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Effects of anodal transcranial direct current stimulation over the ventrolateral prefrontal cortex on episodic memory formation and retrieval

Angela Medvedeva[1], Maurizio Materassi[1], Victorita Neacsu[1,2], Jesssica Beresford-Webb[1], Aya Hussin[1], Naghma Khan[1], Felix Newton[1], *Giulia Galli[1]

Affiliations:

[1] Kingston University, Department of Psychology

[2] University College London, Institute of Cognitive Neuroscience

***Corresponding author:**

Kingston University

Department of Psychology, Faculty of Arts and Social Sciences

Penrhyn Road, Kingston Upon Thames

Surrey KT1 2EE United Kingdom

g.galli@kingston.ac.uk

ABSTRACT

In the past decade, several studies have investigated the effects of transcranial direct current stimulation (tDCS) on episodic memory abilities. However, the specific conditions under which tDCS affects memory remain largely unclear. Here, we report data from four experiments aimed at investigating the effects of anodal tDCS over the left ventrolateral prefrontal cortex (VLPFC) on verbal episodic memory. We evaluated tDCS-induced effects as a function of time of administration, nature of the memory encoding task, and age of the participants. A robust enhancement of memory performance was only found when anodal tDCS was delivered during intentional memorization. This enhancement was evident in young and older adults. tDCS applied during incidental memorization or during retrieval did not induce any modulation of memory performance, and memory was unaffected by offline administration before encoding or retrieval. These results show that the modulation of episodic memory functions by anodal tDCS over the left VLPFC is dependent upon the time of administration and the nature of the memory task. The findings may help profile the optimal stimulation protocols for neurorehabilitation interventions on individuals with memory decline.

KEYWORDS

Episodic memory; non-invasive brain stimulation; recognition memory; transcranial direct current stimulation; ventrolateral prefrontal cortex

INTRODUCTION

Over the past fifteen years, transcranial direct current stimulation (tDCS) has rapidly become one of the most widely used methods of non-invasive brain stimulation among neuroscientists. tDCS involves the delivery of weak electrical currents to the scalp which modulate neuronal transmembrane potentials (Purpura and McMurtry, 1965) and consequently affect motor, cognitive and behavioural processes connected to the stimulated brain regions. The polarity of the stimulation determines the effect of tDCS on cortical excitability, such that anodal stimulation induces depolarization of the resting state of neuron membrane potentials, and cathodal tDCS induces hyperpolarization (Nietsche and Paulus, 2000). The possibility of enhancing cortical excitability with anodal tDCS, together with its relative ease of use, has led researchers to explore the effectiveness of the technique in enhancing and rehabilitating cognitive functions. Beneficial effects of anodal tDCS have been reported across multiple cognitive domains in healthy (Tanoue et al., 2013; Roy et al., 2015) and neuropsychiatric populations (Ferrucci et al., 2008; Brunoni et al., 2011). However, results in the literature are mixed and across different cognitive domains, the administration of anodal tDCS has not consistently resulted in an enhancement of cognitive functions (Sellers et al., 2015; Vannorsdal et al., 2016). In an attempt to resolve these uncertainties, meta-analytical work has surged recently, but the results have not always contributed to clarifying the effects of tDCS (Hill et al., 2015; Horvath et al., 2015; Brunoni and Vanderhasselt, 2016; Dedoncker et al., 2016; Westwood and Romani, 2017).

Such heterogeneity of findings is also evident in the episodic memory literature. Episodic memory, defined as memory for information with specific spatial and temporal details (Tulving, 1983), is of particular interest for neuroscientists given its decline in healthy and pathological ageing (Budson and Price, 2005). The absence of effective pharmacological interventions to counter this decline has encouraged scientists to test the possibility that non-invasive brain stimulation may serve as alternative tool to improve memory abilities, starting with investigations in younger adults. Amongst almost thirty

published articles however, only a few tDCS studies reported enhancing effects of anodal tDCS as evidenced by a higher rate of correct responses, or by changes in combined indices of recognition memory such as d' or the discrimination index Pr (Jacobson et al., 2012; Javadi and Walsh, 2012 Experiment 1; Javadi and Cheng, 2013; Gray et al., 2015; Lu et al., 2015 Experiment 1; Pisoni et al., 2015a). Other studies reported no effects in one or more experimental conditions, or even impairing effects (Zwissler et al., 2014; Nikolin et al., 2015; Pergolizzi and Chua, 2015 Experiment 1; Pisoni et al 2015b Experiment 1; Smirni et al., 2015 Experiment 2; Chen et al., 2016; Manuel and Schneider, 2016; Gaynor and Chua, 2017). The mixed findings are likely due to diversity of stimulation parameters applied, such as the montage, site and duration of administration, the memory phase of administration (encoding vs retrieval), the time of administration with respect to the task (online vs offline), or the specific encoding or retrieval tasks used (incidental vs intentional encoding, recall vs recognition). Given that this heterogeneity of findings has contributed in the past few years to growing scepticism regarding the effectiveness of anodal tDCS, systematic investigations of the stimulation parameters that drive this variability are warranted.

