memory consolidation in older adults

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Abstract

Episodic memory shows the largest degree of age-related memory decline. There is evidence that consolidation, the process that stabilizes memories after encoding, is reduced in older adults. Previous studies have shown that transcranial direct current stimulation (tDCS) applied during intentional encoding or immediately after a contextual reminder enhanced delayed episodic memory performance, suggesting a potential interaction between tDCS and consolidation or reconsolidation processes.

The present randomized, double-blind, sham-controlled study addressed the question whether tDCS applied immediately after verbal encoding enhances episodic memory recall through consolidation in healthy older adults. Twenty-eight participants received tDCS (active or sham) over the prefrontal cortex (anode over the left dorsolateral prefrontal cortex and cathode over the contralateral supraorbital region), a brain region contributing to episodic memory function. Verbal recall was tested two days and one month later.

The results showed that recall performance at one month was enhanced in the active tDCS group relative to the sham group.

These findings suggest that tDCS applied off-line immediately after encoding over the prefrontal cortex interacts with the processes promoting consolidation of episodic memories in healthy older adults. Targeting consolidation by means of tDCS might be a novel strategy for reducing episodic memory decline.

Introduction

From a cognitive perspective, memories are acquired, stored, maintained and later retrieved. For a limited-time after encoding memories are fragile, that is vulnerable to interference, but as time passes, memories stabilize or consolidate and become resistant to interference (McGaugh, 2000). The first type of consolidation process is at cellular level. Morphological changes are critical for the initial stabilization of the memories in the hippocampal circuits. This process takes place in the first few hours (≈6) after encoding. The second type of consolidation is at the system-level. It refers to the gradual reorganization of the brain networks related to memory processes. This process can last from hours to years, depending on the type of memory (Dudai, 2012; Frankland, & Bontempi, 2005).

Episodic memory is the memory for specific events (Tulving, 1983). There is evidence that the prefrontal cortex (PFC) and medial temporal lobe structures, such as the hippocampus, contribute to episodic memory function (Dickerson, & Eichenbaum, 2010; Manenti, Cotelli, Robertson, & Miniussi, 2012; Szczepanski, & Knight, 2014). This type of declarative memory declines with age (Ronnlund et al., 2005), a phenomenon amplified in pathological conditions such as amnestic mild cognitive impairment (aMCI) and Alzheimer's disease (AD). It has been shown that this age-related decline results from a reduction of consolidation (Cherdieu, Reynaud, Uhlrich, Versace, & Mazza, 2014; Kukolja, Goreci, Onur, Riedl, & Fink, 2016; Mander, Rao, Lu, Saletin, Lindquist, Ancoli-Israel, Jagust, & Walker, 2013; Scullin, 2013).

Since pharmacological trials conducted in mild-moderate AD have revealed unsatisfactory results (Karakaya, Fusser, Schroder, & Pantel, 2013), there is a critical need to develop novel interventions for AD prevention (Cotelli, Manenti, Zanetti, & Miniussi, 2012; Gutchess, 2014). Over the last decade, there has been a growing interest in the use of noninvasive brain stimulation techniques as a tool to reduce memory decline in physiological and pathological aging. Among them is transcranial direct current stimulation (tDCS), a safe and well-tolerated neuromodulation technique (Dayan, Censor, Buch, Sandrini, & Cohen, 2013). Based on polarity (anodal or cathodal) and the initial neural activation state of the stimulated regions, tDCS can increase or decrease cortical excitability.

However, evidence of distributed network modulatory effects of tDCS is reported and some investigations showed how connectivity between distant brain areas can change after active stimulation applied over the target areas (Pena-Gomez, Sala-Lonch, Junque, Clemente, Vidal, Bargallo, Falcon, Valls-Sole, Pascual-Leone, & Bartres-Faz, 2012; Polania, Nitsche, & Ruff, 2018; Polania, Paulus, Antal, & Nitsche, 2011).

Among its behavioral applications, tDCS has been shown to enhance delayed episodic memory performance when applied during intentional encoding in older adults (Antonenko, Kulzow, Sousa, Prehn, Grittner, & Floel, 2018; Floel, Suttorp, Kohl, Kurten, Lohmann, Breitenstein, & Knecht, 2012; Medvedeva, Materassi, Neacsu, Beresford-Webb, Hussin, Khan, Newton, & Galli, 2018; Sandrini, Manenti, Brambilla, Cobelli, Cohen, & Cotelli, 2016). Some of these studies (Floel et al., 2012; Sandrini et al., 2016) demonstrated post-tDCS session improvements (i.e. off-line effects), but not within tDCS

session changes (i.e. online effects), suggesting an interaction between tDCS and consolidation processes that contribute more to off-line than online effects (Reis, Schambra, Cohen, Buch, Fritsch, Zarahn, Celnik, & Krakauer, 2009). Other studies in healthy older adults (Sandrini, Brambilla, Manenti, Rosini, Cohen, & Cotelli, 2014) and individuals at risk of developing AD (Manenti, Sandrini, Gobbi, Binetti, & Cotelli, 2018; Manenti, Sandrini, Gobbi, Cobelli, Brambilla, Binetti, & Cotelli, 2017) showed that PFC-tDCS applied after a contextual reminder (i.e. 24 hours after encoding) enhanced delayed verbal episodic memory conceivably through reconsolidation, the processes that re-stabilize memories after reactivation (Lee, Nader, & Schiller, 2017; Sandrini, Cohen, & Censor, 2015). Javadi and Cheng (2013) found similar results in healthy young adults. In addition, a direct comparison of two studies that used a similar protocol (Manenti, Sandrini, Brambilla, & Cotelli, 2016) showed that PFC-tDCS applied after a contextual reminder (Sandrini et al., 2014) induced longer lasting positive effects than PFC-tDCS during intentional encoding (Sandrini et al., 2016).

