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Transcranial direct current stimulation (tDCS) for memory enhancement --Manuscript Draft--

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TITLE:

2 Transcranial direct current stimulation (tDCS) for memory enhancement

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SUMMARY:

A protocol for memory enhancement by using transcranial direct current stimulation (tDCS) targeting dorsolateral prefrontal and posterior parietal cortices, as core cortical nodes within hippocampo—cortical network, is presented. The protocol has been well evaluated in healthy-participant studies and is applicable to aging and dementia research as well.

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ABSTRACT:

Memory enhancement is one of the great challenges in cognitive neuroscience and neurorehabilitation. Among various techniques used for memory enhancement, transcranial direct current stimulation (tDCS) is emerging as an especially promising tool for improvement of memory functions in a non-invasive manner. Here, we present a tDCS protocol that can be applied for memory enhancement in healthy-participant studies as well as in aging and dementia research. The protocol uses weak constant anodal current to stimulate cortical targets within cortico-hippocampal functional network engaged in memory processes. The target electrode is placed either on the posterior parietal cortex (PPC) or the dorsolateral prefrontal cortex (DLPFC), while the return electrode is placed extracranially (i.e., on the contralateral cheek). In addition, we outline a more advanced method of oscillatory tDCS, a natural brain rhythm to promote hippocampus-dependent memory functions, which can be applied in a personalized and non-personalized manner. We present illustrative results of associative and working memory improvement following single tDCS sessions (20 minutes) in which the described electrode montages were used with current intensities between 1.5 mA and 1.8 mA. Finally, we discuss crucial steps in the protocol and methodological decisions that must be made when designing a tDCS study on memory.

INTRODUCTION:

Memory plays a vital role in everyday functioning as it enables one to remember information about people and places, recall past events, learn new facts and skills, as well as to make judgments and decisions. Here we focus on two types of memory – working memory (WM) and associative memory (AM). WM provide us with the ability to temporarily maintain and store information for ongoing cognitive processing ¹, while AM enables us to remember multiple pieces of experience or information bound together. Therefore, these two types of memory underline almost all daily activities. Unfortunately, memory is one of the most vulnerable functions as it declines with normal ageing as well as due to various pathological states and conditions. Both WM and AM decline is prominent in mild cognitive impairment ^{2,3} and dementia ^{4,5} as well as in normal ageing ^{6,7}. Since memory deficits are associated with a high disease burden level ^{8,9} and significantly affect quality of life ^{10–13}, there is a growing need for novel approaches to prevention and treatment of memory decline.

Transcranial direct current stimulation (tDCS) is a promising tool for tackling memory decline ^{14–16} and gaining better understanding of brain functions in general ¹⁷. tDCS is a non-invasive brain stimulation technique that uses weak electric currents (usually between 1 mA and 2 mA) to modulate brain activity by affecting neuronal membrane excitability. The effects of tDCS are polarity-dependent, such that anodal stimulation increases while cathodal decreases neuronal excitability. Namely, anodal tDCS increases the likelihood for action potentials to fire through depolarization of neuronal membranes, thus facilitating spontaneous brain activity under the anode ¹⁸. Moreover, it is shown that the effect of increased activation does not remain localized but tends to spread to other functionally connected areas of the central nervous system. Anodal tDCS is thus expected to promote cognitive functions that rely on targeted brain regions and functionally interconnected brain areas, while cathodal tDCS is expected to have the opposite effect.

The tDCS has several advantages over other brain stimulation techniques: (1) tDCS is safe, i.e., does not pose health risks and does not produce any negative short or long term structural or functional changes ¹⁹; (2) tDCS is characterized by highest tolerability among brain stimulation techniques as it causes minimal discomfort to participants in a form of a mild tingling and itching sensations under the stimulating electrodes ²⁰; (3) tDCS is cost-effective – the price of tDCS devices and application are ten to hundred times lower than other treatment options, which makes it attractive for patients and healthcare system; (4) tDCS is easy to use, and therefore has a high potential to be applied even in home-based settings, which can lead to higher compliance of patients and reduced cost for medical staff and facilities.

