The optimal timing of stimulation to induce long-lasting positive effects on episodic memory in physiological aging

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ABSTRACT

Episodic memory displays the largest degree of age-related decline. A noninvasive brain stimulation technique that can be used to modulate memory in physiological aging is transcranial Direct Current Stimulation (tDCS). However, an aspect that has not been adequately investigated in previous studies is the optimal timing of stimulation to induce long-lasting positive effects on episodic memory function.

Our previous studies showed episodic memory enhancement in older adults when anodal tDCS was applied over the left lateral prefrontal cortex during encoding or after memory consolidation with or without a contextual reminder.

Here we directly compared the two studies to explore which of the tDCS protocols would induce longer-lasting positive effects on episodic memory function in older adults. In addition, we aimed to determine whether subjective memory complaints would be related to the changes in memory performance (forgetting) induced by tDCS, a relevant issue in aging research since individuals with subject memory complaints seem to be at higher risk of later memory decline.

The results showed that anodal tDCS applied after consolidation with a contextual reminder induced longer-lasting positive effects on episodic memory, conceivably through reconsolidation, than anodal tDCS during encoding. Furthermore, we reported, providing new data, a moderate negative correlation between subjective memory complaints and forgetting when anodal tDCS was applied after consolidation with a contextual reminder.

This study sheds light on the best-suited timing of stimulation to induce long-lasting positive effects on memory function and might help the clinicians to select the most effective tDCS protocol to prevent memory decline.

Episodic memory refers to the ability to retrieve information on what has happened and where and when these events took place [1]. This type of declarative memory displays the largest degree of age-related decline, a process that is accelerated in pathological conditions like Alzheimer's disease [2]. Early interventions aiming to prevent or delay memory decline focus on prodromal stages such as physiological aging, subjective memory complaints and amnestic Mild Cognitive Impairment [3, 4].

A noninvasive brain stimulation technique that can be used to modulate memory in physiological aging is transcranial Direct Current Stimulation (tDCS) [5, 6]. TDCS has been used for two main purposes in memory research: (1) to test the causal relationship between activity of a cortical region and a memory function; and (2) to investigate whether tDCS might modulate memory formation and learning, an issue of relevance for research and neurorehabilitation [5, 7-9].

tDCS studies have shown that the left lateral prefrontal cortex (PFC) contributes to verbal episodic memories along the life span [8-13] and enhancement of these abilities has been reported in healthy older adults after the application of tDCS over this brain area [8, 9, 13]. Specifically, Sandrini and coworkers [8, 9] showed that anodal tDCS strengthened memories when applied during the encoding phase or after the consolidation process in elderly individuals. However, an aspect that has not been directly investigated in previous studies is the optimal timing of stimulation to induce long-lasting positive effects on episodic memory function. Furthermore, participants' characteristics must be considered since some researches have highlighted that inter-individual differences might influence the magnitude of tDCS effects [10, 14, 15].

In the present study, we re-analyzed data acquired from two previous studies [8, 9] in which we showed episodic memory enhancement in older adults when anodal tDCS was applied to the left PFC during the encoding phase [9] or after the memory consolidation process [24h after the encoding session, 8] using the same word-learning paradigm.

The aim of this study was to compare the two studies to explore which of the tDCS protocols would induce longer-lasting positive effects on episodic memory function in older adults. In addition, we aimed to determine whether subjective memory complaints would be related to the changes in memory performance (forgetting) induced by tDCS, a relevant issue in aging research since individuals with subject memory complaints seem to be at higher risk of later memory decline [16].

In our first study elderly participants learned a list of words and 24h later (i.e. after consolidation) they received tDCS over the left PFC with or without a contextual reminder (respectively, same or different experimental room as done in previous studies [18,19]). On Day 3 and Day 30 (respectively 48h and 30 days from the learning phase) they were asked to recall the words [8]. In

the successive study, elderly participants learned the list of words while receiving tDCS over the left PFC during encoding. As in the previous study [9] they were asked to recall the words on Day 3 and Day 30 [9]. Both studies employed a between-subject experimental design. The only difference between the two studies was the timing of stimulation (see Figure 1).