However, it remains an open question whether tDCS applied immediately after verbal encoding over the PFC is able to interact directly with the consolidation processes in healthy older adults. This is because in previous studies tDCS was applied over the PFC during encoding (Sandrini et al., 2016) or 24h post-encoding (i.e. after a contextual reminder) without unequivocal evidence of enhanced reconsolidation (Sandrini et al., 2014).

To address this knowledge gap is important because the development of an effective tDCS intervention requires a better understanding not only of the mechanisms

underlying off-line effects but also of the optimal timing of stimulation to induce long-lasting effects.

The aim of this study was to investigate whether Active relative to Sham tDCS applied over the PFC immediately after the encoding session would enhance delayed episodic memory in older adults. Older adults learned a list of 20 words. Immediately after the encoding session, they received tDCS (Active or Sham) over the PFC. Memory recall was tested two days and one month later. It was hypothesized that Active tDCS applied immediately after encoding would enhance delayed verbal recall relative to Sham tDCS.

Methods and materials

Participants

Between October 2017 and November 2018, twenty-eight healthy older adults were enrolled in this randomized, double-blind, sham-controlled study.

The sample size calculation was based on our previous study using a similar paradigm in healthy older adults (Sandrini et al., 2014) with an effect size of 1.49 (Cohen's d) for memory recall performance at one month (Day 30), a significance level (α) of 0.05 and power (1-β) of 0.9 (two-tailed independent t-test). The minimum sample size was eleven participants for each group.

All participants underwent a detailed neuropsychological evaluation in order to verify the absence of any cognitive deficit. In addition, we administered the Cognitive Reserve Index questionnaire, which provides a standardized measure of the cognitive reserve accumulated by individuals through their lifespan (Nucci, Mapelli, & Mondini, 2012). See

Table 1 for details.

Participants were excluded from the study if they had: a) other prior or current neurological or major psychiatric disorders; b) history of traumatic brain injury, brain tumours or stroke; c) a history of alcohol abuse; d) any contraindication to tDCS; e) a pathological score in one or more of the neuropsychological tests. Prior to being enrolled in the study, all participants were informed about the study and the possible risks of tDCS and signed a written informed consent after a safety screening. The local Human Ethics Committee of IRCCS Fatebenefratelli of Brescia approved the protocol and it was conducted in accordance with the Declaration of Helsinki.

Patients were randomized into two groups: a) Active tDCS (anode over the left dorsolateral PFC –cathode over right supraorbital area) or b) Sham tDCS. The tDCS group assigned to each participant was obtained by stratified randomization according to Mini Mental State Examination and age.

Procedure: Memory task and tDCS

On Day 1, the experimenter pulled out one item at a time at random (a word printed on piece of card) from a bag and gave it to the participants. Participants were asked to pay close attention so they could remember the words later and to place them in a different bag when ready. When all 20 words were placed into the bag, the experimenter took it away and asked the participants to recall the words orally. Before of each learning rounds, all the words in the bag were mixed in order to randomize the order of the presentation. This learning procedure was repeated until participants recalled at least

17 of 20 words or a maximum of five learning rounds was reached. We recorded the number of learning rounds (range: 1–5) necessary for participants to recall at least 17 over 20 words, whereas participants who recalled < 17 words during the last learning round were given a number of learning rounds of 6.

Immediately after this encoding session, participants received tDCS (Active or Sham). We applied tDCS after the encoding session because a recent study in older adults showed that only the application of tDCS immediately after the encoding, but not after 1 or 2 hours, enhanced off-line motor consolidation (Rumpf, Wegscheider, Hinselmann, Fricke, King, Weise, Klann, Binkofski, Buccino, Karni, Doyon, & Classen, 2017) . Participants were instructed to remain awake, silent and quiet during tDCS but they were stopped if they started to recall any encoded word.

tDCS stimulator (BrainStim, EMS, Bologna, Italy) delivered constant low intensity (1.5 mA) current for 15 minutes through two saline-soaked sponge electrodes (7cm x 5cm, current density: 0.043 mA/cm2 (with a ramping period of 10 seconds at the beginning and at the end of the stimulation) (Antal, Alekseichuk, Bikson, Brockmoller, Brunoni, Chen, Cohen, Dowthwaite, Ellrich, Floel, Fregni, George, Hamilton, Haueisen, Herrmann, Hummel, Lefaucheur, Liebetanz, Loo, McCaig, Miniussi, Miranda, Moliadze, Nitsche, Nowak, Padberg, Pascual-Leone, Poppendieck, Priori, Rossi, Rossini, Rothwell, Rueger, Ruffini, Schellhorn, Siebner, Ugawa, Wexler, Ziemann, Hallett, & Paulus, 2017). The electrodes were secured using elastic bands, and to reduce contact impedance, an

