effect, path $a \times b$). CI reached
significance when the interval range did not include zero.

Results

Memory and fornix white matter microstructure

Fornix FA showed a positive correlation with percentage of total correct responses (partial
correlation coefficient: $r$ (corrected for age) = 0.39, $p = 0.035$). In order to examine whether this
relationship was specific to a response category, we further examined separate correlations between
Fornix FA and percentage of hits and correct rejections: Fornix FA did not correlate with percentage
of hits ($partial$ $r = -0.001, p = 0.995$) but showed a positive correlation with percentage of correct
rejections (partial $r = 0.403, p = 0.030$, Figure 1).

Memory and DG volume

DG volume showed no strong correlation with percentage of total correct responses (partial
correlation coefficient: $r$ (corrected for age) = 0.291, $p = 0.126$). In order to explore this relationship
in different response categories, we further examined separate correlations between DG volume and
percentage of hits and correct rejections: DG volume did not correlate with percentage of hits
(partial $r = -0.133, p = 0.492$) but showed a positive correlation with percentage of correct rejections
(partial $r = 0.501, p = 0.006$, Figure 1).
Figure 1: Tractography analysis, hippocampal subfields representation, and their correlation with memory performance. The left panel shows a sagittal and a coronal section of the fornix, and the tractography using ROI waypoints (Leemans, Jeurissen et al. 2009) (“SEED” ROI is shown in blue, “AND” ROI is shown in green and “NOT” ROIs are shown in red) for the fornix in the native space of one participant. The scatter plot shows a positive correlation of fornix FA and percentage of correct rejections, corrected for age (partial $r = 0.403$, $p = 0.030$). The right panel shows hippocampal subfields segmentation performed using FreeSurfer v6.0 algorithm. This segmentation resulted in 12 subfields; DG is shown in red and all remaining subfields are shown in colored outline. The scatter plot shows a positive correlation of left DG volume (in mm$^3$) and % correct rejections, adjusted for age (partial $r = 0.501$, $p = 0.006$).

Mediation model analysis

Our model including fornix FA, memory performance and left DG volume met the criteria for mediation, where path a, b and c showed significant associations ($p<0.05$) and path c’ did not show significant associations (Table 2). Single mediation analysis (model A) showed an indirect effect of fornix FA on percentage of correct rejections, mediated by left DG volume, corrected for age ($\beta = 1.50$, 95% CI: 0.03, 4.57, Table 2, Figure 2). The mediation effect (path ab) constituted 48% of the total effect of fornix FA on percentage of correct rejections (path c). Reverse mediation analysis
(model B) showed that fornix FA did not mediate the effect of DG volume on percentage of correct rejections, corrected for age ($\beta = 0.0007, \text{95\% CI: -0.0005, 0.0019, Table 2}$). These findings support the mediation effects of DG volume on the relationship between fornix FA and percentage of correct rejections.

![Mediation Model](image)

**Figure 2:** Overview of results from the simple mediation analysis, including fornix FA, memory performance and left DG volume ($n = 30$). (A) “c” indicates the total effect (direct and indirect) of fornix FA on % correct rejections. (B) “a” indicates the effect of fornix FA on left DG volume, “b” indicates the effect of left DG volume on % correct rejections, adjusted for fornix FA, i.e., direct effect of left DG volume on % correct rejections. “c’” indicates the direct effect of fornix FA on % correct rejections.

**Table 2.** Mediation of fornix FA and percentage of correct rejections by left DG volume ($n = 30$).

<table>
<thead>
<tr>
<th>Effect</th>
<th>Coefficient ± SE (% Mediation)</th>
<th>t</th>
<th>p</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total effect c (fornix FA on % correct rejections)</td>
<td>3.13 ± 1.37</td>
<td>2.28</td>
<td>0.030*</td>
<td></td>
</tr>
<tr>
<td>a (fornix FA on left DG volume)</td>
<td>545.31 ± 189.80</td>
<td>2.87</td>
<td>0.008**</td>
<td></td>
</tr>
<tr>
<td>b (left DG volume on % correct rejections)</td>
<td>0.0028 ± 0.0013</td>
<td>2.10</td>
<td>0.045*</td>
<td></td>
</tr>
<tr>
<td>Mediation effect ab (fornix FA on % correct rejections via left DG volume)</td>
<td>1.50 ± 1.03 (48)</td>
<td>---</td>
<td>---</td>
<td>0.03, 4.57</td>
</tr>
<tr>
<td>Direct effect c’ (fornix FA on % correct rejections)</td>
<td>1.63 ± 1.47</td>
<td>1.10</td>
<td>0.279</td>
<td></td>
</tr>
<tr>
<td><strong>Model B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total effect c (fornix FA on % correct rejections)</td>
<td>0.0035 ± 0.0012</td>
<td>3.0</td>
<td>0.006**</td>
<td></td>
</tr>
<tr>
<td>a (fornix FA on left DG volume)</td>
<td>0.0004 ± 0.0001</td>
<td>2.87</td>
<td>0.008**</td>
<td></td>
</tr>
</tbody>
</table>
b (left DG volume on % correct rejections) & 1.63 ± 1.47 & 1.10 & 0.279 \\
Mediation effect ab (fornix FA on % correct rejections via left DG volume) & 0.0007 ± 0.0006 & — & — & -0.0005, 0.0019 \\
Direct effect c' (fornix FA on % correct rejections) & 0.0028 ± 0.0013 & 2.10 & 0.045* \\

Abbreviations: FA, fractional anisotropy; DG, dentate gyrus; CI, confidence interval, * indicates significance with p<0.05 and ** indicates significance with p<0.01. Significant bias-corrected confidence intervals are shown in bold.

Discussion

The current study investigated the effect of fornix white matter microstructure and DG volume on episodic memory formation in healthy older adults. Fornix FA and DG volume were correlated with correct rejections (i.e., successful retrieval of incorrect associations) during retrieval of previously acquired picture-word pairs. Mediation analysis further showed that the prediction of memory performance by increased fornix microstructure was mediated by higher volume in the left DG, but not vice versa. This finding indicates that reduced fornix microstructure impairs successful memory formation through its impact on DG subfield of the hippocampus.

Association of fornix white matter microstructure and memory performance

Our finding of a positive association between fornix FA and memory performance is in line with previous studies in both young and older adults (Rudebeck, Scholz et al. 2009, Douet and Chang 2014, Ly, Adluru et al. 2016). Ly and colleagues found that variability in fornix microstructure in middle-to-late aged adults was related to face recognition memory and partly explained preserved functional connectivity within hippocampal networks (Ly, Adluru et al. 2016). A study by Metzler-Baddeley and colleagues further suggested that specifically age-related degradation of fornix microstructure as derived from individual fiber tracking was linked to memory recall performance in strategic and visual memory tasks in older adults (Metzler-Baddeley, Jones et al. 2011). Our data further extend the role of the fornix in older adults to verbal episodic memory, in particular to associative memory formation. Moreover, we found a positive link between fornix FA and percentage of correct rejections in older adults, indicating that preservation of fornix fiber pathway integrity with increasing age may be crucial for the ability to detect false associations.
Association of dentate gyrus volume and memory performance

In line with previous studies, age-related preservation in left DG volumes was also associated with superior memory performance (Small, Tsai et al. 2002, Toner, Pirogovsky et al. 2009, Stark and Stark 2017, Bennett, Stark et al. 2018). Our data further suggests that in particular the inhibition of false memories may be sensitive to the effects of age-related atrophy in the DG. This result complements the findings of Shing et al. (2011) who showed that DG volumes in healthy older adults were negatively correlated to false alarm rates in a word-pair learning task. The authors hypothesized that the role of the DG is to enhance the specificity of encoded memories. In order to store overlapping inputs, DG performs pattern separation that enables the correct retrieval of interfering information (Rolls 2010). As such, successful rejection of false associations is based on the efficient representation of differences between the correct and the incorrect associations. Interestingly, older adults show a specific decrease in their pattern separation ability, making them more vulnerable to memory distortions (Toner, Pirogovsky et al. 2009, Stark, Yassa et al. 2010). It is also possible that this specific association between DG volume and correct rejections reveals the function of DG in implementing retrieval strategies to prevent these memory distortions (for review (Kesner, Lee et al. 2004)). Novelty detection is one strategy that has been related to the DG and that allows recognition of newly presented information. Our findings lend further support to this concept, assuming that participants may have implemented this strategy to successfully detect new (i.e., incorrect) associations.

Association of fornix white matter microstructure and memory performance was mediated by dentate gyrus volume

Combining both white matter microstructure and grey matter volume within the structural hippocampal memory network, our mediation analysis showed that the prediction of memory performance by fornix microstructure was partially mediated by the volume of left DG in older adults. Previous studies have shown a link between age-related decrease of fornix integrity and

The directional relationship, however, remains unclear, as it is conceivable that both hippocampal grey matter loss induces fornix white matter fiber degeneration and vice versa (cf. Zhuang, Sachdev et al. 2013). So far, it has been shown that fornix microstructural degradation, and not hippocampal atrophy, served as a biomarker for early amnestic MCI due to Alzheimer’s Disease (Zhuang, Sachdev et al. 2013). Further, using mediation analysis, a recent study provided evidence for a causal effect of age-related fornix white matter damage on hippocampal grey matter volume decline in healthy adults (Metzler-Baddeley, Mole et al. 2019). The current study complements previous evidence by demonstrating that fornix microstructure and DG subfield volume interact to specifically mediate memory for false associations. Our mediation model offered a neurobehavioral model in which preserved fornix white matter microstructure in older age predicts successful memory formation through its protective effect on grey matter volume in the DG. Thus, demyelination, even in healthy aging, may induce grey matter loss reflecting altered intracellular metabolism or neural death in connected structures, and leading to constrained memory performance (Bartzokis 2004, Metzler-Baddeley, Mole et al. 2019). Due to the cross-sectional design of our study that still limits conclusions about causality; future longitudinal studies are needed to support this hypothesis.