Data from 64 older individuals (mean age=67.91 years) were included in the analysis. Participants reported being free of neurological and psychiatric disorders and had no history of seizures. The protocols got ethical approval from the local Human Ethics Committee of Saint John of God Clinical Research Centre, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy. Prior to being enrolled in the experiment, older subjects completed a Mini Mental State Examination (MMSE) and a detailed neuropsychological evaluation to verify the absence of any cognitive deficit. A pathological score in one or more of the tests was an exclusion criterion. In addition, the participants completed the Cognitive Reserve Index questionnaire (CRIq), the Memory strategies questionnaire aiming to record strategies used during the encoding phase, and the Everyday Memory Questionnaire (EMQ) [17, 18] that serves as an easy-to-use instrument to evaluate subjective memory complaints (SMC) (see Table 1).

tDCS was applied over the left PFC (intensity: 1.5 mA, duration: 15 minutes, current density 0.043 mA/cm2, size of electrodes: 5x7 cm2) [8, 9, 13, 19]: the anode was placed over F3 according to the 10-20 EEG international system for electrode placement, and the cathode was placed over the right supraorbital area (see Figure 1). The placement of the reference electrode over the supraorbital region was motivated by the assumption that, since this area is not specifically involved in memory processing, this electrode would not actively contribute to modulation. This montage delivered a diffuse current flow pattern, but seems to be the better choice because it induces a more focused current on the targeted area [20]. In the placebo (sham) stimulation, the tDCS montage was the same, but the current was turned off 10s after the beginning of the stimulation (plus the duration of the fade-in=10s) and was turned on for the last 10s of the stimulation period (plus the duration of the fade-out =10s). Potential tDCS side effects were assessed with a questionnaire at administered at the end of the stimulation session.

In order to re-analyze the data acquired in two previous studies [8, 9] we run a series of analyses including all the 64 participants' data divided in five groups: Anodal tDCS during encoding (N=14), Placebo tDCS during encoding (N=14) [9], Anodal tDCS after consolidation with a reminder (N=12), Anodal tDCS after consolidation without a reminder (N=12), Placebo tDCS after consolidation with a reminder (N=12) [8].

Statistical analyses were performed using Statistica software (version 10; www.statsoft.com) and significance level was set at α=0.05.

The first step included a series of non-parametric (Kruskal-Wallis test) analyses on demographic and neuropsychological variables, tDCS sensations, cognitive reserve, strategies used and EMQ score in order to assess possible differences between the five experimental groups. We found no differences in age (H(4)=4.57, p=0.33), education (H(4)=3.15, p=0.53), standardized neuropsychological tests scores, strategies used (H(4)=2.29, p=0.68), cognitive reserve accumulated (H(4)=0.48, p=0.97) and subjective memory complaints (H(4)=6.93, p=0.14).

Subsequently, in order to compare the learning rate of the five experimental groups, we recorded how many learning trials (1–5) were necessary for each participant to recall at least 17 words (85%) on Day 1. Participants who recalled <17 words during the fifth learning trial were given a score of 6. Participants needed on average 5.1 (Standard Deviation, SD=1.0) learning trials to reach the criterion on Day 1 (Anodal tDCS during encoding: mean, M=4.93 SD=1.0; Placebo tDCS during encoding: M=5.0 SD=0.9; Anodal tDCS after consolidation with a reminder cue: M=5.0 SD=1.1; Anodal tDCS after consolidation without reminder cue: M=5.0 SD=1.1; Placebo tDCS after consolidation with a reminder cue: M=5.2 SD=1.1). There were no significant differences between the groups (H(4)=4.06, p=0.40).

Furthermore, participants recalled on average the 76.6% (SD=12.4) of the words at the last learning trial (Anodal tDCS during encoding: M=75.0 SD=11.6; Placebo tDCS during encoding: M=71.4 SD=11.6; Anodal tDCS after consolidation with a reminder cue: M=81.6 SD=12.4; Anodal tDCS after consolidation without reminder cue: M=77.5 SD=12.4; Placebo tDCS after consolidation with a reminder cue: M=78.3 SD=12.4). No significant differences were found between the groups (H(4)=2.02, p=0.73) (see Figure 2).