179 electroconductive gel (Cogel Lithium One %, Comedical, https://www.comedical.biz/)
180 was applied under the electrodes before the montage (Manenti, Brambilla, Petesi,
181 Ferrari, & Cotelli, 2013; Manenti et al., 2017; Sandrini et al., 2014; Sandrini et al., 2016).
182
183 Active or Sham stimulation mode was selected by entering different codes so that the
184 experimenter that applied tDCS did not know the type of stimulation applied. The
185 targeted region was the PFC. This brain regions plays a causal role in episodic memory
186 (Duarte, Ranganath, & Knight, 2005; Manenti et al., 2012). The anode electrode was
187 placed over F3 (left dorsolateral PFC) and the cathode electrode was located over Fp2
188 (right supraorbital region) according to the 10–20 system for EEG electrode placement
189 as in previous studies (Manenti et al., 2013; Manenti et al., 2017; Sandrini et al., 2014;
190 Sandrini et al., 2016). The anode was placed over F3 with the long side parallel to the
191 sagittal line, while the cathode was positioned above the arcus superciliaris on the right
192 with the long side of the rectangular pad parallel to the horizontal line (DaSilva, Volz,
193 Bikson, & Fregni, 2011). This tDCS cephalic montage has been shown to be effective in
194 enhancing episodic memory retrieval in older adults (Manenti et al., 2013; Manenti et
195 al., 2018; Manenti et al., 2017; Sandrini, & Cohen, 2014; Sandrini et al., 2016).
196 Sensations induced by tDCS were assessed immediately after the stimulation session
197 with the standardized questionnaire developed by Fertonani et al. (2015). At the end of
198 the tDCS session, participants were asked to complete a semi-structured memory
199 strategies questionnaire, which comprises 13 possible strategies that can be used to
200 enhance the learning of information. Participants rated how often they had used each

strategy during the learning session using a 5-point-scale (0, never; 1, rarely; 2, sometimes; 3, often; and 4, always). The total score of this questionnaire ranges between 0 and 52 (Manenti, Cotelli, Calabria, Maioli, & Miniussi, 2010).

On Day 1 no information was given to them regarding the two retrieval sessions (i.e., Day 3 and Day 30).

Free memory recall was tested two days (Day 3) and one month (Day 30) after the encoding session. The experimenter asked the participants to recall the words learned on Day 1 orally, without a new presentation of the words. When participants indicated that they could not remember any more words or after a maximum of five minutes, the experimenter engaged the participants in a figure-copying task for about 30 seconds. In this period, the participants were asked to copy a series of geometric figures (square, circle etc), that had no any relationship with the words to be remembered. This recall procedure was repeated for four consecutive rounds in order to test reliability of recall as in previous studies (Sandrini et al., 2014; Sandrini et al., 2016). The mean percentage of words correctly recalled in the four recall rounds was computed.

See Figure 1 for details.

Statistical analyses

Demographic and neuropsychological variables, sensations induced by tDCS, mean of words correctly recalled during the learning rounds, learning rate and memory strategies were compared between the Active and Sham groups using Mann-Whitney U

test.

We analyzed the changes in memory performance at different time points (Day 1, Day 3 and Day 30) in the two groups (Active vs. Sham). As in previous studies (Sandrini et al., 2014, 2016), the dependent variable was the mean percentage of words correctly recalled at Day 1 (last learning round), Day 3 and Day 30. Considering that the data were not normally distributed (Kolmogorov-Smirnov Test: $d = 0.25$, $p < 0.01$; Skewness +1.5, right skewed), we adopted logarithmic transformation of data and we analysed log-transformed data. Thus, a mixed ANOVA model was adopted to analyze the dependent variable "mean percentage of words correctly recalled" at Day 1, Day 3 and Day 30 including one within-subjects variable "Time" (Day 1, Day 3 and Day 30) and one between-subjects variable "Group" (Active and Sham). Post-hoc analysis was carried out using the Bonferroni correction for multiple comparisons.

Moreover, we analyzed the retention scores normalized with respect to baseline at different time points (Day 3 and Day 30) in the two groups (Active vs. Sham). The retention scores were calculated using the following formula: mean percentage of words correctly recalled at Day 3 or Day 30 divided by the percentage of words correctly recalled at Day 1 (last learning round) and multiplied by 100 (e.g. Retention Day 3= score at Day 3/score at Day 1 x 100).

Thus, a mixed ANOVA model was adopted to analyze the dependent variable "retention scores" at Day 3 and Day 30 including one within-subjects variable "Time" (Day 3 and Day 30) and one between-subjects variable "Group" (Active and Sham). Post-hoc analysis was carried out using the Bonferroni correction for multiple comparisons.