The main challenges for using tDCS for memory enhancement are finding the optimal electrode montage and stimulation protocol that will produce reliable effects on memory. Here we use the term *electrode montage* to refer to the configuration and the positions of the electrodes (i.e., the placement of the target and reference (return) electrode). Due to the nature of the electrical fields, the reference (return) electrode is not neutral – it has the polarity opposite to the target electrode – and thus can also exercise biological (neuromodulatory) effects on the underlying neural tissue. Therefore, careful choice of the reference electrode is essential for

avoiding unwanted additional effects of the stimulation.

When using the term *stimulation protocol*, we refer to the tDCS parameters such as the duration and the intensity of the current being applied as well as the way current intensity changes over time (i.e., whether the intensity is constant throughout the stimulation or changes following a sinusoidal waveform with certain amplitude and frequency). Different stimulation protocols can be applied using the same electrode montage, and the same protocol can be used across different montages.

To optimize the electrode montage, we look at the function-relevant brain areas and how the electric fields induced by various positions of the electrodes would affect those brain areas and consequentially cognitive functions. Several different cortical and subcortical structures play a significant role in memory functions - including areas of the frontal, temporal, and parietal cortex. Namely, WM is supported by a widespread neural network that includes dorsolateral (DLPFC) and ventral lateral prefrontal cortex (VLPFC), premotor and supplementary motor cortices, as well as posterior parietal cortex (PPC)²¹. For AM and episodic memory in general, structures within medial temporal lobe are essential ²². However, associative areas of the parietal, frontal, and temporal cortices, with their convergent pathways to the hippocampus also play a significant role. Due to its anatomical position, the hippocampus cannot be directly stimulated using tDCS, and thus the enhancement of hippocampus-dependent memory functions is done using the cortical targets with high functional connectivity to hippocampus such as posterior parietal cortex. For these reasons, DLPFC and PPC are most frequently used as stimulation targets to enhance memory. Positioning of the electrodes can be further refined based on current flow modeling 23 and validated in studies that combine tDCS with neuroimaging techniques²⁴.

The most usual *stimulation protocol* is a constant anodal current of 1-2 mA that lasts between 10-30 minutes. The assumed mechanism behind this protocol is that the electrode with a positive charge will increase the excitability of the underlying cortical tissue which will than result in enhanced subsequent memory performance. Unlike the constant anodal tDCS, where current intensity stays the same during the whole stimulation period, in the oscillatory tDCS protocol the intensity of the current fluctuates at the given frequency around a set value. Therefore, this type of protocol modulates not only excitability but also entrains neural oscillations of the relevant brain areas. It is important to note that for both constant and oscillatory tDCS the electrodes retain the same current polarity for the whole duration of the stimulation.

Here we present tDCS montages that target nodes within fronto-parieto-hippocampal network to promote memory – both WM and AM: specifically, two electrode montages with the target electrode over either left/right DLPFC or left/right PPC. In addition to constant anodal tDCS protocol we outline a theta oscillatory tDCS protocol.

Study design

Before providing a detailed guide on how to use tDCS for memory enhancement, we will

outline a few essential properties of the experimental design that are important to consider when planning a tDCS study on memory.

131132 Sham control

To assess the effects of tDCS on memory, the study needs to be sham controlled. This implies that in one of the experimental conditions the protocol resembles a real stimulation session, but no treatment is given. This fake or sham session serves as a reference point to compare performance following real tDCS and make inferences about its effectiveness. Commonly, in the sham protocol the current is applied only for a brief period – usually up to 60 seconds at the beginning and at the end of the sham stimulation as a ramp-up followed by immediate ramp-down (i.e., fade-in/fade-out, up to 30 seconds each) fashion. This way it is ensured that the duration of the stimulation is insufficient to produce any behavioral or physiological effects. Since local skin/scalp sensations are usually most pronounced at the beginning and at the end of stimulation (due to changes in the current intensity), the sensations induced in all protocols are comparable and difficult to distinguish ²⁵. This way, the participant is blinded on whether the stimulation is real or not, which is especially important in within-subject designs.