Strengths and limitations of the study

Strengths of the study include the multimodal imaging approach of combined grey matter volumetric analysis and white matter fiber tractography to assess the impact of structural integrity that promotes successful cognitive function in older adults. A robust tractography method was used. CSD overcomes the limitations of other DTI techniques, estimating the orientation of multiple intravoxel fiber populations in regions of white matter structures with crossing fibers like the fornix (Tournier, Yeh et al. 2008, Jeurissen, Leemans et al. 2011). The present study presents two methodological limitations; first, partial volume effects (PVE) affect DTI-based indices (Alexander, Hasan et al. 2001, Szczepankiewicz, Latt et al. 2013). In order to attenuate PVE, we set the FA threshold to 0.2, as a means to eliminate all underestimated FA values. Second, volumes of hippocampal subfields
segmentation was performed on images with standard spatial resolution using a 3-Tesla MRI scanner (Iglesias, Augustinack et al. 2015). Future studies need to confirm age-related DG subfield volume changes and its linkage to white matter microstructure and memory using high resolutions 7-Tesla MRI scans.

**Conclusion**

We investigated the effect of fornix FA and DG volume on episodic memory formation in healthy older adults. Our findings demonstrated that preserved fornix microstructure positively impacts on memory for false associations through a protective effect on DG subfield volume. More generally, the results lend further support to the hypothesis that structural disconnection plays a crucial role in mediating deficits in the course of aging. Our study provides a neurobehavioral model for the linkage between structural memory network properties to explain inter-individual variability behavioral outcomes in older adults. Understanding this relationship is an important prerequisite for the development of interventions to counteract cognitive decline such as aerobic exercise, cognitive training or dietary interventions (Brickman, Khan et al. 2014, Antonenko, Kulzow et al. 2016, Kobe, Witte et al. 2016).

**Acknowledgements**

This work was supported by the “Bundesministerium für Bildung und Forschung” [01GQ1424A]. We thank Justus Netzband for help with data acquisition and Dr. Magdalene Ortmann for statistical support.

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Supplementary Figure S1: Sagittal view of the left fornix in the 30 subjects. Using ExploreDTI toolbox (Leemans, Jeurissen et al. 2009), fornix was manually drawn by author D.H. in native space using color-coded fiber orientation maps for individual subjects while blinded to age and memory performance.
Supplementary figure S2: Coronal view of the left hippocampal subfields in the 30 subjects. Fully automated hippocampal subfields reconstruction and volumetric segmentations were performed using FreeSurfer (version 6.0) (http://surfer.nmr.mgh.harvard.edu/) algorithm, introduced by Iglesias et al. (2015). Legend represents all hippocampal subfields segmented using FreeSurfer algorithm. Hippocampal fimbria, hippocampal fissure and hippocampal tail were not visible. DG: dentate gyrus; CA (1-4): cornu ammonis; ML_HP: molecular layer of the hippocampus.
tDCS-induced episodic memory enhancement and its association with functional network coupling in older adults

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Scientific Reprt 9 (1):2273

Published in 2019

Authors contributions:

D.A. and A.F. designed research. D.A., D.H. and J.N. collected the data. D.A., D.H., J.N. and U.G. analyzed the data. D.H. performed resting state functional analysis. D.A. prepared all figures. D.A. and A.F. wrote the manuscript. All authors reviewed and revised the manuscript.
tDCS-induced episodic memory enhancement and its association with functional network coupling in older adults

Daria Antonenko1,2, Dayana Hayek1,2, Justus Netzband1, Ulrike Grittner3,4,5 & Agnes Flöel1,2

Transcranial direct current stimulation (tDCS) augments training-induced cognitive gains, an issue of particular relevance in the aging population. However, negative outcomes have been reported as well, and few studies so far have evaluated the impact of tDCS on episodic memory formation in elderly cohorts. The heterogeneity of previous findings highlights the importance of elucidating neuronal underpinnings of tDCS-induced modulations, and of determining individual predictors of a positive response. In the present study, we aimed to modulate episodic memory formation in 34 older adults with anodal tDCS (1 mA, 20 min) over left temporoparietal cortex. Participants were asked to learn novel associations between pictures and pseudowords, and episodic memory performance was subsequently assessed during immediate retrieval. Prior to experimental sessions, participants underwent resting-state functional magnetic resonance imaging. tDCS led to better retrieval performance and augmented learning curves. Hippocampo-temporoparietal functional connectivity was positively related to initial memory performance, and was positively associated with the magnitude of individual tDCS-induced enhancement. In sum, we provide evidence for brain stimulation-induced plasticity of episodic memory processes in older adults, corroborating and extending previous findings. Our results demonstrate that intrinsic network coupling may determine individual responsiveness to brain stimulation, and thus help to further explain variability of tDCS responsiveness in older adults.

Research aiming at the facilitation and augmentation of cognitive processes through non-invasive brain stimulation (NIBS) in the course of aging is an area of great current interest1–5. In particular, transcranial direct current stimulation (tDCS) has been suggested to tune ongoing network processes2–5, to increase inter-regional functional communication and to reverse age-related network reorganization6. Beyond its potential for cognitive enhancement, tDCS may also reveal the magnitude of preserved neuroplasticity in older adults7–9. Recently, several studies have reported an age-dependency of neural and behavioral effects of tDCS7,10–12. For instance, Fiori and colleagues showed that verbal learning during temporoparietal anodal tDCS was enhanced in older but not young adults12. Martin and colleagues observed comparable behavioral impact on semantic word fluency induced by tDCS in older and young adults, but found differential task-related network modulation11. Other studies could not corroborate beneficial effects of tDCS on memory performance in older adults13,14. Based on evidence for age-related structural deterioration and associated functional brain-wide network reorganization, interventional techniques may operate upon other neural processes than in young brains1. Importantly, age-related cortical changes not only affect the magnitude of tDCS-induced modulation but also the pattern of...
underlying network reorganization\textsuperscript{10–12,15}. Beside the general variability in tDCS effects\textsuperscript{16}, responsiveness may even vary more among older adults due to large inter-individual differences in age-related deterioration of cognitive performance and brain structure\textsuperscript{8,17}, most likely explaining differential effects of tDCS in young versus older adults. What seems indisputable thus far is that results from young adults regarding efficient stimulation parameters as well as expected interactions with underlying regional brain activity might not be transferable to older brains\textsuperscript{1,17}. In sum, despite the large neuroscientific interest, the understanding of tDCS effects on the aged brain is still incomplete. The heterogeneity of findings further highlights the complexity of underlying mechanisms.

Among cognitive domains, episodic memory processes exhibit the most crucial age-related impairment\textsuperscript{18}, thus representing a core target for interventional strategies such as tDCS. At the same time, these processes are difficult to target directly as they are mainly mediated by medial temporal brain structures, such as the hippocampus\textsuperscript{19}. However, activity in these brain areas may be modulated by stimulating functionally connected cortical regions, as suggested by both functional magnetic resonance imaging (fMRI) studies\textsuperscript{19–21} and previous NIBS studies, using transcranial magnetic stimulation\textsuperscript{22,23} and tDCS with temporoparietal stimulation targets\textsuperscript{12,15,24–26}. In addition, modulation of verbal episodic memory formation has been demonstrated by anodal tDCS over the left prefrontal cortex\textsuperscript{14,27–30}. These findings confirm the involvement of a widespread neural network of medial temporal, temporoparietal and frontal areas in episodic memory processes, with several nodes of the network being susceptible to modulation with brain stimulation\textsuperscript{19,31}. Further, tDCS experiments have highlighted a laterality-dependent benefit for episodic memory in older adults, with right temporoparietal stimulation improving memory for visuospatial\textsuperscript{15,24} and left temporoparietal stimulation for verbal information\textsuperscript{2,27}. No study to date investigated the relationship of individual tDCS-induced episodic memory enhancement with intrinsic network coupling in older adults.

In the present study, we aimed to investigate these open issues, using anodal tDCS over left temporoparietal cortex to modulate episodic memory formation. Specifically, we hypothesized that tDCS would enhance memory as assessed by retrieval performance after picture-word associative learning. In addition, we expected that tDCS would lead to steeper learning curves over multiple blocks. Further, we acquired resting-state functional images to examine the relationship individual memory network coupling with episodic memory formation at baseline as well as with the magnitude of tDCS-induced enhancement. Here, we hypothesized that individual memory network coupling would be positively associated with memory formation and tDCS-induced memory enhancement.

**Results**

**tDCS-induced learning and memory improvement.** An episodic memory task was administered that required participants to learn picture-pseudoword associations during five learning blocks with concurrent tDCS application (1 mA, 20 min). Subsequent retrieval was assessed in two “transfer” blocks (one immediate, one with a 20-min delay) where previously presented pictures were replaced by corresponding words and participants had to identify correct pairs. Performance on immediate retrieval was defined as main outcome. To examine the effects of tDCS on memory performance and learning curves, percentage of correct responses and reaction times for retrieval and learning blocks were subjected to linear mixed model analyses with stimulation condition (sham, anodal) as within-subject factor. Models were adjusted for age and the order of experimental sessions.