Statistical analyses were performed using Statistica software (version 10; www.statsoft.com). Statistical power and effect sizes analyses were estimated using GPower 3.1 (Faul, Erdfelder, Lang, & Buchner, 2007).

Results

No significant differences in age, education, cognitive reserve or in any other standardized neuropsychological test were observed between the experimental groups (Table 1). Moreover, there were no significant differences between the groups in memory strategies (Active tDCS group: 8.8, SD 4.5, Sham tDCS group: 8.6, SD 4.1, $U = 96$, $Z = -0.05$, $p = 0.96$). The strategies more frequently reported by the participants were: to imagine the pictures corresponding to the words displayed (57% of Active Group, 64% of Sham Group); to repeat the words (50% of Active Group, 71% of Sham Group); to create associations of words (86% of Active Group, 86% of Sham Group); and to associate each word to a personal event (50% of Active Group, 36% of Sham Group). Moreover, none of the strategies listed below showed significant differences between Sham and Active groups: (1) to use the first letter of each word: $U = 98$, $Z = 0.00$, $p = 0.99$; (2) to create sentences that includes some of the words displayed: $U = 98$, $Z = 0.00$, $p = 0.99$; (3) to imagine the pictures corresponding to the words displayed: $U = 91$, $Z = -0.30$, $p = 0.77$; (4) to repeat the words: $U = 77$, $Z = -0.94$, $p = 0.35$; (5) to create songs that includes some of the words displayed: $U = 98$, $Z = 0.02$, $p = 0.98$; (6) to create rhymes between the words displayed: $U = 91$, $Z = -0.30$, $p = 0.77$; (7) to translate the words in a foreign language: $U = 91$, $Z = 0.30$, $p = 0.77$; (8) to create associations of words: $U = 98$, $Z = -0.02$,

p = 0.98; (9) to create a brief story that included the words displayed: U = 98, Z = -0.02, p = 0.98; (10) to associate each word to a personal event: U = 84, Z = 0.62, p = 0.54; (11) to classify each word as easy or difficult, abstract or concrete, positive or negative, and so forth: U = 91, Z = 0.30, p = 0.77; (12) to imagine the words' sound, color, shape, and so forth: U = 98, Z = -0.02, p = 0.98; and (13) other strategies: U = 91, Z = 0.30, p = 0.77. Finally, the two groups did not differ on the tDCS-induced sensations (Active tDCS group: 2.1, SD 1.4, Sham tDCS group: 1.9, SD 0.9, U = 88, Z = 0.43, p = 0.66). Hence, there are no reasons to reject the blinded character of this study on the basis of these results.

Experimental memory task

We recorded how many learning rounds (1-5) were necessary for each participant to recall at least 17 words on the learning session of Day 1. Participants who recalled <17 words during the fifth learning round were given a score of 6. There were no significant differences between Active and Sham groups in the number of learning rounds (Active tDCS group: 5.5, SD: 0.9; Sham: 5.9, SD: 0.4; U = 82, Z = -0.71, p = 0.48).

We analyzed changes in memory performance at different time points using one mixed ANOVA with "Group" as the between-subjects variable and "Time" as the within-subjects variable. This analysis showed a significant effect for "Time" ($F(2,52)=78.2$, $p<.001$, $\eta p^2 =0.75$, $1-\beta= 0.99$), showing a decrease of performance from Day 1 to Day 3 ($p<0.001$) and from Day 3 to Day 30, ($p<0.001$), and an effect for "Group" ($F(1,26)=4.6$, $p=.04$, $\eta p^2 =0.15$, $1-\beta= 0.76$), indicating better performance in the Active tDCS group

compared to the Sham Group (see Figure 2). The interaction "Group" x "Recall" was also significant ($F(2,52)=3.9$, $p=.02$, $\eta p^2=0.13$, $1-\beta=0.63$). Interestingly, post hoc comparisons showed no significant difference between Active and Sham Group on Day 3 (Sham Group: 41.3, SD 12.6, Active Group: 50.9, SD 20.4; $p=.90$), whereas Active Group showed a better performance than Sham Group on Day 30 (Sham Group: 24.1, SD 8.9, Active Group: 41.3, SD 14.3; $p=0.026$). No significant difference was found on Day 1 (Sham Group: 63.9, SD 13.0, Active Group: 71.8, SD 14.1; $p=.90$).

Finally, we analyzed retention scores normalized with respect to baseline at different time points using one mixed ANOVA with "Group" as the between-subjects variable and "Time" as the within-subjects variable. The main effect "Time" was significant ($F(1,26)=31.5$, $p<.001$, $\eta p^2=0.55$, $1-\beta=0.98$), showing a reduction of the retention score from Day 3 (Mean 67.2, SD 17.5) to Day 30 (Mean 47.3, SD 17.6). The main effect "Group" was also significant ($F(1,26)=5.1$, $p=.03$, $\eta p^2=0.17$, $1-\beta=0.77$), indicating higher retention scores in the Active tDCS group (Mean 63.2%, SD 19.4) compared to the Sham Group (Mean 51.3%, SD 19.2). The interaction "Group" x "Recall" showed a trend toward statistical significance ($F(1,26)=4.0$, $p=.056$, $\eta p^2=0.13$, $1-\beta=0.62$). To further characterize this trend we run post hoc comparisons showing no significant difference between Active and Sham Group on Day 3 (Sham Group: 64.7, SD 19.1, Active Group: 69.6, SD 19.1; $p=.90$), whereas Active Group obtained a better performance than Sham Group on Day 30 (Sham Group: 37.8, SD 17.2, Active Group: 56.8, SD 17.2; $p=0.026$).