An ANOVA model for repeated measures was adopted to analyse the individual mean percentage of words correctly recalled including one within factor “time” (Day 1, Day 3 and Day 30) and one between factor “group” [Anodal tDCS during encoding, Placebo tDCS during encoding [9]; Anodal tDCS after consolidation with a reminder, Anodal tDCS after consolidation without reminder, Placebo tDCS after consolidation with a reminder [8]]. Post-hoc analyses were carried out by Fisher’s Least Significant Difference (LSD) tests for evaluating pairwise comparisons among levels of ANOVA significant factors in order to discover which of the comparisons were responsible for rejections in ANOVA test.

The main effect “time” was significant ($F(2,59)=169.55$, $p<.001$, $\eta p2 =0.74$). Post-hoc comparisons showed that memory performance decreased from Day 1 to Day 3 (Day 1: M=76.6 SD=12.4; Day

3: M=43.6 SD=20.3; p<0.001) and from Day 1 to Day 30 (Day 30: M=37.9 SD=25.0; p=0.013). The main effect "group" was significant (F(4,59)=3.64, p=.010, ηp2 =0.20). Post-hoc comparisons showed enhanced memory performance in Anodal tDCS after consolidation groups (with or without a reminder) relative to Placebo tDCS after consolidation with a reminder group (Anodal tDCS after consolidation with a reminder group vs. Placebo tDCS after consolidation with a reminder group: p=0.006; Anodal tDCS after consolidation without a reminder group vs. Placebo tDCS after consolidation with a reminder group: p=0.038). The interaction between "group" and "time was also significant (F(8,118)=3.48, p=0.001, ηp2=0.19). Post-hoc comparisons revealed that the groups that received Anodal stimulation recalled more words than Placebo stimulation groups on Day 3 (Anodal tDCS during encoding vs. Placebo tDCS during encoding: p= 0.013; Anodal tDCS after consolidation with a reminder vs. Placebo tDCS after consolidation with a reminder: p= 0.019; Anodal tDCS after consolidation without reminder vs. Placebo tDCS after consolidation with a reminder: p= 0.018) (see Figure 2).

Regarding memory performance on Day 30, Anodal tDCS applied after the consolidation process (with or without contextual reminder) enhanced memory recall relative to placebo tDCS group (Anodal tDCS after consolidation with a reminder vs. Placebo tDCS after consolidation with a reminder: p<0.001; Anodal tDCS after consolidation without reminder vs. Placebo tDCS after consolidation with a reminder: p= 0.004). However, Anodal tDCS applied during encoding did not enhance memory recall relative to placebo tDCS group (Anodal tDCS during encoding vs. Placebo tDCS during encoding: p= 0.10). In addition, Anodal tDCS after consolidation with a reminder enhanced memory recall relative to Anodal tDCS during encoding (p=0.011), but not relative to Anodal tDCS after consolidation without a reminder (p=0.23). There was no significant difference between Anodal tDCS after consolidation without a reminder and Anodal tDCS during encoding (p=0.19).

Interestingly, the group that received Anodal tDCS during encoding showed a significant decrease in memory performance from Day 3 to Day 30 (Anodal tDCS during encoding: Day 3: M=52.1 SD=17.7; Day 30: M=37.6 SD=24.4, p=0.003). None of the other experimental groups showed a significant performance decrease from Day 3 to Day 30 (Placebo tDCS during encoding: Day 3: M=34.5 SD=18.4, Day 30: M=26.0 SD=18.8, p=0.09; Anodal tDCS after consolidation with a reminder: Day 3: M=49.9 SD=24.3; Day 30: Mean=56.3 SD=25.6, p=0.23; Anodal tDCS after consolidation without reminder: Day 3: M=49.9 SD=17.3; Day 30: M=47.2 SD=25.9, p=0.60; Placebo tDCS after consolidation with a reminder: Day 3: M=31.9 SD=16.6; Day 30: M=24.7 SD=17.6, p=0.17). This decreased in memory performance from Day 3 to Day 30 observed

exclusively in the Anodal tDCS during encoding group suggests that the facilitation effects observed up to Day 30 in the Anodal tDCS groups after the consolidation process (with or without a contextual reminder) are conceivably attributable to tDCS over the left PFC during reconsolidation [21] and not to the strengthening effects induced by repeated reactivations of the consolidated memories during retrieval [22] on Day 3 (four recall tests) (see Figure 2).