**Memory performance.** To address the question whether memory performance differed significantly between stimulation conditions after learning (primary endpoint), performance during the immediate retrieval block was compared. Performance after anodal stimulation was on average 2.8% better compared to sham in the 1st retrieval...
block ($\beta = 2.8, 95\%-CI: [0.3, 5.4], F_{(1,89)} = 4.815, p = 0.031$; linear mixed model post-hoc, N = 34, 127 data points; Fig. 1). The main effect of stimulation condition on memory performance indicated superior performance in the anodal compared to the sham stimulation condition of on average 2.3% ($\beta = 2.3, 95\%-CI: [0.5, 4.2], F_{(1,91)} = 6.30, p = 0.014$). There were no condition by retrieval block interaction ($\beta = -1.9, 95\%-CI: [-4.6, 2.6], F_{(1,90)} = 0.30, p = 0.585$) or condition by age interaction effects ($\beta = 0.01, 95\%-CI: [-0.25, 0.27], F_{(1,90)} = 0.01, p = 0.927$). Performance on the 2nd retrieval block was on average 4.6% better compared to the 1st ($\beta = 5.1, 95\%-CI: [2.8, 6.4], F_{(1,89)} = 25.58, p < 0.001$). Effect of age was not statistically significant ($\beta = -0.3, 95\%-CI: [-0.6, 0.1], F_{(1,230)} = 2.43, p = 0.129$). A session effect revealed practice effects over the experimental sessions with an average improvement of 2.9% ($\beta = 0.029, 95\%-CI: [1.1, 4.8], F_{(1,91)} = 9.70, p = 0.002$).

There was no difference between stimulation conditions in reaction times (main effect: $\beta = -1.2, 95\%-CI: [-20.3, 17.9], F_{(1,90)} = 0.02, p = 0.899$). Reaction time on the 2nd retrieval block was shorter compared to the 1st (main effect: $\beta = -29.6, 95\%-CI: [-55.2, -4.0], F_{(1,89)} = 11.05, p = 0.001$). Effect of age was not significant ($\beta = 0.9, 95\%-CI: [-2.5, 4.2], F_{(1,32)} = 0.02, p = 0.899$). A session effect revealed faster responses on the second experimental session ($\beta = 25.0, 95\%-CI: [6.0, 44.1], F_{(1,91)} = 6.80, p = 0.011$). Interactions between condition and retrieval block ($\beta = -2.6, 95\%-CI: [-39.5, 34.3], F_{(1,89)} = 0.02, p = 0.889$) and condition and age ($\beta = -1.4, 95\%-CI: [-4.0, 1.3], F_{(1,91)} = 1.06, p = 0.307$) were non-significant.

Learning performance. Performance accuracy during five learning blocks was analyzed using a linear mixed model with blocks as level-one units nested in different individuals who were level-two units, in order to test for differences in the learning curves between stimulation conditions. The effect of stimulation condition itself was not significant (main effect: $\beta = 0.4, 95\%-CI: [-0.8, 1.6], F_{(1,287)} = 0.52, p = 0.473$; linear mixed model, N = 34, 320 data points; Fig. 1), but the interaction of condition and learning block indicated steeper learning curves in the anodal compared to the sham stimulation condition ($\beta = 1.0, 95\%-CI: [0.2, 1.8], F_{(1,281)} = 6.16, p = 0.014$). Overall, task performance improved over the learning blocks; improvement showed a curvilinear convex shape indicated by a linear increase of approximately 5% per block (for the five learning blocks [centered and linear] = 4.9, 95\%-CI: [4.4, 5.5], $F_{(1,281)} = 687.61, p < 0.001$) and an additional negative coefficient for the square of block order ($\beta$ [squared] = -1.1, 95\%-CI: [-1.4, -0.7], $F_{(1,281)} = 38.00, p < 0.001$). The negative age effect revealed flatter learning curves with higher age (main effect: $\beta = -0.2, 95\%-CI: [-0.5, 0.0], F_{(1,33)} = 4.68, p = 0.038$). A session effect indicated practice effects over the experimental sessions of 2.5% ($\beta = 2.5, 95\%-CI: [1.3, 3.7], F_{(1,287)} = 16.32, p < 0.001$). There was no interaction effect of condition and age ($\beta = -0.02, 95\%-CI: [-0.19, 0.15], F_{(1,294)} = 0.05, p = 0.827$).

Reaction time became shorter over the course of learning blocks ($\beta = -21.1, 95\%-CI: [-27.0, -15.2], F_{(1,279)} = 123.20, p < 0.001$; Fig. 2) as well as experimental sessions ($\beta = -28.3, 95\%-CI: [-40.8, -15.8], F_{(1,284)} = 19.80, p < 0.001$). An age effect on reaction time indicated slower responses with higher age, but was not statistically significant ($\beta = 3.2, 95\%-CI: [0.2, 6.2], F_{(1,32)} = 3.70, p = 0.063$). Effect of stimulation condition ($\beta = 7.5, 95\%-CI: [-5.0, 20.1], F_{(1,284)} = 1.40, p = 0.237$), condition by learning block interaction ($\beta = -6.0, 95\%-CI: [-14.5, 2.6], F_{(1,281)} = 1.90, p = 0.169$), and condition by age interaction ($\beta = -1.0, 95\%-CI: [-2.8, 0.7], F_{(1,290)} = 1.29, p = 0.258$) were not statistically significant.

Functional network correlates. Baseline correlations. To determine whether functional coupling within the memory network at rest predicted memory performance at baseline, seed-to-voxel correlation maps were calculated for the left hippocampus (i.e., Pearson’s r correlation of the blood-oxygenation level-dependent (BOLD) time course of the hippocampus with all other brain voxels). Subsequent general linear model analysis for the dependant variable baseline memory performance revealed an association of task performance with left-hemisphere hippocampo-temporoparietal coupling (significant cluster in left angular gyrus, peak coordinates: $x = -40, y = -52, z = 18, T = 4.45, k = 232$ voxel, cluster-$p$-FDR = 0.040, cluster-$p$-unc < 0.001, Fig. 3, corrected for age).
We then aimed to investigate whether the individual left-hemisphere hippocampo-temporoparietal coupling at baseline was also associated with the magnitude of responsiveness to tDCS. The latter was defined as difference in memory performance between anodal and sham stimulation (“percentage of correct responses during immediate retrieval in anodal stimulation” minus “percentage of correct responses during immediate retrieval in sham stimulation”). Pearson’s r correlation coefficients between BOLD time series in the left hippocampus and in the significant cluster in the left angular gyrus were extracted to obtain individual memory network coupling. We performed a linear regression adjusted for sham stimulation performance, session order and age. Sham performance was included as a covariate in the statistical model because we wanted to account for “performance without stimulation” in the evaluation of individual improvement through anodal stimulation. We found that individual responsiveness to tDCS was positively associated to functional connectivity (standardized $\beta = 0.31$, $p = 0.036$; see Table 1 for all model coefficients, overall model $R^2 = 0.58$, adjusted $R^2 = 0.52$).

### Table 1. Multiple linear regression analysis for difference in memory performance (anodal minus sham) (dependent variable) (n = 34). Note. $R^2 = 0.58$, adjusted $R^2 = 0.52$.

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>B</th>
<th>SE</th>
<th>Standardized $\beta$</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>0.626</td>
<td>0.187</td>
<td>3.251</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Sham performance</td>
<td>−0.706</td>
<td>0.140</td>
<td>−0.826</td>
<td>−5.044</td>
<td>0.000</td>
</tr>
<tr>
<td>Session order</td>
<td>−0.004</td>
<td>0.028</td>
<td>−0.022</td>
<td>−0.142</td>
<td>0.888</td>
</tr>
<tr>
<td>Functional connectivity</td>
<td>0.182</td>
<td>0.083</td>
<td>0.305</td>
<td>2.195</td>
<td>0.036</td>
</tr>
<tr>
<td>Age</td>
<td>−0.002</td>
<td>0.002</td>
<td>−0.135</td>
<td>−1.035</td>
<td>0.309</td>
</tr>
</tbody>
</table>

**Figure 3.** Scatterplot of functional connectivity and memory performance. Functional connectivity between the left hippocampus and left temporoparietal cortex correlated with task performance at baseline (R, retrieval). The significant cluster that emerged from whole-brain seed-to-voxel analyses had peak coordinates of $x = −40$, $y = −52$, $z = 18$, $T = 4.45$, cluster size $k = 232$ mm$^3$, cluster-$p$-FDR = 0.040. The scatterplot illustrates the relationship (baseline performance is plotted over individual functional connectivity that was extracted from the significant cluster).

**Association of functional connectivity with individual tDCS-induced improvement.** We then aimed to investigate whether the individual left-hemisphere hippocampo-temporoparietal coupling at baseline was also associated with the magnitude of responsiveness to tDCS. The latter was defined as difference in memory performance between anodal and sham stimulation (“percentage of correct responses during immediate retrieval in anodal stimulation” minus “percentage of correct responses during immediate retrieval in sham stimulation”). Pearson’s r correlation coefficients between BOLD time series in the left hippocampus and in the significant cluster in the left angular gyrus were extracted to obtain individual memory network coupling. We performed a linear regression adjusted for sham stimulation performance, session order and age. Sham performance was included as a covariate in the statistical model because we wanted to account for “performance without stimulation” in the evaluation of individual improvement through anodal stimulation. We found that individual responsiveness to tDCS was positively associated to functional connectivity (standardized $\beta = 0.31$, $p = 0.036$; see Table 1 for all model coefficients, overall model $R^2 = 0.58$, adjusted $R^2 = 0.52$; Fig. 4).