Discussion

This study shows for the first time that, relative to Sham, Active tDCS applied immediately after encoding to the PFC enhanced episodic memory recall (percentage of words correctly recalled and retention score). Importantly, there were no differences between groups in the learning rate, words correctly recalled during the learning rounds and memory strategies used.

Previous studies that applied tDCS during the encoding session found off-line (but not online) positive effects, suggesting an interaction between tDCS and consolidation processes (Floel et al., 2012; Sandrini et al., 2016). The current study provides evidence for the conclusion that stabilization of episodic memories may be facilitated by direct interaction of tDCS with the mechanisms of consolidation. In support of our results, a recent study showed that active tDCS applied immediately after training to the motor cortex enhanced motor memory consolidation in healthy older adults (Rumpf et al., 2017). The findings of these studies suggest that tDCS applied off-line immediately after encoding/training to critical brain regions may interact with early processes promoting consolidation in healthy older people.

The fact that the effect emerged after one month is consistent with a recent anodal transcutaneous spinal direct current stimulation study on motor learning (Awosika, Sandrini, Volochayev, Thompson, Fishman, Wu, Floeter, Hallett, & Cohen, 2019). Stabilization of learning often develops over time, requiring more than a couple of days to fully consolidate (Abe, Schambra, Wassermann, Luckenbaugh, Schweighofer, & Cohen, 2011; Awosika et al., 2019). In addition, Antonenko et al., (2018) showed that

331 the effects of anodal tDCS on a training task (i.e. object-location) and on a transfer task
332 (i.e. words list) were not evident on the day after the intervention, but one month later.
333 This study suggests that PFC-tDCS applied after encoding (during early consolidation)
334 can induce longer-lasting effects than PFC-tDCS applied during encoding, effect explored
335 in our previous study (Sandrini et al., 2016).
336
337 Regarding the neural mechanisms underlying the long-lasting positive effect observed in
338 our study, it has been proposed that the Default Mode Network (DMN) may support the
339 off-line processing and system-level consolidation of memories (Huo, Li, Wang, Zheng, &
340 Li, 2018; Miall, & Robertson, 2006). DMN is a large-scale brain network mediating
341 episodic memory function (Jeong, Chung, & Kim, 2015; Kim, Cha, Lee, Shin, Jung, Kim,
342 Choe, Lee, Kim, Kim, Lee, Na, & Seo, 2016; Pievani, Pini, Ferrari, Pizzini, Boscolo Galazzo,
343 Cobelli, Cotelli, Manenti, & Frisoni, 2017). Changes in DMN connectivity have been
344 shown in normal and pathological aging (Jones, Machulda, Vemuri, McDade, Zeng,
345 Senjem, Gunter, Przybelski, Avula, Knopman, Boeve, Petersen, & Jack, 2011).
346 Considering the idea that tDCS may act by modulating functional connectivity (Keeser,
347 Meindl, Bor, Palm, Pogarell, Mulert, Brunelin, Moller, Reiser, & Padberg, 2011; Krause,
348 Zanos, Csorba, Pilly, Choe, Phillips, Datta, & Pack, 2017; Meinzer, Lindenberg, Phan, Ulm,
349 Volk, & Floel, 2015), tDCS after encoding might have changed the intrinsic DMN
350 functional connectivity (Antonenko et al., 2018; Keeser et al., 2011). Future studies
351 combining tDCS with resting state fMRI (Kukolja et al., 2016; Shafi, Westover, Fox, &
352 Pascual-Leone, 2012) might help gain insights into the brain networks mechanisms

353 promoting consolidation of episodic memories.

354 Strengthening of the consolidation processes might be the mechanism acting during the
355 hours or days after tDCS (Au, Karsten, Buschkuehl, & Jaeggi, 2017). The current work
356 and previous studies (Javadi, & Cheng, 2013; Manenti et al., 2018; Manenti et al., 2017;
357 Rumpf et al., 2017; Sandrini et al., 2014; Tecchio, Zappasodi, Assenza, Tombini, Vollaro,
358 Barbati, & Rossini, 2010) showed enhanced consolidation after to the application of
359 tDCS during quiet wakefulness, specifically during early consolidation (Rumpf et al.,
360 2017; Tecchio et al., 2010) or reconsolidation (Javadi, & Cheng, 2013; Manenti et al.,
361 2018; Manenti et al., 2017; Sandrini et al., 2014). Since the reactivation of newly
362 encoded memories (or "replay") during subsequent waking state may be critical for
363 memory stabilization (consolidation) (Karlsson, & Frank, 2009; Sirota, & Buzsaki, 2005),
364 tDCS applied during awake periods might have facilitated neural reactivation and
365 consequently enhanced system-level consolidation for long-term memory retention (Au
367 et al., 2017).