Here, in four experiments we set out to use a systematic approach to profile the circumstances under which anodal tDCS effectively alters episodic memory functions. All experiments examined verbal episodic memory and used word stimuli. We focused our interest on the left ventrolateral prefrontal cortex (VLPFC). Although not a traditional target region in tDCS studies of episodic memory, the left VLPFC has consistently been associated with episodic memory encoding and retrieval in functional magnetic resonance imaging (fMRI) studies (Badre and Wagner, 2007). In addition, recent Transcranial Magnetic Stimulation (TMS) studies (Blumenfeld et al., 2014; Galli et al., 2017) have shown that episodic memory performance is more effectively modulated by the stimulation of the left VLPFC, as opposed to the left dorsolateral prefrontal cortex (DLPFC), which is a more common target of tDCS episodic memory studies.

We first examined the optimal time of anodal tDCS administration to induce effects on episodic memory abilities using an intentional memory task, in which participants were told to memorize the words while at the same time performing a pleasantness task on the words. We examined the time of administration with respect to the task (online during the task vs offline immediately before the task), and with respect to the memory phase (encoding vs retrieval). Existing findings in the literature do not allow establishing the optimal time of tDCS administration to induce episodic memory effects. Some studies targeting the DLPFC reported facilitatory effects when anodal tDCS was administered at encoding online (Penolazzi et al., 2010; Balzarotti and Colombo, 2016; Manuel and Schnider, 2016) or offline (Javadi and Walsh, 2012; Lu et al., 2015; Pisoni et al 2015a), while others reported impairing or no effects (Zwissler et al., 2014; Gaynor and Chua, 2017). Facilitatory effects of anodal tDCS over the PFC at retrieval were found with offline stimulation (Boggio et al., 2009; Javadi and Cheng, 2013; Sandrini et al., 2014; Gray et al., 2015) but other studies failed to find a significant effect (Nikolin et al., 2015; Smirni et al., 2015). We used a between-subjects, sham-controlled design to compare the effects of online vs offline stimulation when the stimulation was delivered at encoding (Experiment 1) or retrieval (Experiment 2). In Experiment 3, we examined tDCS effects using an incidental memorization task. To this aim, we administered anodal tDCS to the left VLPFC while subjects encoded words using a deep or shallow encoding task (Craik and Lockart, 1972), and assessed the effects of the stimulation on a later surprise recognition memory task. To account for the difference in the brain regions involved in deep and shallow episodic encoding (Galli, 2014), Experiment 3 also included a group that received anodal stimulation on the left parietal cortex. In Experiment 4 we capitalized on the results with young adults and examined whether the stimulation parameters that successfully enhanced memory performance in the previous experiments were equally effective in a sample of older adults. This question is of potential clinical relevance, because as mentioned previously episodic memory declines with age (Budson and Price, 2005).

EXPERIMENT 1: EFFECTS OF ANODAL tDCS DURING INTENTIONAL ENCODING

Methods

Participants

Fifty-four participants (40 females; mean age ± standard deviation: 24 ± 5 years; range: 19-41 years) were recruited for this experiment. Participants were randomly assigned to one of three groups: Online tDCS, Offline tDCS or Sham (see below for differences in the stimulation protocol between the three groups). The three groups did not differ in age (P = 0.713). Five participants took part in the study phase but did not return the following day for the test phase. This resulted in a sample of 49 participants (17 in the Online tDCS group, 15 in the Offline tDCS group and 17 in the Sham group). All participants had normal or corrected-to-normal vision, no recent history of major psychiatric disease and were native English speakers. Participants received course credits or £13 for their participation. All participants gave written informed consent. The study was approved by the Kingston University Ethics Committee.