**Control variables, mood ratings and adverse effect questionnaire.** In order to control for effects of attention or working memory capacity, we conducted digit span forward and backward both before and after experimental sessions (Table 2). Forward and backward digit span did not differ between stimulation conditions (forward: $\beta = 0.06$, 95%-CI: [−0.38, 0.50], $F_{(1,100)} = 0.07$, $p = 0.790$; backward: $\beta = 0.1$, 95%-CI: [−0.38, 0.47],
Positive and negative affect ratings before and after the experimental sessions are shown in Table 3. Positive, and negative affect did not differ between stimulation conditions (positive: $\beta = -0.05$, 95%-CI: $[-0.17, 0.07]$, $F_{(1,100)} = 0.60$, $p = 0.440$; negative: $\beta < 0.01$, 95%-CI: $[-0.02, 0.03]$, $F_{(1,100)} = 0.03$, $p = 0.855$) and time points (positive: $\beta = 0.10$, 95%-CI: $[-0.02, 0.22]$, $F_{(1,100)} = 2.87$, $p = 0.093$; negative: $\beta = -0.01$, 95%-CI: $[-0.04, 0.02]$, $F_{(1,100)} = 0.54$, $p = 0.466$; linear mixed models; N = 34, 136 data points).

Number of participants who reported the respective adverse effect are shown in Table 4. Tingling during stimulation was most commonly reported in both stimulation conditions (in total by 47% of the participants). However, the occurrence of all adverse effects did not differ between conditions (all chi-square's $\leq 3.1$, all $p$'s $\geq 0.08$).

### Discussion
The present study investigated tDCS-induced modulation of episodic memory processes in older adults as well as the relationship with functional network coupling on an individual level. Memory performance was enhanced after anodal tDCS over left temporoparietal cortex, i.e., participants retrieved significantly more correct pairings of newly acquired picture-pseudoword associations in the transfer task. Additionally, older participants exhibited steeper learning curves during anodal compared to sham stimulation. Functional memory network coupling between left hippocampus and left temporoparietal brain area was positively associated with the magnitude of individual tDCS-induced cognitive enhancement.

Previous studies that used anodal tDCS over the left temporoparietal cortex to modulate associative learning and memory have reported benefits for performance of young adults. For example, Flöel et al. found accelerated learning and improved retrieval of newly acquired picture-word pairs when learning was accompanied by anodal tDCS, using identical task and stimulation parameters. In the present study, older adults likewise
exhibited faster learning success during stimulation compared to sham and enhanced subsequent memory. Thus, our findings extend previous results from young adults to the aging population, and indicate preserved responsiveness of episodic memory networks in older adults. Anodal tDCS over left temporoparietal cortex could have enhanced memory formation by modulating synchronous activity or connectivity within the memory network comprising temporoparietal and hippocampal structures. In older adults, beneficial effects for verbal learning of anodal tDCS over left temporoparietal cortex were supported by Fiori et al. who further suggested that simultaneous cathodal tDCS over right homologue may even be superior to a unilateral electrode montage. Modulation of episodic memory functions has been also shown with prefrontal stimulation targets in older adults. Anodal tDCS over left dorsolateral prefrontal cortex enhanced memory for words and reduced forgetting when applied during the encoding, reconsolidation or recall phase. A recent study showed that also ventrolateral prefrontal tDCS during intentional encoding enhanced delayed recognition memory in older adults. These findings corroborate the hypothesis that hippocampus-dependent memory processes are mediated by the inter-regional interaction within a distributed network in older adults, and are susceptible to modulation through lateral frontal and parietal brain stimulation. In sum, the group-level comparison between anodal temporoparietal and sham stimulation conditions in our study corroborated and extended previous findings by demonstrating preserved plasticity of episodic memory processes in older adults.

As we observed beneficial effects on both learning curves and subsequent retrieval, the question whether memory enhancement in our study was caused primarily by modulation of encoding, of consolidation, or of retrieval processes remains open. However, from previous reports, there is more evidence favoring the hypothesis that tDCS preferentially enhances post-encoding processes, such as consolidation and retrieval of acquired material. Offline effects may thus be stronger than online effects and of particular relevance as they may also persist after a period of consolidation. In older adults, beneficial effects for verbal learning of anodal tDCS, and further studies are needed to approach this question.

Recent studies have highlighted the need to report and examine individual data in tDCS studies in order to better understand inter-subject variability in responsiveness and its neurobiological correlates. Given the complex mechanisms underlying tDCS effects, including interacting state-dependent brain activity, task demands, stimulation parameters and a set of inter-individual factors, it seems not surprising that findings between studies are heterogeneous and also depend on the study sample. In older cohorts, the topic may even be more relevant as, in addition to variability in behavioral performance, variability of the rate of structural decline in aging likely also affects individual sensitivity to plasticity-inducing mechanisms. As in Brosnan et al., we observed a large range of variability in individual responsiveness to tDCS within our sample of older adults. As sources of variability still need to be determined, detailed report of sample characteristics, individual factors and its correlative relationships within the sample under study will help to further advance our understanding of underlying mechanisms.

By showing a positive correlation between memory performance and intrinsic hippocampo-temporoparietal functional coupling, our data further support the hypothesis that successful memory formation may be dependent on the connection between posterior inferior parietal and medial temporal brain regions. Thus, in our group of older adults, variability in task performance may be predicted by individual intrinsic connectivity within the memory network. This finding corroborates previous evidence for the crucial role of hippocampal-cortical

### Table 3. Mood ratings. Mean (SD) values.

<table>
<thead>
<tr>
<th></th>
<th>Anodal stimulation</th>
<th>Sham stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>3.31 (0.82)</td>
<td>3.32 (0.84)</td>
</tr>
<tr>
<td>Post</td>
<td>3.27 (0.81)</td>
<td>3.16 (0.92)</td>
</tr>
<tr>
<td>Negative affect</td>
<td>1.07 (0.11)</td>
<td>1.07 (0.10)</td>
</tr>
<tr>
<td>Post</td>
<td>1.08 (0.12)</td>
<td>1.09 (0.14)</td>
</tr>
</tbody>
</table>

### Table 4. Adverse effect ratings after the last experimental session. Number of participants. Total N = 34.

<table>
<thead>
<tr>
<th></th>
<th>Anodal stimulation</th>
<th>Sham stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Tingling</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>Itchiness</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Burning</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Fatigue</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Tension</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Loss of concentration</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Headache</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Discomfort</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
networks for memory functions. Our finding further emphasizes the importance of anatomical connection, and coordinated activity, between medial temporal and parietal areas for successful memory retrieval in older adults. This link would then allow to modulate hippocampus-dependent processes by targeting connected cortical sites.

Further, we assessed whole-brain hippocampal functional connectivity to evaluate if individual tDCS responsiveness was predicted by baseline functional network coupling in older adults. We found that hippocampo-temporoparietal coupling was positively associated with the magnitude of individual tDCS-induced memory enhancement. This finding is in line with prior research suggesting a pivotal role of intrinsic hippocampal coupling in age-related memory decline. Specific connections, including synchronous activity between hippocampus and angular gyrus may constitute central mechanisms underlying functional decline. Modulation of synchronous activity within and between networks appears not only to be crucial in the course of brain aging, but has been also suggested as main underlying mechanisms of tDCS effects. Our data supports the notion that functional connectivity at rest can be used as a predictor for individual response to brain stimulation and that posterior brain areas may be viable target regions for neuromodulatory techniques in the context of age-related deficits. Here, the combination of brain stimulation and neuroimaging to study neuromodulatory effects during and after tDCS will have to further elucidate its complex interaction with brain activity and age-related modulation of memory processes.

The present study does not allow drawing firm conclusions about spatial specificity of the applied electrode configuration due to the large size of the temporoparietal electrode as well as the lack of testing other electrode configurations. Conventional dual-electrode tDCS set-ups stimulate networks rather than brain regions. In particular, the targeted temporoparietal region has a heterogeneous organization with regard to its cytoarchitectonic, connectional and functional diversity, playing a role in several higher-order cognition processes in humans. Therefore, it is conceivable that other brain networks not primarily involved in memory processes were stimulated as well. Our computational modeling result confirms that maximal electric field strengths are distributed over the left lateral temporal and parietal areas, with high intensities around the intended target. Please note that spatial specificity of tDCS is thought to be increased by concurrent task-related activity, indicating that the interaction of brain stimulation with a task induces more focused effects. Moreover, the correlative functional connectivity analysis suggested that intrinsic synchronous activity in hippocampus and angular gyrus may be involved in successful memory performance in older adults. Thus, even though we cannot draw firm conclusions about spatial specificity, our set-up demonstrated tDCS-induced tuning of ongoing memory network processes and preserved plasticity in older adults. Further, our data does not allow concluding whether the observed effects are specific to older adults, as no young group was included. However, we believe that at this point, detailed examination of older cohorts is paramount, to better understand variability in this group.

Prospectively, persistent benefit of tDCS is essential to promote the development of clinical applications in the context of age-related diseases and may be achieved with combined tDCS-training interventions. Studies should therefore evaluate additional biomarkers determining individual responsiveness and prove the efficiency of tDCS to produce sustained plasticity induction in older adults, for which our current mechanistic study provides important groundwork.

Materials and Methods

Participants and experimental procedure. Thirty-four healthy older adults participated in the study (16 female, min/max age: 51/80 years, see Table 1 for participant characteristics). All were native German speakers, were right-handed, and had no history of neurological or psychiatric disorders. Neuropsychological testing was administered prior to study inclusion in order to assure normal cognitive functioning (CERAD-Plus, http://memoryclinic.ch). Performances on all subtests were not below 1.5 SDs according to age- and education-related normative scores. The study was approved by the ethics committee of the Charité University Medicine and conducted in accordance with the Helsinki Declaration. Written informed consent was obtained from all participants prior to participation.