368

369 The relative small sample size of this study represents a limitation and it needs to be
370 acknowledged. Another limitation of the present work is the lack of a control
371 stimulation site. This control condition is critical to ensure that changes in memory
372 performance are indeed specific for tDCS over a given brain area. In addition to the
373 optimal timing of stimulation, location is another relevant variable for treatment
374 optimization. In addition, we are not able to definitely discuss age-related changes in
375 consolidation processes due to the lack of a young healthy control group.

Future work should determine whether tDCS applied after encoding to other cortical regions facilitates consolidation of episodic memory. For instance, it has been shown that tDCS applied over the posterior parietal cortex during encoding or retrieval enhances memory performance (Bjekic, Colic, Zivanovic, Milanovic, & Filipovic, 2018; Jacobson, Ezra, Berger, & Lavidor, 2012; Jones, Gozenman, & Berryhill, 2014; Manenti et al., 2013).

Finally, since the weak induced electric fields reaching the human brain contrast with the numerous behavioral and clinical effects reported (Voroslakos, Takeuchi, Brinyiczki, Zombori, Oliva, Fernandez-Ruiz, Kozak, Kincses, Ivanyi, Buzsaki, & Berenyi, 2018), future work should also consider how tDCS can affect brain activity indirectly (Liu, Voroslakos, Kronberg, Henin, Krause, Huang, Opitz, Mehta, Pack, Krekelberg, Berenyi, Parra, Melloni, Devinsky, & Buzsaki, 2018).

Conclusions

These findings suggest that tDCS applied off-line immediately after encoding interacts directly with the processes promoting consolidation of verbal episodic memories in healthy older people.

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Captions

Figure 1. Experimental Paradigm. Participants were required to learn 20 words on Day 1 and memory retrieval (four free recall rounds) was tested on Day 3 and on Day 30. tDCS (Active or Sham) was applied with the anode over the left dorsolateral prefrontal cortex immediately after the learning session 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. Active tDCS enhanced memory recall on Day 30 relative to Sham tDCS. Dotted lines describes individual participants data. The table shows the mean percentage of words correctly recalled for each tDCS group. Standard deviations are reported between parentheses.

Table 1. Demographical, clinical and neuropsychological data.

	Active tDCS (n=14)	Sham tDCS (n=14)	Cut-off	p-value
Age (years)	68.6 (6.9)	67.1 (5.8)		0.75
Gender (male/female)	3/11	2/12		0.77
Education (years)	12.9 (5.0)	11.9 (3.3)		0.45
EHI (%)	89.2 (21.4)	89.6 (10.2)		0.49
Mood and Anxiety Assessment				
Geriatric Depression Scale (GDS) [1]	3.5 (3.4)	6.1 (4.1)	<11	0.06
State-Trait Anxiety Inventory (STAI) [2]				
STAI-State	29.8 (5.1)	31.1 (7.5)		0.93
STAI-Trait	34.9 (8.5)	35.3 (7.7)		0.89
Subjective Memory Complaints Questionnaire				
Everyday Memory Questionnaire (EMQ) [3]	48.1 (11.7)	44.3 (15.2)		0.48
Cognitive Reserve				
Cognitive Reserve Index – questionnaire (CRI – q) [4]				
CRI – Total Score	117.4 (18.6)	120.0 (10.5)		0.89
CRI – Education	114.4 (16.4)	112.9 (12.2)		0.77
CRI – Working Activity	105.2 (17.2)	107.1 (13.9)		0.61
CRI – Leisure Time	119.8 (21.7)	125.4 (13.5)		0.40
Screening for dementia				
MMSE [5]	29.3 (0.8)	28.9 (0.8)	≥24	0.18
Non-Verbal Reasoning				
Raven's coloured progressive matrices [6]	31.6 (3.3)	29.9 (4.8)	>17.5	0.29
Memory				
Digit Span (forward) [7]	6.0 (1.6)	5.9 (1.0)	>4.25	0.99
Story Recall [8]	14.1 (4.2)	15.9 (4.9)	>7.5	0.93
Rey-Osterrieth Complex Figure, recall [9]	19.8 (4.3)	17.9 (5.0)	>9.46	0.45
Auditory Verbal Learning Test – AVLT: [10]				
AVLT, Immediate recall	52.4 (8.1)	49.4 (5.6)	>28.52	0.21
AVLT, Delayed recall	11.5 (2.3)	10.4 (3.0)	>4.68	0.34
Language				
Token Test [11]	34.5 (1.0)	34.3 (1.1)	>26.25	0.80
Verbal Fluency, phonemic [12]	41.0 (12.2)	40.9 (11.7)	>16	0.66
Verbal Fluency, semantic [12]	49.7 (10.9)	48.9 (8.1)	>24	0.98
Praxis				
Rey-Osterrieth Complex Figure, copy [9]	32.0 (1.7)	30.5 (2.3)	>28.87	0.08
Attentional and Executive Functions				
Digit Span (backward) [7]	4.6 (1.1)	4.5 (0.9)	>2.64	0.37
Trial Making Test, section A (seconds) [13]	42.4 (19.4)	43.4 (16.0)	<94	0.68
Trial Making Test, section B (seconds) [13]	114.6 (50.5)	112.2 (52.7)	<283	0.73
Stroop test: [14]				
Interference effect on time (seconds)	23.8 (8.5)	25.4 (9.2)	<36.92	0.35
Interference effect on accuracy (errors)	1.2 (1.8)	0.6 (0.7)	<4.24	0.80
Wisconsin Card Sorting Test (WCST): [15]				
WCST – Global score	63.9 (38.6)	58.2 (42.0)	<90.6	0.87
WCST – Perseverative responses	24.2 (15.5)	17.9 (12.9)	<42.7	0.30
WCST – Non Perseverative errors	18.5 (11.7)	17.3 (14.2)	<30.0	0.82
WCST – Failure to maintain the set	1.1 (1.2)	1.9 (1.5)	<4	0.19