Materials

Stimuli were 248 words (mean number of letters in words 6.17, standard deviation 1.96; mean word frequency 27.47, standard deviation 46.46; Kucera and Francis 1967) extracted from the MRC psycholinguistic database (Coltheart, 1981). For each subject, 160 words were randomly selected from this pool to be presented as old items during the study phase, and 81 words were randomly selected to be presented as new items in the test phase. Seven words were used to create practice lists for the study and test tasks.

Procedure

The procedure was identical for the three groups, except from the time of administration of tDCS during encoding (see below). On the first day, the experimenter applied the electrodes, gave instructions and run the practice trials for the study phase. Participants were then

asked to read a weekly magazine for ten minutes. The study phase started immediately after the ten minutes elapsed. During the study phase, participants saw words appearing on the screen one by one in four blocks of 40 words. Each trial started with a fixation mark shown for 500 ms, followed by the presentation of the word for 1000 ms. There was an interval of 1000 ms between the offset of the word and the onset of the following fixation mark, during which a blank screen was presented. Participants were asked to try to memorize each word for a subsequent memory test, and to press the letter A on the keyboard if they thought the word referred to a pleasant object, or the letter L if they thought the word referred to an unpleasant object. This task ensured that they attended to the words for the whole duration of the study phase. Given the subjective nature of this task, performance at this task was not analysed.

Participants returned to the lab after approximately 24 hours for the test phase. The same procedure of the study phase (electrode application, instructions, practice trial, magazine reading for ten minutes) was repeated to allow comparison with Experiment 2 in which the stimulation was delivered at retrieval. In the test phase, participants were presented with 201 words in three blocks of 67 words. Each block consisted of 40 old words and 27 new ones. To ensure that study and test phases had equal duration while accommodating for the inclusion of new trials, only old words presented in the second, third and fourth block of the study phases were repeated in the test phase. The presentation of blocks followed the same order of the study phase (e.g., words that were presented in the second block in the study phase, were presented in the first block of the test phase). Each trial started with a fixation mark shown for 500 ms, followed by the presentation of the word for 500 ms, and an intertrial interval of 1000 ms. Participants were asked to discriminate between previously-presented and new words by pressing one of two keys with their left or right index fingers. The response hand was counterbalanced across participants. At both study and test, words were presented in a white uppercase Helvetica on a grey background. At a viewing distance of approximately 55 cm, words subtended a visual angle of 1.6° vertically, and 4.3° to 11.6° horizontally.

tDCS

tDCS was delivered by a battery-driven current stimulator (DC-STIMULATOR PLUS, Neuroconn, Germany) through a pair of 5 x 7 cm saline-soaked sponge electrodes. The anode was placed over site F7 according to the 10-20 EEG system for electrode placement. This site has been used in previous studies to stimulate the VLPFC (e.g., Chrysikou et al., 2013). The cathode electrode was placed extracranially over the contralateral deltoid muscle to avoid opposite polarization in another brain area (Wolkenstein and Plewnia, 2013). The active stimulation was delivered with a current of 2 mA. In the Offline tDCS group, the stimulation started at the same time as the reading task and lasted until the start of the encoding phase (ten minutes). In the Online tDCS group, the stimulation started with the onset of the encoding phase and covered its whole duration (approximately nine minutes). In the sham group, the stimulation lasted for 30 seconds. Offline, online and sham stimulations included a 10-second ramp-up. For sham stimulation, this elicits a transient tingling sensation on the scalp that fades after a few seconds, mimicking the sensations felt at the beginning of the anodal stimulation and therefore ensuring blinding of participants to the stimulation condition (Gandiga et al., 2006). In this group, the stimulation started with the onset of the encoding phase in half participants, and with the onset of the reading task in the other half. The study was a single-blind experiment: participants were not aware of the stimulation they received, but the experimenter was fully informed.

Results

For statistical analyses, the accuracy of recognition memory judgements was established with the discrimination index Pr (the proportion of hits minus the proportions of false alarms; Snodgrass & Corwin, 1988). Accuracy was significantly above chance in the three groups (all *t*s > 3.451). We used the bias index Br (Snodgrass and Corwin, 1988) to evaluate response bias (False Alarms/[1-(Hits-False Alarms]). Significant interactions were followed