Older adults participated in three experimental sessions. The first session (baseline) included MRI scanning (acquisition of resting-state data) and subsequent administration of the episodic memory task. In the two subsequent experimental sessions, tDCS was applied (either anodal or sham in counterbalanced order, separated by one week) over the left temporoparietal cortex during the learning phase of the task.

tDCS. A battery-driven stimulator (neuroConn DC-Stimulator Plus; neuroCare Group GmbH, Munich, Germany) was used to deliver direct current to the scalp via two sponge-electrodes soaked into saline solution. The anodal electrode (5 x 7 cm²) was placed centrally over CP5 according to the 10–10 EEG system, while the cathode (10 x 10 cm²) was placed over the right supraorbital area (centered over the right anterior frontal cortex, or AF4, respectively). The larger size of the sponge (10 x 10 cm²) has been shown to reduce current density to a level (0.01 mA/cm²) that does not exert functional neurophysiological effects. Due to these results, it is assumed that the effects underneath the cathode are functionally less efficient (but not "inert") (1). Episodic memory processes as assessed in our study have been shown to be mediated by distributed networks including medial temporal areas such as the hippocampus and lateral temporoparietal areas. As anodal tDCS over the left temporoparietal cortex has been shown to modulate performance in similar tasks as the one used in our study, it was chosen as target area for stimulation. The software SimNIBS 2.1.1 was used to estimate the electric field induced by tDCS, based on the finite element method and individualized tetrahedral head meshes generated from T1- and T2-weighted structural MR images of one healthy older participant (http://simnibs.org; see Supplementary Information for details on current field modeling). Modeling results showed that maximal electric field strengths are distributed over left lateral temporal and parietal areas, with high intensities around the intended target area, i.e., in the vicinity of the left angular gyrus (Figure S1). Electrodes were fixed with two...
rubber bands and impedance was kept below 5 kOhm. Duration of anodal stimulation was 20 min (starting with the first learning block of the task, covering the first four learning blocks of the task) whereas in sham stimulation current was turned off after 30 sec. Stimulation intensity was 1 mA with 10 sec fade in and fade out. Before and after each stimulation condition, positive and negative affect schedule (PANAS) was administered. Participants had to rate their current mood on 10 positive and 10 negative items (on a Likert scale with a range of 1 to 5)\(^65\). Change of ratings between time points was compared between conditions. After the completion of the last experimental session, participants were asked to retrospectively report the occurrence of adverse effects during the last session in a standardized questionnaire\(^66\).

**Episodic memory task.** The episodic memory paradigm was adapted from previous studies\(^20,26,67,68\) and programmed using the software Presentation (Neurobehavioral Systems, [http://www.neurobs.com/](http://www.neurobs.com/), version 18.1). The paradigm consists of the presentation of pseudoword-picture pairs. For each participant, a set of 30 pseudowords and 30 pictures of daily life objects were randomly matched to 30 “correct” pairings to create different correct (and, therefore, also incorrect) pseudoword-picture combinations.

Three different sets of stimuli (A, B, C) were used for three sessions (baseline, sham, anodal), containing different pseudowords and pictures\(^26\). The order of the set presentation was counterbalanced across participants. In each session, five learning blocks followed by an immediate retrieval block were presented. In the stimulation conditions (sham, anodal), an additional retrieval block was administered after a delay of 20 min.

During the learning blocks, a total of 600 trials were presented (120 trials per block). “Correct” pairings occurred ten times in total (i.e., twice in each block). In addition, each of the 30 pictures was presented ten times with varying “incorrect” pseudowords (i.e., two different “incorrect” pairings for each picture per block). Each “incorrect” pairing was presented only once over the course of learning. The order of trial presentation was randomized. Participants were not informed about the underlying frequency principle, and were instructed to decide as quickly as possible if word and picture match. Thus, the learning principle of this associative learning phase involves higher co-occurrences of “correct” arbitrary couplings compared to “incorrect” couplings (ratio 10:1)\(^68\).

In each learning trial, the picture was presented 200 ms after the onset of the auditory spoken pseudoword (delivered over headphones) and remained on the screen for 1500 ms\(^67\). During picture presentation, participants had to decide whether the pairing was “correct” or “incorrect” by pressing one of two response buttons (using their left or right index finger, respectively).

During retrieval blocks, learning success was measured in a “transfer” task. Here, instead of presenting a picture, corresponding German spoken words were delivered with the pseudowords. Stimulus count, the underlying frequency principle (i.e., two “correct” and two “incorrect” pairings per block), and trial timings were identical to those in the learning phase. Percentage of correct responses and mean reaction time of each block were assessed. Main outcome measure for memory performance was percentage of correct responses during the immediate retrieval block\(^26\).

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>63.1</td>
<td>7.7</td>
</tr>
<tr>
<td>Education, years</td>
<td>15.3</td>
<td>2.3</td>
</tr>
<tr>
<td>LQ(^a)</td>
<td>93.5</td>
<td>9.8</td>
</tr>
<tr>
<td>GDS(^b)</td>
<td>1.3</td>
<td>1.4</td>
</tr>
</tbody>
</table>

**Table 5.** Sample characteristics. \(^a\)LQ, laterality quotient\(^73\). \(^b\)GDS, Geriatric Depression Scale\(^74\). \(^c\)75.
MRI acquisition. Brain imaging data were acquired at the Berlin Center of Advanced Neuroimaging using a 3T Siemens Trio Scanner with a standard 12-channel head coil. A three-dimensional structural scanning protocol was applied using high-resolution T1-weighted magnetization-prepared rapid gradient echo imaging (1 × 1 × 1 mm³ voxel size; flip angle = 9 deg, repetition time = 1900 ms, echo time = 2.52 ms, 192 slices). Acquisition of resting-state fMRI was performed using an echo-planar imaging sequence (3 × 3 × 4 mm³ voxel size; flip angle = 78 deg, repetition time = 2070 ms, echo time = 30 ms, descending acquisition, 172 volumes). Participants were instructed to keep their eyes closed, not to fall asleep or think of anything in particular. None of the participants fell asleep during the scanning interval as per self-report. An additional fluid attenuated inversion recovery sequence was acquired for neuroradiological assessment and to exclude structural abnormalities.

fMRI analysis. Functional connectivity analysis was performed using the CONN toolbox version 17f (www.nitrc.org/projects/conn, RRID:SCR_009550)96,70. We used the default data preprocessing pipeline provided by the toolbox which includes functional realignment, slice-time correction, structural segmentation and normalization to the Montreal Neurological Institute (MNI) template, functional segmentation and normalization, and smoothing (with 6-mm Gaussian kernel). The realignment confound (derived from the estimated motion parameters) was defined by 6 rigid-body dimensions plus 6 first-order temporal derivatives using Artifact Detection Toolbox (ART, www.nitrc.org/projects/artifact_detect/). Confounds in the blood oxygenation level-dependent (BOLD) signal from physiological and other spurious sources of noise were estimated and regressed out using the anatomical CompCor method implemented in the toolbox97. Anatomical images were segmented into grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF) masks using SPM12. To minimize partial voluming with GM the WM and CSF masks were eroded by one voxel70. Functional data were temporally filtered (at high-pass threshold 0.01 Hz). First-level (within-subjects) seed-to-voxel correlation maps were calculated for the left hippocampus (i.e., Pearson’s r between the residual BOLD time-course of the seed mask and the time course of all other voxels in the brain were computed).

For second-level (between-subjects) general linear model analyses, correlation coefficients were transformed to normally distributed z scores (using Fisher’s transformation). To examine the association of hippocampal connectivity with baseline task performance, first-level connectivity maps for each participant were entered into a whole-brain regression analysis with age as covariate. The reported cluster survived a height threshold of uncorrected p < 0.005 (positive contrast) and an extent threshold of FDR-corrected p < 0.05 at the cluster level. To obtain individual ROI-to-ROI connectivity values of hippocampal-temporoparietal coupling, Pearson’s r correlation coefficients between BOLD time series in the left hippocampus and in the significant cluster in the angular gyrus were extracted.

Statistical analysis. Statistical analyses were conducted using IBM SPSS Statistics 24 (http://www-01.ibm.com/software/uk/analytics/spss/). Linear mixed models (random intercept models) were conducted for dependent variables with the factor stimulation condition (anodal, sham). Models were adjusted for age and experimental session. Linear trends were tested with an index variable (centered) for task blocks (i.e., five for learning and two for retrieval). For learning, an additional quadratic term for learning blocks was entered into the model. Linear regression analysis was conducted for association between functional connectivity and individual responsiveness to tDCS (defined by performance in anodal minus performance in sham stimulation condition), adjusted for age, order of experimental sessions and sham stimulation performance. Logistic regression models were computed for the comparison of adverse event occurrences. No corrections for multiple comparisons were applied. A two-sided significance level of α = 0.05 was used.

Data Availability
The datasets generated during the current study are available from the corresponding author on reasonable request.

References
Acknowledgements

This work was supported by the “Bundesministerium für Bildung und Forschung” [01GQ1424A].

Author Contributions

D.A. and A.F. designed research. D.A., D.H. and J.N. collected the data. D.A., D.H., J.N. and U.G. analyzed the data. D.A. prepared all figures. D.A. and A.F. wrote the manuscript. All authors reviewed and revised the manuscript.

Additional Information

Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-019-38630-7.

Competing Interests: The authors declare no competing interests.