Reactivation of consolidated memories during retrieval or by a reminder triggers reconsolidation, a time-limited period during which consolidated memories may be modified by behavioral means (e.g. repeated reactivations, extinction), noninvasive brain stimulation or pharmacological interventions [21].

Previous reconsolidation studies in young adults showed that consolidated memories are automatically reactivated if the subjects are in the same context (i.e. same experimental room) of Day 1 [23, 24]. The pattern shown by older adults suggests that their consolidated memories might have been reactivated by other factors (e.g., the context may have been encoded at a general level but without distinctive detail [8, 21, 25]) and subsequently strengthened through the effect of anodal tDCS over the left PFC during reconsolidation.

Finally, we analyzed unpublished data regarding individual SMC, formally assessed with the EMQ, to investigate the relationship between SMC and forgetting on Day 3 and Day 30. A measure of forgetting was obtained by calculating for each participant the change in performance (delta) between the first recall test on Day 3 or on Day 30 and the last learning trial on Day 1. The correlation analyses (Pearson correlation coefficient) were performed between EMQ total scores and forgetting delta scores. The data set was preliminary examined for outliers and this analysis showed no evidence of significant outliers.

A significant moderate negative correlation was found between EMQ scores and forgetting delta scores in the Anodal tDCS after consolidation with a reminder group ($r=-0.59$, $p=0.043$), indicating that higher EMQ scores (higher SMC) were associated with less forgetting after the application of Anodal tDCS after the consolidation process (see Figure 3B). A significant moderate positive correlation was found between EMQ scores and forgetting delta scores in the Anodal tDCS during encoding group ($r=0.53$, $p=0.049$), suggesting that higher EMQ scores (higher SMC) were associated with greater forgetting after the application of Anodal tDCS during the encoding phase (see Figure 3A). No other significant correlations were found on Day 3 and on Day 30.

Thus, even if we did not find any significant correlation between SMC and forgetting on Day 30, the correlation analysis suggests that Anodal tDCS after consolidation with a reminder should be applied to older adults with higher subjective memory complaints in order to maximize the positive

effects on memory function (i.e. reduced forgetting on Day 3). Since all groups received a single session of Anodal tDCS and the same number of retrieval sessions, we suggest that the association observed when Anodal tDCS was applied after consolidation with a reminder might be explained by the importance of the contextual reminder to reactivate the existing memories and subsequently trigger the reconsolidation process.

Nevertheless, some limitations to this pilot study need to be acknowledged. This study compared two distinct studies and did not assess directly the timing of stimulation. The relative small number of subjects represents also a limitation. Further studies, based on larger samples, should be conducted to better investigate the inter-individual differences that could influence the magnitude of the long-lasting effects of this intervention. Moreover, another limitation is represented by the lack of a control stimulation site. This type of control condition is important to ensure that changes in memory performance are topographically specific.

In conclusion, Anodal tDCS applied after consolidation with a contextual reminder induced longer-lasting positive effects (i.e. up to 30 days) than Anodal tDCS during encoding on verbal episodic memory, conceivably through reconsolidation. Importantly, the behavioral effects observed were not influenced by differences in the learning rate, number of words correctly recalled in the last learning trial, tDCS sensations, cognitive reserve accumulated and memory strategies used.
In addition, when Anodal tDCS was applied after consolidation with a contextual reminder we observed a moderate negative correlation between subjective memory complaints and forgetting on Day 3. The higher the subjective memory complaints were reported, the less forgetting was exhibited.
The findings of this study shed light on the best-suited timing of stimulation to induce long-lasting positive effects on verbal episodic memory and might pave the way for the use of a more effective tDCS protocol aimed at preventing memory decline in the elderly.

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Captions

Table 1. Demographic characteristics and neuropsychological assessment of older individuals grouped according to experimental conditions.

Figure 1. Experimental Paradigms. In both studies elderly participants were required to learn 20 words on Day 1 and memory retrieval (four free recall trials) was tested on Day 3 (48h later) and on Day 30. A) In the first study [8] tDCS was applied over the left DLPFC after receiving or not receiving a contextual reminder on Day 2. B) In the second study [9] tDCS was applied over the same brain area during the encoding phase on Day 1.