Raw scores are reported. Standard deviations (SD) are presented in parentheses. EHI: Edinburgh Handedness Inventory; MMSE: Mini Mental State Examination; p-value: comparison between groups. Cut-off scores according to Italian normative data are reported.

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Figure 1

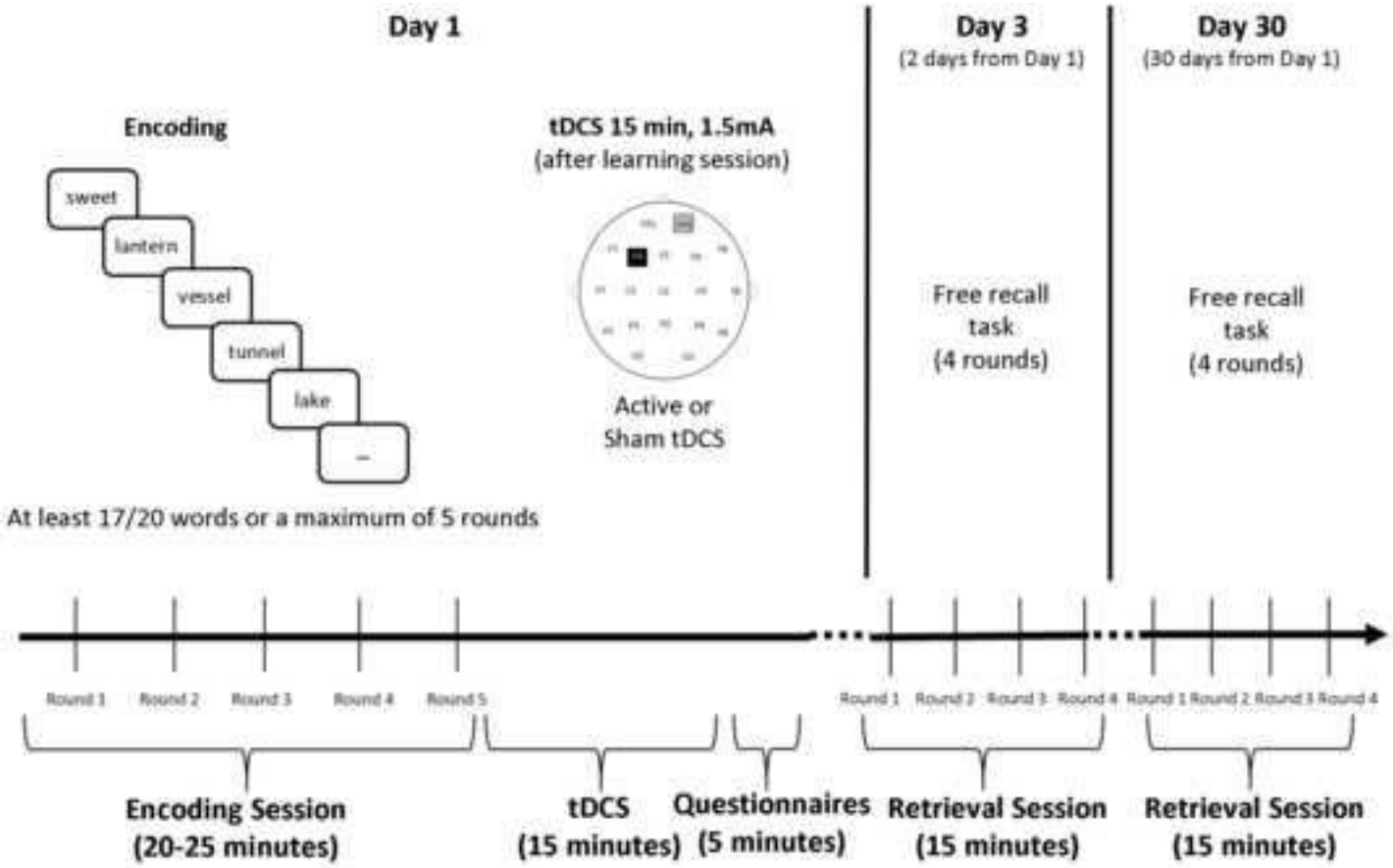
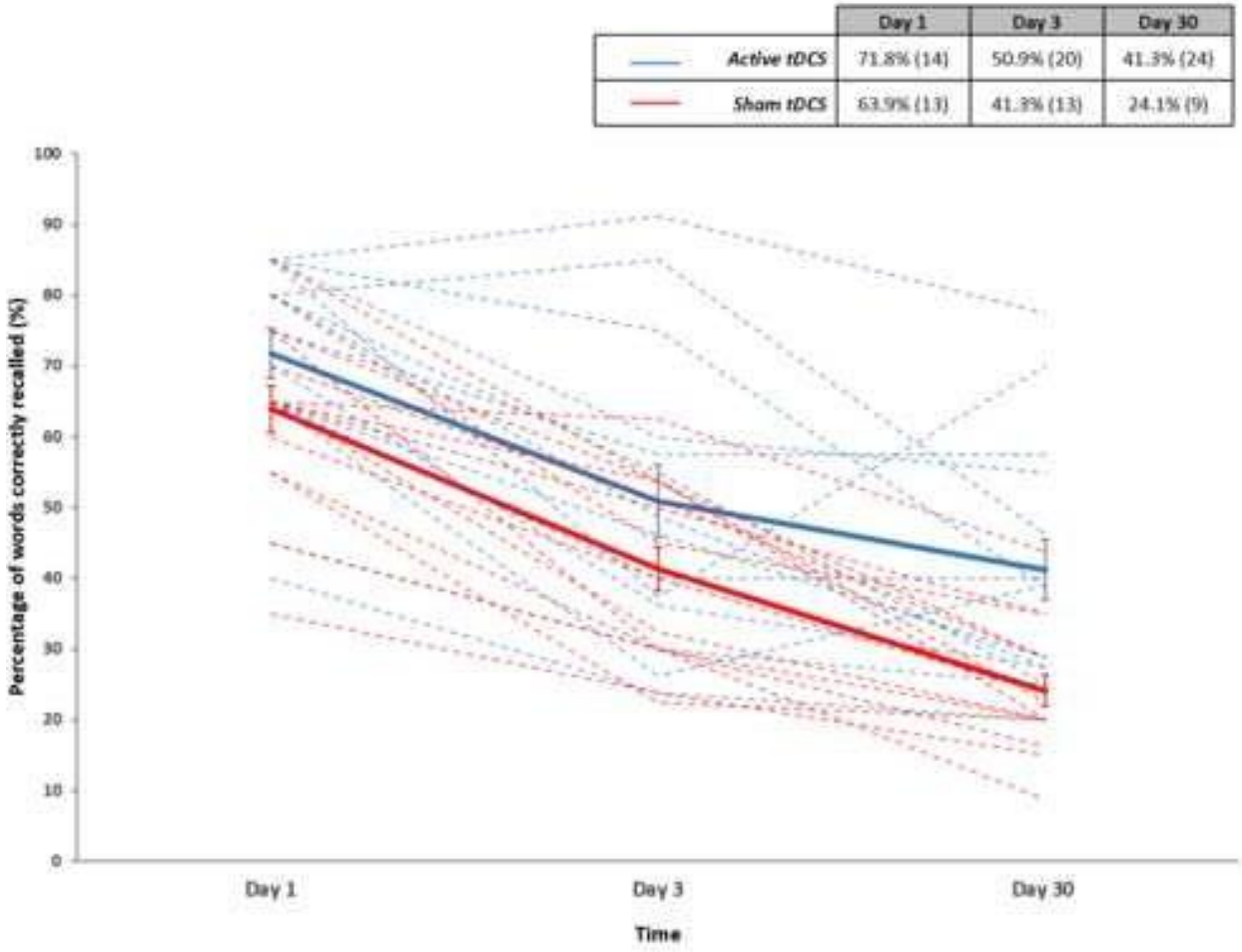

Day 1
Day 3
(2 days from Day 1)
Day 30
(30 days from Day 1)
Encoding
sweet
lantern
vessel
tunnel
lake
_
At least 17/20 words or a maximum of 5 rounds
tDCS 15 min, 1.5mA
(after learning session)
Active or
Sham tDCS
Free recall
task
(4 rounds)
Free recall
task
(4 rounds)
Round 1
Round 2
Round 3
Round 4
Round 5
Round 1
Round 2
Round 3
Round 4
Round 1
Round 2
Round 3
Round 4
Encoding Session
(20-25 minutes)
tDCS
(15 minutes)
Questionnaires
(5 minutes)
Retrieval Session
(15 minutes)
Retrieval Session
(15 minutes)

Figure 2


Day 1
Day 3
Day 30
Active tDCS
71.8% (14)
50.9% (20)
41.3% (24)
Sham tDCS
63.9% (13)
41.3% (13)
24.1% (9)
100
90
80
70
60
50
40
30
20
10
0
Percentage of words correctly recalled (%)
Day 1
Day 3
Day 30
Time