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Manuscript 3

Role of Sensorimotor Cortex in Gestural-Verbal Integration

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Frontiers Human Neuroscience 12:482

Published in 2018

Authors contributions:

DH, AF and DA designed research; DH and DA performed research; DH, AF and DA analyzed data;

DH, AF and DA wrote the paper.
Role of Sensorimotor Cortex in Gestural-Verbal Integration

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Action comprehension that is related to language or gestural integration has been shown to engage the motor system in the brain, thus providing preliminary evidence for the gestural-verbal embodiment concept. Based on the involvement of the sensorimotor cortex (M1) in language processing, we aimed to further explore its role in the cognitive embodiment necessary for gestural-verbal integration. As such, we applied anodal (excitatory) and sham transcranial direct current stimulation (tDCS) over the left M1 (with reference electrode over the contralateral supraorbital region) during a gestural-verbal integration task where subjects had to make a decision about the semantic congruency of the gesture (prime) and the word (target). We used a cross-over within-subject design in young subjects. Attentional load and simple reaction time (RT) tasks served as control conditions, applied during stimulation (order of three tasks was counterbalanced). Our results showed that anodal (atDCS) compared to sham tDCS (stDCS) reduced RTs in the gestural-verbal integration task, specifically for incongruent pairs of gestures and verbal expressions, with no effect on control task performance. Our findings provide evidence for the involvement of the sensorimotor system in gestural-verbal integration performance. Further, our results suggest that functional modulation induced by sensorimotor tDCS may be specific to gestural-verbal integration. Future studies should now evaluate the modulatory effect of tDCS on semantic congruency by using tDCS over additional brain regions and include assessments of neural connectivity.

Keywords: brain stimulation, embodiment, gestural-verbal association, cognition, language processing

INTRODUCTION

The engagement of the sensorimotor system in word comprehension has been an intriguing question in brain research (Hauk et al., 2004; Tettamanti et al., 2005; Pulvermüller and Fadiga, 2010; Vukovic et al., 2017). Gestures were found to enhance language comprehension of listeners (Hostetter, 2011), possibly via embodiment. The embodiment concept, also referred to as grounded cognition, is based on involuntary mimicry (Barsalou, 2008). Parzuchowski et al. (2014) used embodied cognition to show that hand gestures enhance language comprehension. According to Hostetter and Alibali (2008), the embodiment approach suggests that language understanding is based on perceptual experience, that is, words start to have meaning when linked to real world perception.
Gestural-verbal integration has been studied at both the behavioral and the neural level. It has been suggested that language comprehension is facilitated by gestures due to the presence of common neural substrates for processing language and gesture (Holle et al., 2008; Dick et al., 2009; Hubbard et al., 2009; Straube et al., 2012). These neural substrates include both inferior frontal gyrus (IFG; Straube et al., 2011; Dick et al., 2014; He et al., 2018b) and motor cortex (for review Ozyurek, 2014). Studies have reported activation in the primary motor cortex during word comprehension when words involved sensorimotor features (Willems and Hagoort, 2007; Pulvermüller and Fadiga, 2010), using various methodological approaches. These approaches include functional magnetic resonance imaging (fMRI; Kemmerer et al., 2008; Kana et al., 2012, 2015), electroencephalography (Mollo et al., 2016; Schaller et al., 2017) and magnetoencephalography (Klepp et al., 2015; Mollo et al., 2016). The findings have raised the intriguing question why language comprehension is processed in a sensorimotor area (De Marco et al., 2015). One explanation might be that mirror neurons are responsible for this interaction (Caramazza et al., 2014). Arid and Mukamel (2016) found that observing someone else performing a task enhances one's own performance. Simultaneously acquired fMRI data showed a significant positive correlation between blood oxygen level dependent (BOLD) fMRI response within left sensorimotor cortex (M1) during action observation and the execution rate of the subjects. Thus, activation of the same fronto-parietal sensorimotor areas in both observing and executing an action enables an individual to understand the observed action more easily (Rizzolatti et al., 2001).

In addition to neuroimaging, non-invasive brain stimulation is a promising approach to investigate the involvement of the stimulated cortex in the respective function (Jacobson et al., 2012; Parkin et al., 2015; Tremblay et al., 2016; Polania et al., 2018). Moreover, stimulation may modulate performance in the task under study. In particular, transcranial direct current stimulation (tDCS) was found to modulate cognitive functions and underlying neuronal activity and connectivity (Meinzer et al., 2012; Parkin et al., 2015; Lavidor, 2016; Strobach and Antonenko, 2017; Yavari et al., 2017). More recent studies have used tDCS to reveal a role for M1 in language comprehension, especially for action-related words (Meinzer et al., 2016; Branscheidt et al., 2018). These results provided evidence for an interaction of motor cortex activity with language processing. A more recent study found that for healthy individuals, anodal tDCS (atDCS) over the motor cortex improved semantic word retrieval performance (Martin et al., 2017). In terms of gesture comprehension, studies have used tDCS over the IFG to investigate the role of this region in processing gestural-verbal stimuli (Cohen-Maximov et al., 2015; Schulke and Straube, 2017, 2018). Gesture prime clips were implemented for word targets. Participants were instructed to make a semantic decision of the prime-target congruency. Subjects responded faster under atDCS of the right IFG compared to sham tDCS (stDCS). The study suggested that inferior frontal atDCS may enhance gestural-verbal integration, which in turn enhances gesture comprehension. However, no previous study, to our knowledge, explored the role of left M1 for gestural-verbal integration, using tDCS.

In our study, we therefore assessed the impact of atDCS over left M1 on gestural-verbal integration (adapted from Cohen-Maximov et al., 2015). In order to exclude that the effect on gestural-verbal task would be based on improved attentional and motor processes, we included two control tasks during stimulation [attentional load task and simple reaction time (RT) task]. Given the role of left M1 in language processing, we hypothesized that tDCS-induced upregulation of M1 will improve the performance of associating gestures and word comprehension.

**MATERIALS AND METHODS**

**Participants**

Twenty-two right-handed healthy young adults (14 female; mean/SD/range age: 24/3/19–30 years; mean/SD/range Handedness score: 91/10/70–100) participated in the study. All were native German speakers and had no history of neurological or psychiatric disorders. The study was carried out in agreement with the Helsinki Declaration, and was approved by the ethics committee of the Charité Universitätsmedizin. Participants signed an informed consent form before participating.

**Study Design**

In a within-subjects design, all young adults participated in two sessions where either anodal or sham tDCS was applied. Participants were blind to the stimulation condition. During the stimulation interval, participants were exposed to three different tasks that were presented in a counterbalanced order: gestural task, attention load task and simple RT task. Order of stimulation conditions was counterbalanced across subjects and sessions were separated by at least 1 week (Figure 1).

**Stimuli**

The tasks were administered on the computer screen using the software Presentation (Neurobehavioral Systems1, version 18.1).

**Gestural Task**

The task was adapted from previous studies (Vainiger et al., 2014; Cohen-Maximov et al., 2015 for detailed description). Participants were exposed to a set of videos followed by set of written German words (prime, target). The duration of the task was 10 min. They were asked to choose whether the words describe the video or not, by clicking either the button “V” or “N” on the keyboard. They were instructed to use their right index finger and rest it on the “B” button between answers. Each trial began with the presentation of a fixation cross for 500 ms, followed by the 1,520 ms video clip (the prime). Gestures were grouped into instrumental and symbolic categories: instrumental gestures are those that imitate commonly known actions such as brushing teeth;
symbolic gestures are those that carry figurative meaning such as “goodbye.” A third type of video consisted of landscape scenes such as an erupting volcano. All videos were followed by a short written German sentence comprising of a maximum of three words; the sentence was either congruent or incongruent in the preceding video. A total of 108 videos, grouped into two sessions with a 90 s break in-between, were presented. Of the 108 videos, 22 were instrumental congruent, 22 instrumental incongruent, 16 symbolic congruent, 16 symbolic incongruent, 16 landscapes congruent and 16 landscapes incongruent (Figure 2). RT was defined as main dependent variable. In addition, percentage of correct responses was assessed. Only correct answers with RT less than 2 s were included in the analyses.

**Attentional Load Task**

We adapted the Visual Attention—Flanker Compatibility Task 

Version 4 paradigm described elsewhere (Green and Bavelier, 2003). The duration of the task was 7 min. The task consisted of 12 practice trials and 96 experimental trials. Each trial in the attentional load task began with 500 ms of a central white fixation cross. Participants searched for two possible target shapes (“Square” or “Diamond”) among central non-target shapes. Participants were asked to indicate whether one of the Shapes was a “Square” or a “Diamond” by pressing “V” or “N” buttons on the keyboard. They were instructed to use their right index finger and rest it on the “B” button between answers. Attentional load was manipulated randomly between trials. Target position (1–6), target identity and distractor compatibility were counterbalanced when the trials were constructed. Two load conditions were presented, the low load (low competition condition), where the circle was composed of the target shape with no competing central shapes and the high load (high competition condition), where the circle was composed of the target shapes along with five competing shapes. A flanker appeared to the right or left of the circle in equal probabilities. The flankers were a “Square” or a “Diamond” and could be compatible with the target shape or not. Participants were instructed to ignore the flankers. The stimulus was presented for 100 ms. The interstimulus duration was 1,000 ms. RT and percentage of correct responses were assessed.

**Simple Reaction Time Task**

The duration of the task was 3 min. The task was adapted from Neurobehavioral Systems1; (e.g., Vieluf et al., 2017). Participants were instructed to click the “space” button on the keyboard as soon as they see a red square on the screen. The experiment consisted of eight practice trials and 100 experimental trials. The stimulus was presented for 500 ms with a rectangular distribution of inter-stimulus duration (between 1,000 ms and 2,000 ms). RT and percentage of correct responses were assessed.

**tDCS**

Direct current stimulation was delivered through a battery-driven stimulator (neuroConn DC-Stimulator Plus, neuroCare Group GmbH, Munich, Germany) using two electrodes inserted in saline-soaked synthetic sponges. The anode (5 × 7 cm²) was centered over left sensorimotor cortex (left M1) and the reference electrode (10 × 10 cm²) was positioned over the contralateral (right) supraorbital region. Due to its larger size (10 × 10 cm²), the effect underneath the cathode is thought to be functionally less efficient (Nitsche et al., 2007; Nitsche and Paulus, 2011; Antal et al., 2017). Electrode positions were individually determined according to the 10-20 EEG system (active electrode centered over C3, reference over Fp2). The stimulation started with the beginning of the tasks. In the atDCS condition, stimulation was delivered continuously for 20 min (with 10-s fade in/out intervals) with a constant current of 1 mA. The duration of the stimulation was equal to the total duration of the three tasks. In the stDCS condition, stimulation was turned off after 30 s.