Figure 2. The plot shows the mean percentage of words correctly recalled in each group on Day 1, Day 3 and Day 30. Anodal tDCS enhanced memory recall on Day 3 (48h) relative to Placebo tDCS when applied during encoding and after consolidation (with or without a contextual reminder). Interestingly, Anodal tDCS after consolidation with a reminder enhanced memory recall on Day 30 relative to Placebo stimulation groups and Anodal tDCS during encoding. The table shows the mean percentage of words correctly recalled for each tDCS group. Standard deviations are reported between blankets.

Figure 3. These plots show the correlations between the Everyday Memory Questionnaire (EMQ) and forgetting on Day 3 for Anodal tDCS after consolidation with a contextual reminder (A) and Anodal tDCS during encoding (B).

Table 1.

Demographic characteristics and Neuropsychological assessment	AtDCS after consolidation with a reminder (*n*=12)	AtDCS after consolidation without a reminder (*n*=12)	PtDCS after consolidation with a reminder (*n*=12)	AtDCS during encoding (*n*=14)	PtDCS during encoding (*n*=14)	Cut-off	P-value
Age (years)	67.6 (4.3)	67.5 (2.7)	66.4 (4.0)	68.6 (4.2)	69.1 (3.4)		ns
Gender (male/female)	5/7	4/8	3/9	5/9	6/8		
Education (years)	11.3 (3.9)	11.8 (5.0)	13.2 (4.4)	12.4 (3.9)	10.4 (5.0)		ns
EHI	85.3 (14.3)	88.3 (9.9)	80.0 (16.1)	80.3 (12.1)	92.8 (11.7)		ns
Cognitive Reserve Index (CRI)							
CRI-Total Score	119.3 (17.0)	118.4 (20.7)	123.7 (21.9)	121.1 (17.5)	117.6 (20.8)		ns
CRI-Education	104.8 (10.3)	110.1 (15.6)	117.2 (12.8)	113.3 (12.0)	106.1 (16.3)		ns
CRI-Working Activity	103.8 (17.3)	108.8 (13.7)	106.7 (20.2)	108.6 (13.3)	104.9 (19.6)		ns
CRI-Leisure Time	130.0 (22.1)	122.7 (28.9)	129.7 (22.1)	125.7 (26.5)	129.0 (22.5)		ns
Strategies questionnaire (total score)	6.4 (3.3)	8.6 (4.0)	7.4 (3.8)	7.7 (3.6)	8.2 (3.7)		ns
Everyday Memory Questionnaire (EMQ)	38.8 (4.8)	33.0 (7.7)	40.6 (8.2)	39.1 (11.3)	35.1 (5.3)		ns
Screening for dementia							
MMSE	29.0 (1.2)	28.8 (1.1)	28.9 (0.9)	29.1 (1.0)	29.1 (0.9)	≥24	ns
Non-Verbal Reasoning							
Raven's colored progressive matrices	28.9 (4.5)	30.6 (3.6)	30.2 (3.8)	30.4 (3.1)	29.4 (4.7)	>17.5	ns
Language							
Fluency, phonemic	43.1 (11.5)	39.2 (10.2)	38.0 (10.5)	40.6 (11.1)	41.1 (11.7)	>16	ns
Fluency, semantic	48.3 (7.7)	45.9 (8.4)	44.5 (8.3)	47.7 (6.8)	45.5 (9.0)	>24	ns
Memory							
Digit Span	5.8 (1.0)	5.8 (0.9)	6.3 (0.5)	6.3 (0.7)	5.6 (0.9)	>3.5	ns
Story Recall	13.3 (3.9)	13.6 (4.2)	13.9 (3.0)	14.1 (1.7)	13.5 (4.4)	>7.5	ns
AVLT (Immediate recall)	48.5 (9.5)	45.6 (6.6)	46.7 (6.4)	47.0 (5.9)	43.9 (8.8)	>28.52	ns
AVLT (Delayed recall)	10.5 (3.2)	8.8 (3.4)	10.1 (1.3)	9.9 (2.9)	9.3 (2.7)	>4.68	ns
Rey-Osterrieth complex figure, recall	13.9 (4.7)	15.3 (6.5)	14.5 (5.8)	15.1 (6.1)	13.7 (4.6)	>9.46	ns
Praxis							
Rey-Osterrieth complex figure, copy	31.6 (2.8)	32.4 (2.7)	33.6 (2.1)	32.4 (2.3)	32.0 (2.6)	>28.87	ns
De Renzi test-right upper limb	69.7 (1.5)	70.0 (1.7)	69.5 (2.2)	70.1 (2.0)	69.0 (2.1)	>52	ns