up by planned pairwise comparisons between each active stimulation group and the Sham group. A one-way ANOVA showed that discrimination accuracy differed across the three groups ($F_{2,46}$ = 5.69, P = 0.006, η^2 = 0.198). Pairwise comparisons revealed that compared to sham memory performance was higher in participants who received the stimulation online (t_{32} = 3.74, P = 0.001, d = 1.286; Figure 1), but not in participants who received the stimulation offline (t_{30} = 0.66). Response bias did not significantly differ between the three groups ($F_{2,46}$ = 2.83; Table 1). Next, we ran separate ANOVAs on the proportion of hits and false alarms, and found that the three groups differed in the proportion of false alarms ($F_{2,46}$ = 5.38, P = 0.008, η^2 =0.190). The false alarm rate was lower in the Online tDCS group compared to the Sham group (t_{32} = 3.18, P = 0.003, d = 1.092, Table 1). There was no significant difference in the proportion of hits across the three groups ($F_{2,46}$ = 0.17; Table 1). This indicates that the increase in memory accuracy in participants who received the stimulation during encoding was mainly driven by a decrease in false alarms. The proportion of hits was not affected by the pleasantness judgement in the study phase (ts < 0.74).

The three groups differed in reaction times ($F_{2,46}$ = 4.54, P = 0.016, η^2 =0.165). Accurate memory judgements were slower for the Online compared to the Sham group (t_{32} = 2.79, P = 0.009, d = 0.961), but did not differ between the Offline and the Sham group (t_{30} = 0.29).

EXPERIMENT 2: EFFECTS OF ANODAL tDCS DURING RETRIEVAL

Methods

Participants

Fifty-four participants (42 females; mean age ± standard deviation: 22 ± 3 years; range: 19-30 years) were recruited for this experiment. Participants were randomly assigned to one of three groups: Online, Offline or Sham. The three groups did not differ in age (P = 0.833).

Data from five participants were excluded from statistical analysis because (i) participants did not return for the second experimental session (one participant in the Online group) (ii) participants felt discomfort during the stimulation (one participant in the Online group and one participant in the Offline group) and (iii) technical failures (two participants in the Offline group). The remaining 49 participants (16 in the Online group, 15 in the Offline group and 18 in the Sham group) had normal or corrected-to-normal vision, no recent history of major psychiatric disease and were native English speakers. Participants received course credits or £13 for their participation. All participants gave written informed consent. The study was approved by the Kingston University Ethics Committee.

Materials, Procedure and tDCS

Stimuli, procedure and tDCS administration were identical to Experiment 1, with the exception that for the active stimulation groups tDCS was delivered on the second day for ten minutes at the start of the reading task (Offline Retrieval group) or at the onset of the memory phase (Offline Retrieval group).

Results

Accuracy was significantly above chance in the three groups (all *t*s > 3.141). There was no significant difference between the groups in any accuracy measure, or response times (Table 1, *F*s < 1.45).

EXPERIMENT 3: EFFECTS OF ANODAL tDCS DURING INCIDENTAL ENCODING

Methods

Participants

Thirty-six participants (21 females; mean age ± standard deviation: 23 ± 5 years; range 19-39 years) were recruited for this experiment. Participants were randomly assigned to the frontal or parietal stimulation group. The two groups did not differ in age (P = 0.462). Data from five participants were excluded from statistical analysis because participants (i) did not return for the second experimental session (three in the frontal and one in the parietal group) and (ii) did not complete the first experimental session for technical problems (one participant in the parietal group). The remaining 31 participants (15 in the frontal stimulation group, 16 in the parietal stimulation group) had normal or corrected-to-normal vision, no recent history of psychiatric disease and were native English speakers. Participants received course credits or £30 for their participation. All participants gave written informed consent. The study was approved by the Kingston University Ethics Committee.

Materials

Stimuli were 504 words (mean number of letters in words 6.51, SD 2.15; mean word frequency 32.82, SD 62.59; Kucera and Francis 1967) extracted from the MRC psycholinguistic database (Coltheart, 1981). Half of the words contained the letter “e”, the other half did not contain the letter “e”, with an equal number of words referring to animate and inanimate entities. For each subject, 252 words were randomly selected from this pool to be presented during active stimulation, the other 252 were selected for the sham stimulation. In each stimulation condition, 150 words were randomly designated as old items for the study phase, and 102 as new items for the test phase. An additional 12 words were selected from the MRC database to create practice lists for the study and test tasks.