Before and after each stimulation condition mood ratings were assessed using the Positive and Negative Affect Schedule (PANAS; Watson et al., 1988). Participants rated their positive and negative affect (10 items each) on a scale ranging from 1–5, where higher values describe more positive or negative feelings, respectively. After completion of the second experimental session, participants were asked to retrospectively report the

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**FIGURE 1** | Illustration of experimental design. All subjects underwent atDCS and stDCS separated by at least 1 week, in a counterbalanced order. The duration of the main task (gestural task) and of control tasks [attentional load and simple reaction time (RT) tasks] are shown. Order of task presentation was counterbalanced between subjects. G, gestural task; A, attentional load task; SRT, simple RT task; atDCS, anodal transcranial direct current stimulation (tDCS); stDCS, sham tDCS.

**FIGURE 2** | Examples of congruent and incongruent stimuli. (A) A gesture congruent with the word meaning and (B) a gesture incongruent with the word meaning.

"Lampe Wechseln" (Lamp change) "Ohne Salz" (Without salt)
occurrence of adverse effects (pain, tingling, itching, burning, fatigue, tension, headache, discomfort) during stimulation in a standardized questionnaire (Poreisz et al., 2007). The questionnaire included the adverse effect with its corresponding intensity scale that ranges from 1–5 (with 1 as “very low” and 5 as “very high”).

Statistical Analysis
IBM SPSS Statistics 24 was used for statistical analyses. In order to test for differences between atDCS and stDCS, repeated-measures ANOVAs were performed, separately for all dependent variables. For the gestural task, congruency was added as within-subject factor. The interaction of congruency and stimulation effect was further assessed with subsequent ANOVAs conducted per congruency, in order to study the effect of stimulation on congruent and incongruent stimuli separately. In addition, an exploratory analysis was conducted by adding the stimuli type (instrumental, symbolic, landscapes) as an additional factor and subsequent within-subjects t-tests were performed to compare RT of atDCS and stDCS in the three different incongruent stimuli types. Both percentage of correct responses and RT were analyzed separately as dependent variables for each task. Repeated-measures ANOVA were used for mood ratings with stimulation type (atDCS and stDCS) as within-subject factor. Generalized estimating equations were performed for adverse events to compare the frequencies of their occurrence under atDCS compared to stDCS. A two-sided significance level of α = 0.05 was used.

RESULTS

Task Versions Validation
Two versions of the task were created. The similarity of the versions was validated in a pilot study. Ten native German speakers (6 females, Mean/SD age = 27.3/3.9) performed the two versions in a counterbalanced order. No difference in RTs was found between the two versions, version A (Mean/SD RT = 864/135 ms) and version B (Mean/SD RT = 809/113 ms), U = 41.5, p = 0.529. Likewise, no difference in percentage of correct responses was found between version A (Mean/SD percentage of correct responses = 91.7/3.2%) and version B (Mean/SD percentage of correct responses = 89.8/3.1%).

<table>
<thead>
<tr>
<th>Reaction Times (RTs)</th>
<th>Percentage of Correct Responses (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>atDCS Mean SD</td>
</tr>
<tr>
<td>Gestural task</td>
<td></td>
</tr>
<tr>
<td>Cong</td>
<td>876.0, 101.8</td>
</tr>
<tr>
<td>Incong</td>
<td>859.5, 97.1</td>
</tr>
<tr>
<td>Attentional load task</td>
<td>900.2, 133.3</td>
</tr>
<tr>
<td>Simple reaction time task</td>
<td>267.5, 20.0</td>
</tr>
</tbody>
</table>

In both control tasks, there was no significant main effect of stimulation condition on RT (for attentional load task: F(1,21) = 1.33, p = 0.262; for simple reaction time task: F(1,21) = 0.01, p = 0.926; Figure 3).

Percentage of Correct Responses
In the gestural task, a 2 × 2 ANOVA showed that there was no significant main effect of stimulation condition on percentage of correct responses (F(1,21) = 1.14, p = 0.298). There was no significant interaction between stimulation and congruency, (F(1,21) = 0.61, p = 0.807; Table 1). However, there was a significant main effect of congruency on percentage of correct responses (F(1,21) = 44.31, p < 0.001, partial eta squared = 0.68), indicating more correct responses for incongruent compared to congruent stimuli.

In both control tasks, there was no significant main effect of stimulation condition on percentage of correct responses (for
attentional load task ($F_{(1,21)} = 1.01, \ p = 0.327$), and for simple reaction time task ($F_{(1,21)} = 4.10, \ p = 0.056$).

**Mood Rating and Stimulation Side Effects**

Table 2 shows mood ratings before and after stimulation for both atDCS and stDCS. Repeated-measures ANOVA showed that there was no significant difference in mood changes between atDCS and stDCS for both positive ($F_{(1,21)} = 1.623, \ p = 0.217$) and negative ($F_{(1,21)} = 0.062, \ p = 0.806$) affect.

All subjects tolerated the stimulation with only few subjects reporting adverse effects; Table 2 shows the number of participants reporting each adverse effect in the corresponding condition. From participants who reported adverse effects in atDCS, more than 50% scaled the intensity as less than 2 (out of 5). Only 4 of 22 subjects noticed a difference and reported a slightly stronger tingling sensation due to atDCS, indicating an overall efficient placebo condition. Generalized estimating equation did not show any significant differences in atDCS compared to stDCS ($p \ values > 0.106$). PANAS and adverse events (AEs) of tDCS are presented in Tables 2, 3 respectively.

**DISCUSSION**

This study assessed the effect of excitatory atDCS over left M1 on gestural-verbal integration. We found that atDCS led to significantly faster correct answers compared to stDCS, but only for incongruent and not for congruent associations. Performance on attentional load and simple reaction time tasks was not affected by the stimulation.

**Neural Correlates of Gestural-Verbal Integration**

In the present study, we observed faster responses during atDCS compared to stDCS in the gestural-verbal task. This result is consistent with previous studies that showed a tDCS-induced improvement in cognitive functions (Radman et al., 2018; Wang et al., 2018; Yang et al., 2018). Further studies investigated gestural-verbal integration and gesture processing using atDCS over frontal (Cohen-Maximov et al., 2015; Schulke and Straube, 2017) and parietal cortices (Bianchi et al., 2015). Results showed that stimulation of frontal area decreased RTs of processing gestural-verbal associations (Cohen-Maximov et al., 2015; Schulke and Straube, 2017). Similarly, stimulation of the left parietal cortex improved motor test scores in apraxia patients (Bianchi et al., 2015). Taking together, atDCS over frontal and parietal cortices has been shown to improve both gestural- verbal integration and gesture comprehension. More recently, Schulke and Straube (2017) applied tDCS during a speech-gesture semantic task. They found that inhibitory cathodal tDCS (ctDCS) decreased task performance and atDCS enhanced it. As for motor cortex, previous studies investigated the role of motor cortex in language processing using atDCS (Meinzer et al., 2016; Branscheidt et al., 2018). For example, Meinzer et al. (2016) applied atDCS over left motor cortex twice daily at the beginning of a naming therapy and suggested that stimulating this area enhanced naming ability of patients with post-stroke aphasia. Branscheidt and colleagues followed up on Meinzer et al.’s (2016) findings by also applying atDCS over left motor cortex showing that atDCS improved lexical decision accuracy selectively for action-related words and not for object-related ones (Branscheidt et al., 2018). More specifically, we observed faster responses in atDCS compared to stDCS for incongruent associations. This indicates the involvement of motor areas in the processing of language and gesture when the information conveyed by the gesture does not describe the information conveyed by language. Our results corroborate previous studies showing activation of left IFG and its adjacent motor areas during the processing of incongruent speech-gesture associations (Willems et al., 2007; Green et al., 2009; Kircher et al., 2009). For instance, Willems et al. (2007) measured brain activity using fMRI during performance of a gestural-verbal semantic task in healthy young subjects. They

**TABLE 2 | Participants’ mood ratings before and after stimulation.**

<table>
<thead>
<tr>
<th></th>
<th>atDCS</th>
<th></th>
<th>stDCS</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td><strong>Positive affect</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>2.02</td>
<td>0.18</td>
<td>2.10</td>
<td>0.24</td>
</tr>
<tr>
<td>After</td>
<td>2.50</td>
<td>0.48</td>
<td>2.46</td>
<td>0.55</td>
</tr>
<tr>
<td><strong>Negative affect</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>1.88</td>
<td>0.27</td>
<td>1.78</td>
<td>0.26</td>
</tr>
<tr>
<td>After</td>
<td>1.15</td>
<td>0.13</td>
<td>1.14</td>
<td>0.16</td>
</tr>
</tbody>
</table>

atDCS, anodal tDCS; stDCS, sham tDCS.