In addition to sham-control, to assess the specificity of the effects of oscillatory protocols, it is advisable to have an *active control condition*, too. For instance, the active control for oscillatory protocol can be constant anodal stimulation of the same intensity^{26,27}, or oscillatory stimulation in different frequency e.g. theta *vs* gamma ²⁸.

Within- or between-subjects design.

In within-subjects design each participant undergoes both real and sham tDCS, while in between-subjects design one group of participants receives real, and the other group receives sham tDCS. The main advantage of within-subject design is better control of subject-specific confounds. That is, individual differences in anatomy and cognitive abilities are best controlled for when each participant is compared to their self. However, since within-subject design needs to be applied in cross-over fashion (i.e., half of the participants receive real tDCS in the first session and sham in the second session, while the other half of participants of receive sham first and real tDCS second) this design may not be optimal for clinical and training studies as well as studies involving several tDCS sessions over consecutive days, because crossover design may result in unequal baselines between crossover arms. Therefore, within-subject design is suited the best when assessing either behavioral or physiological effects of a single tDCS session, and when unequal baselines are not considered an issue for the research hypothesis. In within-subject design assessing the effects of single tDCS session, it is a good practice to keep 7 days between real and sham tDCS session to avoid carryover effects (however some studies suggest even shorter wash-out periods do not significantly affect the outcomes ^{29,30}) and to use parallel forms of memory tasks in counterbalanced order to minimize training and betweensession learning effects.

When between-subjects design is used, the control group should be carefully matched for baseline performance, as well as other relevant characteristics known to be of relevance for tDCS effectiveness. Random group assignment may not be the best approach in small sample

sizes (e.g., <100) as it may lead to suboptimal matching. In either case, baseline performance should be accounted for in statistical analysis.

176 Sample size.

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One of frequently asked questions is "how many participants does one need to detect tDCS effects". The answer to this question depends on several aspects of the study including experimental design, expected effects sizes, type of statistical analysis, etc. The sample sizes in the brain stimulation experiments are often too small, and it is estimated that studies in this field miss around 50% of true positive results because they are underpowered 31. Power analysis enables determining adequate sample size for each specific experiment based on the study design and expected effect size for planned statistical analysis. The power analysis can be performed in R environment or using free specialized software such as G*Power 32, and it should always be performed a priori (i.e., before the experiment). The power should be set at >.80 (ideally.95) and expected effect size on memory tasks following a single tDCS session is usually between 15-.20 (η^2) i.e., Cohen f 0.42-0.50. Therefore, one typically needs to enroll 20-30 participants in total for within-subject experiment and 30-40 participants per group for between-subject study, to achieve satisfactory power and thus diminish type II error. However, the sample size depends on the number of other factors including the planned analysis, and sensitivity of the behavior measures that are used. Therefore ideally, one would run an initial experiment to understand the effect sizes for the specific design and use those data as an input for power analysis. However, it is important to note that running a pilot experiment on just a few participants will lead to faulty and unreliable estimates of the effect sizes. Therefore, if resources are limited it is better to rely on the previous studies with comparable outcomes, and take slightly more conservative approach i.e., by estimating for somewhat smaller effect sizes than reported in the literature.