Procedure

The task consisted of an incidental memory task followed by a recognition memory test after a delay of approximately one hour. Participants completed two study-test cycles, one for active stimulation and one for sham. To avoid the expectation of a memory test in the second session, participants were recruited for a word judgement experiment and were told

that the tasks were randomly selected, so that the tasks on the first and second session would likely be different. At study, participants viewed a total of 150 words, presented one at a time. Each word was preceded by a cue, which consisted of the presentation of the letter *O* or the letter *X*. When an *O* appeared, subjects were instructed to report whether the following word referred to a living or a non-living entity (animacy judgement, deep encoding task). When an *X* preceded a word, subjects had to decide whether the word contained the letter “e” or not (alphabetical judgement, shallow encoding task). Animacy and alphabetical judgements were equiprobable. In both tasks, subjects responded by pressing one of two buttons on the keyboard with their right or left index fingers. The hand with which each judgement was made was counterbalanced across participants to prevent rule effects. In the test phase, the 150 words from each study block were interspersed with 102 new words and presented again for the recognition memory task. For each word, participants had to decide whether or not they had seen the word during the study phase by pressing one of two keys with their right or left index fingers. The assignment of old responses to the left or right hand was counterbalanced across subjects.

At both study and test, each trial started with the presentation of a fixation mark for 500 ms, followed by the presentation of the word, which remained on the screen for 500 ms. In the study phase, each word was preceded by the presentation of the cue, which had a duration of 2600 ms. There was an interval of 2800 ms plus a random delay between 0 and 1000 ms between the offset of the word and the onset of the following trial, during which a blank screen was presented. The order of study and test words was randomized anew for each participant. Cues and words were presented in a white uppercase Helvetica on a grey background. At a viewing distance of approximately 55 cm, words subtended a visual angle of 1.6° vertically, and 4.3° to 11.6° horizontally. Cues measured 1.6° x 1.4° of visual angle.

tDCS

tDCS was delivered by a battery-driven current stimulator (DC-STIMULATOR PLUS, Neuroconn, Germany) through a pair of 5 x 7 cm saline-soaked sponge electrodes. In the

frontal stimulation group, the anode was placed over site F7 according to the 10-20 EEG system for electrode placement. In the parietal stimulation group, the anode was placed over site P3. In both groups, the cathode electrode was placed extracranially over the contralateral deltoid muscle. Sham or active stimulation was delivered during encoding in two separate sessions spaced one week apart. In the anodal stimulation session, stimulation lasted 15 minutes including a 10-second ramp-up (therefore covering the whole duration of the encoding phase) with a current of 1.5 mA. In the sham session, the stimulation lasted for 30 seconds including a 10-second rump-up. The stimulation order was counterbalanced across subjects, so that in each group half subjects started with anodal stimulation, and the other half with sham. The study was a single-blind experiment: participants were not aware of the stimulation they received, but the experimenter was fully informed.

Results

Encoding task

Accuracy and response times of encoding judgements were analysed with a mixed-model ANOVA with the within-subjects factors Stimulation (Active, Sham) and Encoding Task (Deep, Shallow), and the between-subjects factor Site (Frontal, Parietal). As expected, deep encoding judgements were more accurate than shallow encoding judgements ($F_{1,29} = 17.45$, $P < 0.001$, $\eta^2 = 0.376$). There were no significant main effects or interactions involving the factor Stimulation (all Fs < 1.06). No significant effects emerged from the analysis of response times (Fs < 2.42).

Memory task

Accuracy was significantly above chance in both groups (ts > 8.471). A mixed-model ANOVA with the within-subjects factors Stimulation (Active, Sham) and Encoding Task (Deep, Shallow), and the between-subjects factor Site (Frontal, Parietal), revealed a main effect of Encoding Task on the discrimination index Pr ($F_{1,29} = 91.11$, $P < 0.001$, $\eta^2 = 0.759$), response bias Br ($F_{1,29} = 88.96$, $P < 0.001$, $\eta^2 = 0.754$) and response times ($F_{1,29} = 12.34$, P

= 0.001, η^2 = 0.29). This indicated that in both groups words encoded with the deep encoding task yielded higher memory accuracy, less conservative response bias and faster response times compared to words encoded with the shallow encoding task (all *P*s < 0.001). The stimulation did not modulate memory performance in either group, as evidenced by the non-significant main effect of Stimulation, and the lack of significant interactions involving this factor in all measures (Pr, Hits, FAs, Br and RTs, all *F*s < 1.66; Table 1 and Figure 2).