De Renzi test-left upper limb	70.8 (1.0)	70.8 (1.3)	70.8 (1.2)	70.6 (1.2)	70.4 (1.6)	>52	ns
Executive functions							
Trial Making Test-A (seconds)	40.3 (20.0)	48.8 (10.1)	39.8 (12.8)	40.8 (11.3)	36.9 (13.3)	<94	ns
Trial Making Test-B (seconds)	105.8 (34.0)	100.7 (35.7)	118.3 (43.8)	119.3 (50.5)	111.5 (42.0)	<283	ns

* Raw scores are reported (standard deviation between blankets). AtDCS: Anodal tDCS, PtDCS: Placebo tDCS, EHI: Edinburgh Handedness Inventory, MMSE: Mini Mental State Examination, AVLT: Rey Auditory Verbal Learning Test, p-value: comparison between the five experimental groups, ns: not significant. Cut-off scores according to Italian normative data are reported.

Figure 1

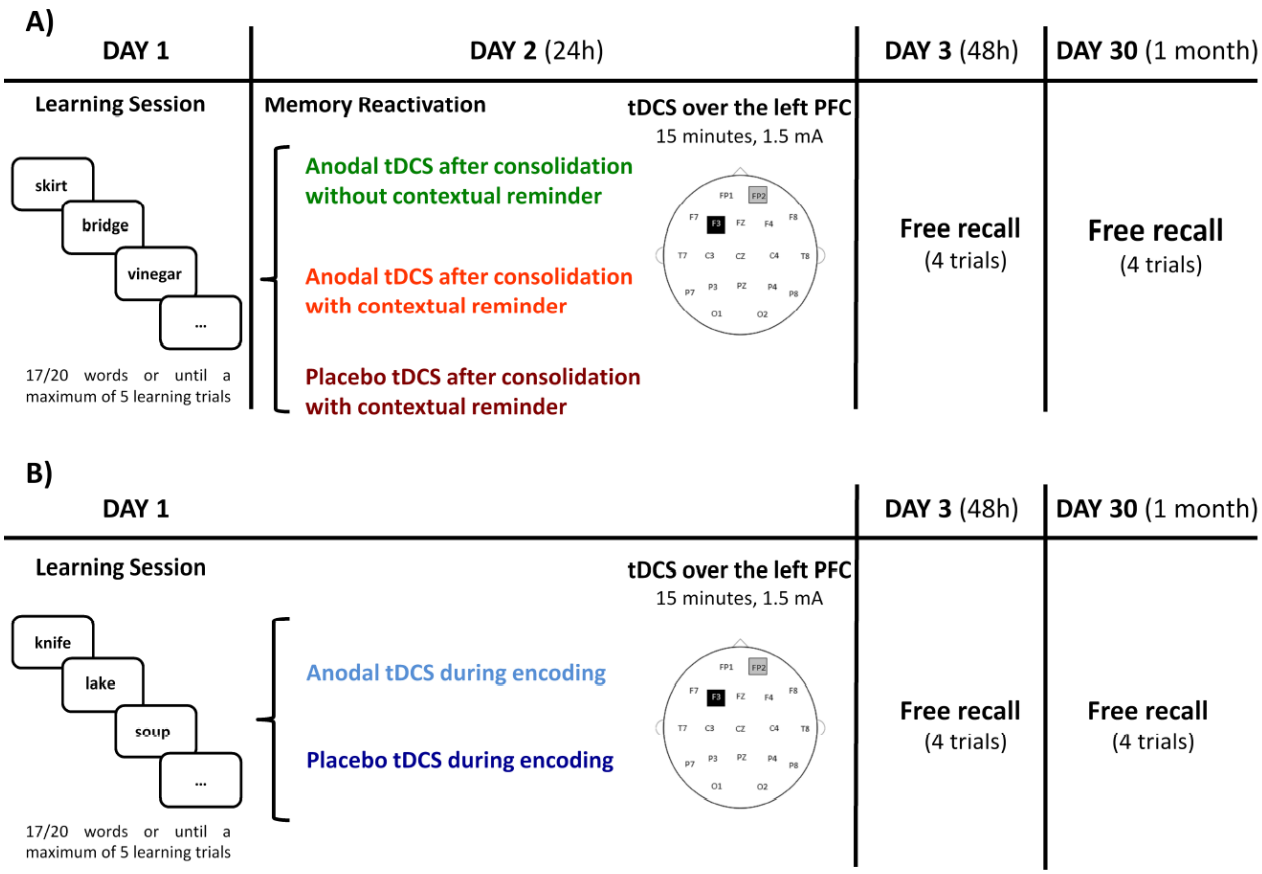
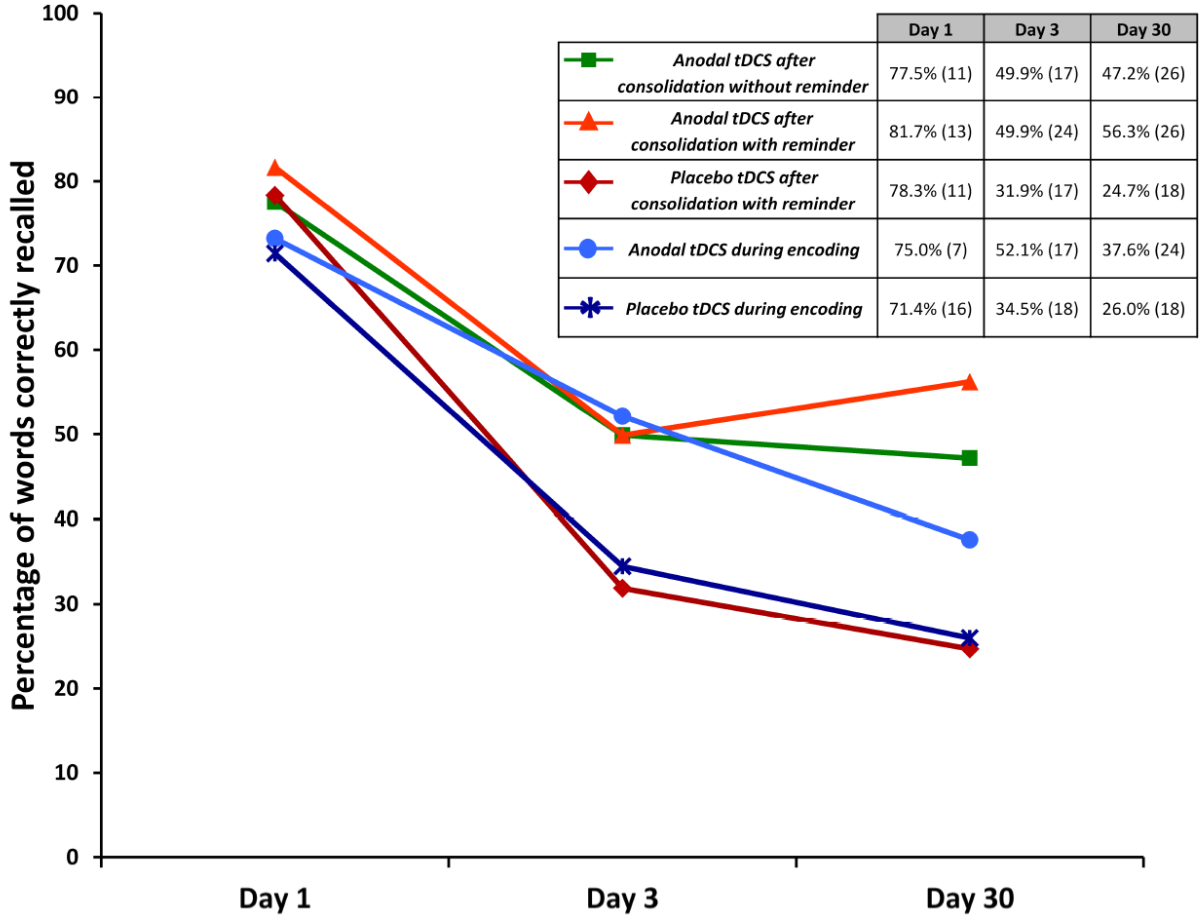
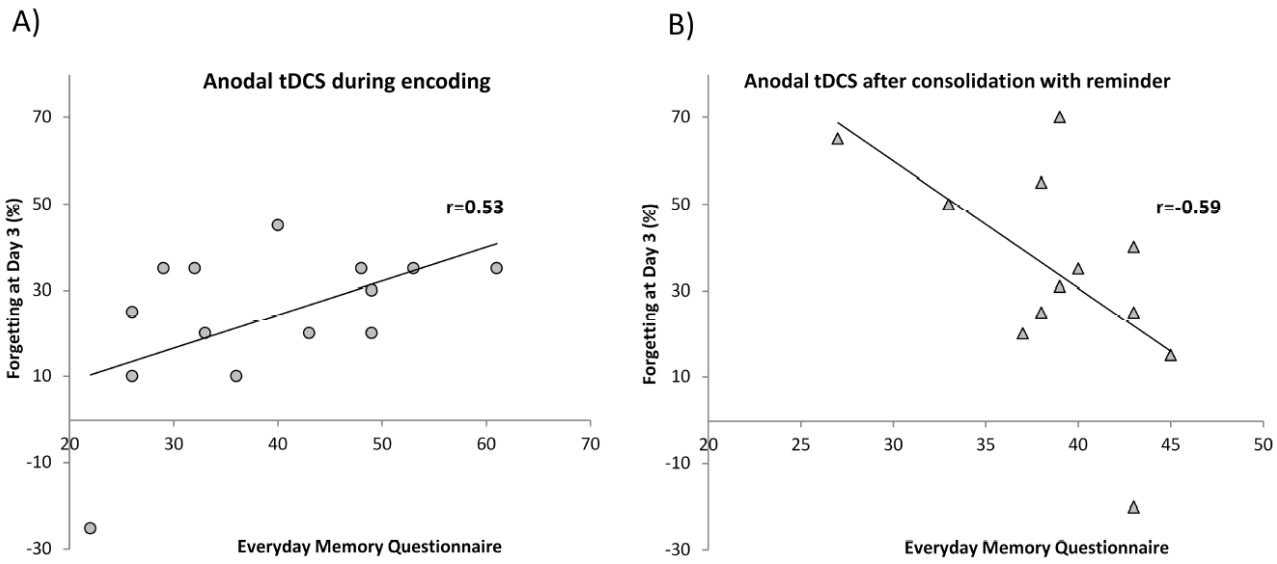