Outcome measures

To assess the effectiveness of tDCS on memory one needs to select adequate behavioral tasks. In fact, the choice of the memory task is one of the crucial aspects of the study design, because the ability to detect the tDCS effect directly depend on the sensitivity of the task. The challenge here is that most standardized memory assessment tools or classical neuropsychological tasks may not be sensitive enough to detect tDCS effects in specific populations. Furthermore, most of the standardized tasks are not available in two or more parallel forms and therefore cannot be used in within-subjects designs. For that reason, most of the tDCS memory studies use custom-build tasks. When designing or selecting outcome measure one should ensure that the task is: (1) focal/selective measure of the memory function of interest; (2) sensitive (i.e., that the scale is fine enough to detect even small changes); (3) challenging for the participants (i.e., that the task difficulty is sufficient and thus to avoid celling effects); (4) reliable (i.e., that the measurement error is minimized as much as possible). Therefore, one should use empirically validated strictly parallel forms of memory tasks, which have a sufficient number of trials – both to ensure sensitivity of the measure as well as to maximize its reliability. Ideally, the tasks should be pre-tested on a group sampled from the same population as the experiment participants to ensure that maximum performance is not achievable, and that the task-forms have equal indices of difficulty. Finally, it is best to use computerized tasks whenever possible

as they allow for controlled duration and precise timing. This way researchers can ensure that all participants undergo memory assessment at the same time in respect to the timing of stimulation (either during or following tDCS). The duration of each task or task block should not be longer than 10 minutes, to avoid fatigue and fluctuation in attention levels; the cognitive assessment should not be longer than 90 minutes in total (including tasks both during and after tDCS).

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PROTOCOL

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This procedure has been approved by the Institutional Ethics Committee and is in line with the Declaration of Helsinki and guidelines for human research.

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1. Materials

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NOTE: For each tDCS session prepare the following materials (**Figure 1**).

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233 1.1. Obtain a tDCS device – use only a battery driven tDCS device or a mains-connected optically isolated tDCS device. The device should function as a constant current stimulator with a maximum output limited preferably to a few milliampere range. The device must have regulatory approval for human use.

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238 1.2. Obtain rubber electrodes – use either 5 cm x 5 cm square-shaped or 25 cm² round-239 shaped electrodes. These electrodes will have the current densities between 0.06 mA/cm² and 240 0.08 mA/cm² for currents of 1.5 mA-2 mA, respectively.

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242 1.3. Prepare sponge pockets that fit the rubber electrodes. If the sponge pocket is too large it will increase the contact surface to the skin.

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245 1.4. Prepare saline solution (standard 0.9% NaCl).

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1.5. Prepare alcohol (70%).

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249 1.6. Obtain an adjustable silicone cap – head straps can be used as well, however EEG
 250 silicone caps can be better adjusted to the size and the shape of participants' head and are
 251 therefore more comfortable for electrode placement.

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253 1.7. Obtain measuring tape (flexible; plastic or ribbon).

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255 1.8. Obtain a skin marker – skin marker pencils or various makeup products (e.g., eye pencil or eyeshadow crayon), the later can be even more convenient as they are dermatologically tested and easily removable.

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259 1.9. Obtain cotton pads.

261 1.10. Obtain comb and single-use mini silicon hair bands.

263 1.11. Obtain a syringe or plastic pipette.

265 1.12. Prepare a protocol sheet – fill-in form for basic information about the session i.e., participants ID, study ID, date, times, notes, etc (see **Appendix** for an example).

1.13. Prepare a table with pre-calculated head measures to help with electrodes' placement.

NOTE: To speed-up the process and to reduce the possibility for errors, it is advisable to have this table ready in advance. The measurement is based on 10-20 EEG electrode placement system; the values used for calculations are nasion-inion/left-right-preauricular distances (see below). The table gives 20% values for a range of distance values. We have found it as the most convenient to have the table embedded into the protocol sheet (Appendix).

1.14. Prepare questionnaires. For each session, collect data on sensations and side effects before and after tDCS; sensations and the level of (un)pleasantness during tDCS; mood and general subjective state i.e., freshness/tiredness.

[Place Figure 1 here].