EXPERIMENT 4: EFFECTS OF ANODAL tDCS DURING INTENTIONAL ENCODING IN ELDERLY ADULTS

Methods

Participants

Twenty-six participants (17 females; mean age ± standard deviation: 73 ± 6 years; range 65-88 years; mean education ± standard deviation: 14 ± 2 years, range 10-17) were recruited for this experiment. Participants were older adults with no evidence of pathological age-related cognitive decline, as assessed by the Mini Mental State Examination (MMSE, Folstein et al.,1975) administered upon arrival to the laboratory (mean score ± standard deviation: 28.6 ± 1.4; range 25-30). Participants were randomly assigned to the Online stimulation or the Sham group. The two groups did not differ in age, years of education or MMSE scores (*P* = 0.414, *P* = 0.416 and *P* =0.265, respectively). Data from four participants were excluded from statistical analysis because (i) participants did not return for the second experimental session (one participant in the Online group) (ii) participants quit the experiment (one participant in the Online group and one participant in the Sham group) and (iii) technical failures (one participant in the Sham group). The remaining 22 participants (11 in the Online group and 11 in the Sham group) had normal or corrected-to-normal vision, were native English speakers and in good general health. All participants gave written informed consent. The study was approved by the Kingston University Ethics Committee.

Materials, Procedure and tDCS

Stimuli, procedure and tDCS administration were identical to Experiment 1, with the exception that only an Online stimulation group was included, and that words were presented for 1.5 sec instead of 1 sec, to account for the general age-related slowing in processing speed (Salthouse, 1993).

Results

Accuracy was significantly above chance in both groups (*t*s > 4.229). An independent-samples t-test revealed that discrimination accuracy was higher in participants in the active stimulation group compared to the Sham group (t_{20} = 2.30, *P* = 0.033, *d* = 1.007; Figure 3). There was no difference between the two groups in hits and false alarm rates, response bias or response times (*t*s < 1.09).

DISCUSSION

We examined the circumstances under which anodal tDCS over the left VLPFC effectively improves episodic memory abilities. We showed a robust enhancement of memory performance only when anodal tDCS was delivered online during intentional memorization. This finding was replicated in a sample of older adults. tDCS administered online during incidental memorization or during retrieval did not induce any modulation of memory performance, nor did tDCS administration offline.

The observation that anodal tDCS delivered to the left VLPFC during encoding enhanced later memorability is in line with a number of fMRI and TMS studies that showed an involvement of this brain region in the formation of verbal memory traces (Henson et al., 1999; Kirchhoff et al., 2000; Blumenfeld et al., 2014; Galli et al., 2017). The analysis on hits and false alarms clarified the specific mechanisms of action of tDCS upon encoding. False alarms were reduced by 20% by anodal tDCS, whereas hits and response bias were not affected by the stimulation. Therefore, tDCS enhanced memory accuracy by acting upon

processes that decrease later false recognition of new items, rather than strengthening memory traces of old ones. One might think that any such process would be triggered by the presentation of new words at retrieval. However, neuroimaging and behavioural evidence suggest that the encoding process is critical in generating false remembering (Gallo et al., 2001; Kim and Cabeza, 2007). One hypothesis is that anodal tDCS enhanced distinctive processing during encoding. This idea is consistent with the suggested role of the VLPFC in the goal-relevant selection of item information during encoding, which contributes to distinctive processing (Blumenfeld and Ranganath, 2007). An enhancement of distinctive processing would certainly benefit memory performance in the context of the present experiments because the long word lists induced a high degree of semantic similarity and consequently a high baseline false alarm rate (see Table 1). More specifically, we suggest that anodal tDCS during intentional encoding boosted distinctiveness by emphasising features specific to individual items in the word list. Interestingly, this item-specific processing at encoding (Hunt and Einstein, 1981; Arndt and Reder, 2003) has been shown to decrease false recognition while leaving hit rates and response bias unaltered (McCabe et al., 2004), a pattern of results that mirrors the tDCS effects observed in Experiment 1.

We did not find any effect of anodal tDCS when the stimulation was delivered offline, or online during retrieval or incidental encoding. This may indicate that in all these cases the left VLPFC was idle or not extensively activated, thereby preventing any effect of tDCS on memory performance. This is comprehensible on the assumption of state-dependency of tDCS effects. tDCS-induced effects are sensitive to the state of the network and modulate the firing of those neurons that are already activated by a given task (Miniussi et al., 2013). Consequently, if the left VLPFC was not particularly activated while participants were performing the recognition task (online condition in Experiment 2), or the depth of processing task during incidental encoding (Experiment 3), one would not expect any reliable effect of tDCS. It could be that brain regions other than the left VLPFC were active during memory retrieval in Experiment 2, such as the posterior parietal cortex (Wagner et al., 2005), the

hippocampus (Rugg and Vilberg, 2012), or the dorsolateral prefrontal cortex (Rugg et al., 2002). With respect to Experiment 3, it should be noted that although several fMRI studies revealed an involvement of the left VLPFC in incidental memory formation, activations of this brain region were specifically associated with memory formation for deeply encoded items (Galli, 2014). In Experiment 3, tDCS did not selectively modulate the encoding or retrieval performance for deeply encoded items, hence the exact contribution of the left VLPFC in this experiment is not entirely clear.