A)
DAY 1
DAY 2 (24h)
DAY 3 (48h)
DAY 30 (1 month)
Learning Session
Memory Reactivation
tDCS over the left PFC
15 minutes, 1.5 mA
skirt
bridge
vinegar
...
17/20 words or until a maximum of 5 learning trials
Anodal tDCS after consolidation without contextual reminder
Anodal tDCS after consolidation with contextual reminder
Placebo tDCS after consolidation with contextual reminder
Free recall
(4 trials)
Free recall
(4 trials)
B)
DAY 1
DAY 3 (48h)
DAY 30 (1 month)
Learning Session
tDCS over the left PFC
15 minutes, 1.5 mA
knife
lake
soup
...
17/20 words or until a maximum of 5 learning trials
Anodal tDCS during encoding
Placebo tDCS during encoding
Free recall
(4 trials)
Free recall
(4 trials)

Figure 2



	Day 1	Day 3	Day 30
Anodal tDCS after consolidation without reminder	77.5% (11)	49.9% (17)	47.2% (26)
Anodal tDCS after consolidation with reminder	81.7% (13)	49.9% (24)	56.3% (26)
Placebo tDCS after consolidation with reminder	78.3% (11)	31.9% (17)	24.7% (18)
Anodal tDCS during encoding	75.0% (7)	52.1% (17)	37.6% (24)
Placebo tDCS during encoding	71.4% (16)	34.5% (18)	26.0% (18)

Figure 3


A)
Anodal tDCS during encoding
r=0.53
Forgetting at Day 3 (%)
Everyday Memory Questionnaire
70
50
30
10
-10
-30
20
30
40
50
60
70
B)
Anodal tDCS after consolidation with reminder
r=-0.59
Forgetting at Day 3 (%)
Everyday Memory Questionnaire
70
50
30
10
-10
-30
20
25
30
35
40
45
50