2. Programing stimulation protocols

NOTE: Exact steps in programming tDCS protocol differ across tDCS systems/devices. However, all tDCS devices provide basic features – the ability to produce constant current with desired stimulation intensity, the ability to gradually ramp up and down, and a method to set the duration of stimulation. The more advanced protocols such as theta-oscillatory tDCS require devices/systems that allow for custom-built stimulation protocols.

2.1. Constant anodal tDCS

2.1.1. Define the standard constant anodal tDCS protocol (**Figure 2A**) as: (1) fade-in period of 30 seconds, when current intensity is gradually ramped up from 0 mA to the target intensity (we typically use 1.5 mA, but other intensities can be used as well, providing they stay within safety limits); (2) stimulation period during which the constant current of the target intensity (e.g., 1.5 mA) is delivered; and (3) fade-out period of 30 seconds when current intensity is gradually decreased to 0 mA.

[Place Figure 2 here].

2.2. Theta oscillatory tDCS

2.2.1. Theta-oscillatory tDCS delivers current of varying intensity but does not switch polarities (Figure 2B). Therefore, define the waveform in which the current is delivered as following: (1)

fade-in period of 30 seconds, when current intensity is gradually ramped up from 0 mA to the target intensity (e.g., 1.5 mA); (2) the stimulation period of 19 minutes in which the current oscillates around the target intensity within a pre-defined amplitude range (we use oscillations of \pm 0.5 mA of the target intensity) in a selected frequency (we typically use 5 Hz frequency as representative for theta rhythm); and (3) fade-out period of 30 seconds to bring the current intensity to 0 mA.

NOTE: This protocol can be generated by any experimental control software (e.g., CED Signal) and delivered through an intelligent interface (e.g., CED 1401 range of devices) that is compatible with tDCS device which is to be used. Some more advanced dedicated transcranial electric stimulation (tES) systems besides tDCS can deliver alternating current (tACS) and random noise stimulation (tRNS) too. They can also be used to generate the oscillatory tDCS protocol. For example, in StarStim the theta-oscillatory tDCS protocol are defined as a linear combination of tDCS (1.5 mA) and tACS (±0.5 mA, 5 Hz). This type of protocol can be personalized in a sense that not all participants receive oscillatory stimulation in the same frequency (i.e., 5 Hz), but that the frequency is adjusted to the dominant frequency within theta band for each person (e.g., Person 1: 5 Hz, Person 2: 6 Hz, Person 3: 4.5 Hz, etc.).

2.3. Sham tDCS

2.3.1. Use a sham protocol with the same duration as constant/oscillatory tDCS (**Figure 2C**). Namely, define it as: (1) first fade in/out period in which the current is gradually ramped up to target intensity (e.g., 1.5 mA) and gradually ramped down to 0 mA during the first 60 seconds (2) 18 minutes of 0 mA, and (3) the second fade in/out period which again lasts 60 seconds.

NOTE: An alternative approach would be to use very low current intensity over the entire stimulation period (20 min). This type of sham protocol is programmed the same as the anodal stimulation (only the current intensity is set to (0.1 mA) and is designed to produce cutaneous sensations but the intensity is too week to produce any physiological effects ³³.

3. Electrode placement (Figure 3)

3.1. DLPFC electrode montage: For stimulation of the DLPFC, place the target (anodal) electrode on either F3 (left) or F4 (right) of the international 10-20 EEG system. Place the return electrode (cathodal) on the contralateral cheek - i.e., right cheek for F3 anode and left cheek for F4 anode.

3.2. PPC electrode montage: For stimulation over PPC, place the target (anodal) on either P3 (left) or P4 (right) of the international 10-20 EEG system. Place the return electrode (cathodal) on the contralateral cheek same as in DLPFC montage.

3.3. The target electrode placement

348 3.3.1. To locate F3 on participants' head

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- 3.3.1.1. Use the measuring tape to measure the distance between nasion (the deepest point of the nasal bridge) and inion (the most pronounced point of the external occipital
- protuberance) going over the top of the head. Mark the halfway distance with the skin marker
- with a thin line.