One further observation regarding Experiment 3 is that memory was probed after a delay of one hour. This is considerably shorter than the delay in Experiment 1 and 4 which also involved tDCS during encoding. One could speculate that the interval between the end of the stimulation and the memory test in Experiment 3 was not long enough to induce long-term consolidation of the encoded material. We cannot rule out this possibility on the basis of our dataset. However, given that tDCS-induced improvements are thought to be based on long-term-potentiation-like increases of synaptic strength which occur relatively early after learning (Liebtanz et al., 2002), it is reasonable to assume that synaptic consolidation processes had at least partly occurred during the retention interval of Experiment 3. In addition, a retention interval of one hour or less was sufficient in previous PFC anodal tDCS studies to demonstrate an increase of episodic memory performance (Penolazzi et al., 2010; Javadi and Walsh, 2012; Gray et al., 2015; Pisoni et al., 2015b), although not consistently (Zwissler et al., 2014; Nikolin et al., 2015; Smirni et al., 2015). More systematic approaches are needed to understand the pattern of tDCS effects over time.

Finally, our finding that the offline condition in Experiment 1 and 2 did not affect performance suggests that, at least in the episodic memory domain and the left VLPFC, tDCS effects take place during the stimulation rather than after its termination. This contradicts the results of previous studies which systematically compared online and offline stimulation in other domains and found prominent offline effects (Pirulli et al., 2013; Santarnecchi et al., 2014), and episodic memory studies that found no effects of tDCS on

learning rate during the stimulation, but found effects offline after a delay (Floel et al., 2012; Sandrini et al., 2014). In summary, we suggest that there is no general indication as to whether tDCS is more effective online or offline. Rather, the temporal specificity of tDCS varies as function of the involvement of the stimulated brain region during a specific stage of processing and associated cognitive functions.

In Experiment 4 we showed that the tDCS effects found in younger adults in Experiment 1 could be replicated in a sample of older individuals. Memory performance of older adults was enhanced by anodal tDCS administered online during the intentional encoding task. This effect was specific to the discrimination index. We found a numerical but not statistical difference in the false alarm rate between active stimulation and sham, mimicking the response pattern observed in younger adults (see Table 2). Whereas our sample size enabled sufficient power to detect the large effect on the discrimination index, it may not have been large enough to detect the smaller effect on the false alarm rates. Further studies will need to establish whether the tDCS modulation of memory accuracy in the elderly is driven by changes in false recognition. Our result of improved discrimination is broadly in line with two previous studies which showed an improvement of episodic memory abilities following anodal tDCS in the elderly (Manenti et al., 2013; Sandrini et al., 2014). Both studies targeted the DLPFC, and the effects were evident when the stimulation was delivered during retrieval (Manenti et al., 2013) or during a reconsolidation session between encoding and retrieval (Sandrini et al., 2014). At present, these findings are not easily reconciled with our tDCS encoding effects. In general, however, the results of Experiment 4 show one way in which episodic memory functions in older adults can be improved by anodal tDCS application. This result paves the way for future studies aimed at investigating the effects of tDCS in pathological aging conditions characterized by a loss of episodic memory abilities, such as Alzheimer's disease.

We have previously mentioned that the left DLPFC is a more common target area of episodic memory studies. It may then be worth examining how our results compare with

verbal episodic memory studies that delivered anodal tDCS over this region. We have identified seven studies (Elmer et al., 2009; Javadi and Walsh, 2012; Javadi and Cheng, 2013; Manenti et al., 2013; Nikolin et al., 2015; Pergolizzi and Chua, 2015; Gaynor and Chua, 2017), all of them using an intentional encoding task. When the stimulation was delivered at encoding, online tDCS decreased recognition memory accuracy (Gaynor and Chua, 2017), and offline tDCS increased it (Javadi and Walsh, 2012 Experiment 1). The stimulation between encoding and retrieval did not modulate recognition memory (Javadi and Cheng, 2013, Nikolin et al., 2015), or increased it only in conjunction with a consolidation session (Javadi and Cheng, 2013). Finally, the stimulation online during retrieval or covering both encoding and retrieval decreased memory accuracy (Manenti et al., 2013), or did not induce any effect (Elmer et al., 2009; Pergolizzi and Chua, 2015). The lack of a comparison within the same experimental set-up prevents a straightforward conclusion on the optimal timings of left DLPFC stimulation, but the studies reviewed above seem to suggest that offline effects are stronger than online effects. It will be of considerable interest to test this assumption directly in future studies.