**TABLE 3 | Number of participants who reported adverse effects (total $N = 22$).**

<table>
<thead>
<tr>
<th>Adverse effect</th>
<th>atDCS</th>
<th></th>
<th>stDCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td>5</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Tingling</td>
<td>2</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Itchiness</td>
<td>7</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Burning</td>
<td>4</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Fatigue</td>
<td>4</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Tension</td>
<td>–</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Loss of concentration</td>
<td>3</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Discomfort</td>
<td>1</td>
<td></td>
<td>–</td>
</tr>
</tbody>
</table>

for incongruent and not for congruent associations. Performance on attentional load and simple reaction time tasks was not affected by the stimulation.
showed that premotor cortex was activated only when the gesture did not match the word. More recently, studies have shown that IFG is involved in gestural-verbal integration in case of semantic conflict (Willems et al., 2007; Green et al., 2009; Zhao et al., 2018). In addition, IFG and premotor cortex are anatomically connected and are both activated in gestural-verbal integration (Marstaller and Burianova, 2015). It is thus possible that activation of one region is affected by the activation of the other, creating an extended network sensitive to the congruency of gestural-verbal association. However, negative findings were also reported: for example, Siciliano et al. (2016) applied anodal tDCS over left IFG during language learning and found no difference between anodal and sham conditions when words were coupled with gestures. In contrast to our study, the authors only included gestures that were congruent with the presented word in their task.

Our subsequent explorative results showed that faster responses under atDCS compared to stDCS were found only for gestural stimuli (instrumental and symbolic), and not for landscape stimuli. In accordance with previous studies suggesting that motor and premotor cortices are activated only in action-related gestures like instrumental gestures (He et al., 2018a), and that left frontal cortex is specifically implicated in the understanding of symbolic gestures (Rapp et al., 2007, 2012; Straube et al., 2013), our initial hypothesis was that only instrumental gestures would be affected. However, our results are in line with previous studies suggesting no difference in the processing of instrumental and symbolic gestures (He et al., 2015, 2018b), indicating that both gesture types may be supported by left motor activity. Nevertheless, these findings should be interpreted with caution due to the relatively small number of stimuli within the stimulus categories.

Involvement of M1 in Cognitive Control Mechanism

In line with our results, tDCS has been previously implicated in modulating task performance in incongruent associations; this hypothesis was investigated using paradigms that include an interference effect like the flanker task and Stroop task (Ouellet et al., 2015; Zmigrod et al., 2016). For instance, a recent study applied ctDCS during a flanker task to examine the effect of stimulation on performance and showed that ctDCS led to slower RTs when the flanker was incongruent with the target (Zmigrod et al., 2016). Thus, tDCS was shown to affect performance of incongruent stimuli associations. Further, Botvinick et al. (2001) suggested that the brain responds to interference (in this case incongruent associations) by implementing a cognitive control mechanism. It was suggested that the cognitive control mechanism is not defined as a simple task, related to one brain region, but rather it is recognized as a cascade of distinct control types performed by distinct brain regions. For instance, in a brain imaging study, dorsolateral prefrontal cortex (DLPFC) was shown to engage its control through the top-down modulation of task-dependent information processing in M1, also referred to as sensory control (Koechlin et al., 2003). Based on these studies, the improvement of subjects’ ability to detect incongruent gestural-verbal associations, or in other words cases of semantic conflicts, might result from increased cognitive control mechanisms following modulation of brain circuitry underlying sensory control in M1 region. In addition, a previous study used high-definition tDCS over DLPFC or over M1 during a flanker task and showed that tDCS over M1 region did not show any significant effect on conflict adaptation effect which is a hallmark of cognitive control mechanism (Gbadeyan et al., 2016). This result complements our results in assuming that the effect we found might be specific to the gestural-verbal task, since the flanker task used in the previous study does not include a motor component. Moreover, given that atDCS did not affect attentional load task, which is a classical conflict task, this result further supported our assumption that the cognitive control mechanism generated by the M1 might be specific to motor action, i.e., gestural-verbal integration and rather than simply affecting attention. It might be interesting to compare our results with DLPFC stimulation of the same task, to test direct cognitive control effects on gestural-verbal associations. Taken together, our findings suggest that atDCS over left M1 might have affected cognitive control mechanisms, leading to better performance in the detection of incongruent associations. Regardless of stimulation condition, we found a significantly higher accuracy for incongruent associations compared to congruent ones. Previous studies observed a similar pattern (Proverbio et al., 2014, 2015; Kelly et al., 2015), possibly due to the fact that semantic matching decision may be easier when it is obviously violated by incongruent associations.

In addition, our results go along with a recent study that used tDCS over right temporo-parietal junction followed by transcranial magnetic stimulation application over left M1. Participants were instructed to perform an action while observing either a congruent or incongruent action, while motor evoked potentials (MEPs) were elicited. Results showed that only during observation of incongruent actions, MEPs were significantly higher under atDCS compared to stDCS. This study suggested that up-regulation of the appropriate motor action leads to suppression of the congruency effect, possibly via interaction between the temporo-parietal junction and M1 (Bardi et al., 2017). The process of action observation and action execution in M1 region is related to gestural-verbal integration in humans, a process that was found to be related to mirror neuron system (for review Rizzolatti et al., 2014). Despite the fact that our paradigm included gesture observation and not production, our findings suggest that atDCS may have improved gestural-verbal integration by facilitating mirror neuron system mapping activity.

Several limitations should be considered when interpreting the results of the present study. First, the sample size was relatively modest and larger future studies be conducted to confirm these results. Second, we used tDCS, a stimulation device with low spatial resolution (Polania et al., 2018). To infer specificity of left M1 for gestural-verbal integration, a control tDCS site would have to be included. Nevertheless, specificity of left M1 atDCS for the gestural task was
demonstrated by the absence of effects on the control tasks. Finally, the sequential presentation of gesture and words might be considered less natural than the integration of auditory input and visual gestural information, so future studies should include paradigms with auditory presentation of words, to more closely resemble the natural setting of language and gesture processing.

CONCLUSIONS AND OUTLOOK

In sum, we found that enhancing left M1 improves semantic processing by specifically enhancing performance of incongruent stimuli, possibly mediated by facilitation the action perception sensitivity of the MNS. In addition, the excitatory effect of tDCS on M1 might have increased its cognitive control potential, leading to lower interference. Future studies should in more detail investigate the neural correlates of semantic congruency with tDCS and EEG exploring also the interaction between the M1 and other brain regions involved in cognitive control like the DLPFC (Koechlin et al., 2003; Gbadeyan et al., 2016), or brain regions know to closely interact with the MNS like the IFG (for review Buccino et al., 2004; Jean and Lee, 2018). In the clinical context, tDCS effects on speech and gesture processing may be relevant for patients with schizophrenia who suffer from severe deficits in speech and gesture processing (Schulke and Straube, 2018).

AUTHOR CONTRIBUTIONS

DH, AF and DA designed the research. DH and DA performed the research. DH and DA analyzed the data. DH, AF and DA wrote the article.

FUNDING

The project was funded by the German-Israeli Foundation for Scientific Research and Development Nb. I-1299-105.4/2015.

ACKNOWLEDGMENTS

We thank Prof. Michal Lavidor who provided insight and expertise during research question development and data analysis, and Dr. Magdalene Ortmann for statistical support.

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**Conflict of Interest Statement**: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix B: Anteil Aller Autoren bei Kumulativen Dissertationen

**Manuscript 1: Hayek, D., Thams, F., Floel, A., and Antonenko, D., (submitted) "Dentate gyrus volume mediates the relation between fornix microstructure and episodic memory performance in older adults"**

D.H, D.A. and A.F. designed research. D.H., F.T., and D.A. analyzed the data. D.H. performed tractography and volumetric analysis. D.H. prepared all figures. D.H. and D.A. wrote the manuscript. All authors reviewed and revised the manuscript.


D.A. and A.F. designed research. D.A., D.H. and J.N. collected the data. D.A., D.H., J.N. and U.G. analyzed the data. D.H. performed resting state functional analysis. D.A. prepared all figures. D.A. and A.F. wrote the manuscript. All authors reviewed and revised the manuscript.


DH, AF and DA designed research; DH and DA performed research; DH, AF and DA analyzed data; DH, AF and DA wrote the paper.
Appendix C: List of Publications  
(Peer-reviewed journal articles)


**Hayek, D., Thams, F., Flöel, A., Antonenko, D.,** Dentate gyrus volume mediates the effect of fornix microstructure on memory formation in older adults (submitted)

**Hayek, D, Antonenko, D. et al.,** The impact of COMT and APOE Polymorphisms on Brain Stimulation Improvement Effect (in prep)

Acknowledgments

"No one can whistle a symphony. It takes a whole orchestra to play it." – H.E. Luccock

*Prof. Dr. Agnes Flöel:* I highly appreciate your continuous support and immense knowledge that added great assets to my research profile.

*Dr. Daria Antonenko:* I don’t think I can find the right words to thank you enough for your patience, motivation, and trust. Your guidance helped me in every step of the way, both on the personal and the professional level.

My thesis committee and reviewers: *Prof. Dr. Alfons Hamm, Prof. Dr. Martin Lotze* and *Prof. Michael Nitsche* for their insightful comments and assistance.

*My lovely colleagues:* *Friederike Thams, Robert Malinowski, Annick Wüsten, Viola von Podewils, Hamza Moussa, Angelica Soussa, Gloria Benson, Claudia Schwarz, Hui Wang, Laura Goeschel, Theresa Köbe, Torsten Rackoll.* Thank you for your cooperation and friendship!

*Katholischer Akademischer Ausländer-Dienst (KAAD)* for funding my studies over the three years: *Dr. Christina Pfestroff, Mrs. Nora Kalbarczyk, Mrs. Santra Sontowski.* Your support was not only financial, but also moral and personal.

Last but not least, to the reason of my strength: *Dori Chbib, Almaza & Joseph Hayek, David and Elsy Hayek, Joe Hayek, Yara Hayek, Kamal & Vera Chbib, Joelle Chbib, Dani Chbib.* I am sure that I wouldn’t have done this without you by my side. Whatever level of success I reach, it would mean nothing without your presence.