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3.3.1.2. Measure the distance between the ears (use preauricular points as references) going over the top of the head and mark the halfway distance with a thin line.

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3.3.1.3. Find the vertex or midline central position, referred as Cz, at the intersections of the two midlines. Mark it clearly with the skin marker.

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3.3.1.4. Measure again the nasion-inion distance, but this time going over Cz, and note the distance as measure A. Measure again the distance between the ears, this time going over Cz, and note the distance as measure B.

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3.3.1.5. Calculate 20% of distance A, and 20% of distance B (or see Protocol sheet for pre-calculated values).

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368 3.3.1.6. Move 20% of distance A forward from Cz along the nasion-inion line to reach Fz (midline frontal) and mark the spot.

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3.3.1.7. Move 20% of distance B leftward from Cz along the inter-auricular line to reach C3 (left central) and mark the spot.

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3.3.1.8. Move 20% forward form **C3** (in parallel with the nasion-inion line), and 20% leftward form Fz (in parallel with the inter-auricular line), to reach F3 at the intersection. Mark **F3** with the skin-marker and place the center of the electrode at the spot.

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3.3.2. To locate **F4**, follow the same procedure only on the right side of the head.

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380 3.3.3. To locate P3 on participants' head

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382 3.3.3.1. Follow the steps 3.3.1.1-3.3.1.5 as outlined above (find Cz, note distance A and B, calculate 20%).

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385 3.3.3.2. Move 20% of distance A backward from Cz along the nasion-inion to reach Pz (midline parietal) and mark the spot.

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388 3.3.3.3. Move 20% of distance B leftward from Cz along the inter-auricular line to reach C3 and mark the spot.

- 391 3.3.3.4. Move 20% backward from C3 (in parallel with the nasion-inion line), and 20%
- leftward from Pz (in parallel with the inter-auricular line), to reach P3 at their intersection.
- 393 Mark **P3** with the skin-marker and place the center of the electrode at the spot.

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395 3.3.4. To locate **P4**, follow the same procedure only on the right side of the head.

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397 3.4. Return electrode placement

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3.4.1. After securing the target electrode with the adjustable silicon cap (see step-by step procedure), insert the return electrode below the chin band to secure the contact of the electrode with the contralateral cheek.

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4. Step-by-step procedure

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405 4.1. Before the tDCS session

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4.1.1. Check if each participant satisfies the inclusion criteria as defined in ethical approval for the study (see **Appendix** for the most common inclusion/exclusion criteria).

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4.1.2. Ask the participant to fill in the Participant information sheet (including all the relevant information such as age, gender, nicotine/alcohol consumption, etc.³⁴).

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4.1.3. Following the institutional review board ethical guidelines, and ask participant to sign informed consent. Use this opportunity to explain the basic aspects of the procedure they will undergo and answer any questions the participants may have.

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4.1.4. Depending on the study design, perform baseline cognitive assessment (memory and/or other cognitive functions).

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420 4.2. tDCS set-up and stimulation

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422 4.2.1. Seat the participant comfortably in a chair.

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4.2.2. Ask participant to fill out pre-tDCS sensations checklist and to report on overall state (i.e., current mood; freshness/tiredness – these can be assessed as either as a single Likert-type item or using standardized questionnaires such as Brief Mood Introspection Scale ³⁵).

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4.2.3. Take head measures using a measuring tape.

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4.2.4. For locating the DLPFC or PPC follow the procedure described above (electrode placement). Write down the measures in the Protocol sheet for each participant. These can be used to check against when taking measurements in subsequent sessions.

4.2.5. To increase conductance, move away participant's hair from stimulation site (use comb and hair bands for participants with long hair).