Two limitations of the current set of studies should be mentioned. First, although we aimed to stimulate the left VLPFC, the size of the stimulating electrode cannot rule out that adjacent areas of the PFC were also affected by the stimulation. In addition, because of the lack of a control stimulation site aspecific effects of the stimulation cannot be ruled out. However, any such effect would be diffcult to reconcile with the observation that tDCS effects were found in some experimental conditions, but not others.

The results of the current studies help to clarify the optimal set-up that future rehabilitation studies could adopt to enhance episodic memory abilities in patients. We also believe that besides their potential clinical relevance, our findings help refine our knowledge of the conditions under which anodal tDCS is and - equally importantly - is not effective in modulating memory functions.

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Time of tDCS administration on the VLPFC			
	Online tDCS	**Offline tDCS**	**Sham**
Encoding (Intentional - Experiment 1)			
Discrimination Pr	0.28 (0.12)	0.16 (0.18)	0.12 (0.13)
Response bias Br	0.51 (0.15)	0.60 (0.16)	0.63 (0.17)
Proportion of Hits	0.65 (0.10)	0.67 (0.15)	0.68 (0.15)
Proportion of False Alarms	0.37 (0.16)	0.51 (0.17)	0.56 (0.19)
Retrieval (Intentional - Experiment 2)			
Discrimination Pr	0.15 (0.15)	0.14 (0.17)	0.16 (0.11)
Response bias Br	0.53 (0.17)	0.61 (0.19)	0.52 (0.15)
Proportion of Hits	0.60 (0.17)	0.68 (0.14)	0.60 (0.12)
Proportion of False Alarms	0.45 (0.16)	0.54 (0.22)	0.44 (0.16)
Encoding (Incidental - Experiment 3)			
Discrimination Pr	0.35 (0.16)		0.34 (0.10)
Response bias Br	0.39 (0.19)		0.38 (0.16)
Proportion of Hits	0.60 (0.15)		0.58 (0.12)
Proportion of False Alarms	0.25 (0.15)		0.24 (0.13)

Table 1: Memory performance across the three experiments on young adults. In Experiment 3, performance is collapsed across deep and shallow encoding. Discrimination Pr and response Bias Br: proportion of hits minus proportion of false alarms and false Alarms/[1-(Hits-False Alarms], respectively (Snodgrass and Corwin, 1988). Standard deviations are displayed in parentheses.

	Online tDCS	Sham
Discrimination Pr	0.27 (0.11)	0.15 (0.12)
Response bias Br	0.57 (0.18)	0.58 (0.21)
Proportion of Hits	0.68 (0.16)	0.64 (0.18)
Proportion of False Alarms	0.41 (0.14)	0.49 (0.17)

Table 2: Memory performance in Experiment 4. Discrimination Pr and response Bias Br: proportion of hits minus proportion of false alarms and false Alarms/[1-(Hits-False Alarms], respectively (Snodgrass and Corwin, 1988).Standard deviations are displayed in parentheses.

CAPTIONS

Figure 1: Effects of anodal tDCS in Experiment 1. Memory accuracy (Pr Hits – Pr False alarms) in young participants who received the stimulation online during encoding, offline during encoding, and in participants in the Sham group. ****P*=0.001, significant by independent samples *t*-test. Effect sizes for group differences are shown as Cohen's *d*. Error bars depict standard error.

Figure 2: Effects of anodal tDCS in Experiment 3. Memory accuracy (Pr Hits – Pr False alarms) in the left frontal (A) and left parietal (B) group, as a function of depth of encoding. Error bars depict standard error.

Figure 3: Effects of anodal tDCS in Experiment 4. Memory accuracy (Pr Hits – Pr False alarms) in elderly participants. **P*=0.033, significant by independent samples *t*-test. Effect sizes for group differences are shown as Cohen's *d*. Error bars depict standard error.