4.2.6. Inspect for any signs of skin damage at the place of stimulation. Avoid positioning electrodes over damaged skin.

4.2.7. Clean the surface of the skin where electrodes will be placed using alcohol-soaked cotton pads to remove grease, dirt, etc. and let it dry (use a makeup removal product if the participant has heavy makeup on the cheek).

4.2.8. Put the silicon cap on the participant's head and secure it with the chinstrap. Do not make the cap tight (this will be done later).

4.2.9. Soak the sponge pockets with saline solution and put the electrodes inside them. The sponges should be moist but not dripping; usually 10-15 mL of saline solution per sponge is enough. If the sponges are too dry this will cause high resistance and result in poor conductivity, even losing the circuit connection.

NOTE: Most of the tDCS devices have the resistance indicators; however, the sponges should be occasionally inspected for moisture. On the other hand, if the sponges are excessively wet it can cause the current to shift across the head during stimulation. It is recommend having sponges medium wet and use a syringe to add more saline solution during the experiment if the sponges become too dry.

 4.2.10. Put the sponge electrode under the silicon straps and position the center of the target electrode on the marked head-location. Set the return electrode on the contralateral cheek. Use the silicon straps to adjust the cap to the participant's head-size and shape. The cap should be tight so the electrodes cannot move, but still comfortable for the participant.

4.2.11. Turn on the stimulator, select and run predefined tDCS protocol (active anodal stimulation or sham).

4.2.12. Ask the participant to relax and let them report how they feel during the first few minutes of stimulation (1-3 minutes). Explain that the sensations will slowly fade away as they get used to it or when they start focusing their attention on some other activity.

4.2.13. To avoid unstructured activities that can interfere with the stimulation effects, use light cognitive engagement during tDCS. For example, participants can perform practice trials of cognitive tasks or engage in easy memory games during stimulation (starting after 3-5 minutes of stimulation). This type of cognitive engagement during stimulation has the potential to promote tDCS effects and will help participants to keep the mind off the tDCS-induced skin sensations.

4.2.14. Ask the participant to report how they feel multiple times during stimulation (e.g., to 479 report the level of unpleasantness on a 10-point scale every 5 minutes of stimulation, 1 -480 completely absent, 10 - very intensive). Higher levels of unpleasantness (>6) could be expected 481 during fade-in fade-out periods in some participants. If the level of unpleasantness remains 482 high after 5 minutes abort the stimulation.

483

484 4.2.15. After the pre-defined protocol run has elapsed, turn off the stimulator.

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486 4.2.16. Remove the sponge electrodes first, and then remove the silicon cap.

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488 4.2.17. Ask the participant to fill out post-tDCS sensations checklist and to report for any side effects not already listed.

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- 4.2.18. Clean the skin on the places where it was marked and inspect the skin for any changes.
- If there is a skin reaction (e.g., local vasodilation i.e., skin redness on the cheek), monitor as it fades away as it is usually a transient reaction in participants with sensitive skin, and should
- 494 disappear within 10-15 minutes.

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4.3. Memory assessment

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4.3.1. To standardize the assessment across participants, use computerized assessment tools i.e., memory tasks with automatic scoring. Several WM tasks (e.g., verbal and spatial 3-back task) and AM tasks (verbal paired learning; face-word cued recall, object location, etc.) can be found here: https://osf.io/f28ak/?view_only=f8d5e8dd71d24127b3668ac3d8769408

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4.3.2. To evaluate the specificity of tDCS effects on memory it is advisable to include control task(s) i.e., tasks tapping other cognitive or motor function.

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4.4. Ending the experimental session/study

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4.4.1. After the (last) experimental session in the study ask participant to try to guess the sessions in which they have received real and sham stimulation. Note all responses and see whether obtained proportions are higher than chance probability. If not, the blinding was successful. If participants were able to differentiate real from sham stimulation analyze the data for those that guessed correctly and those that did not to check if the unsuccessful blinding affected the tDCS effects.

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515 4.4.2. In line with ethical guidelines, debrief the participants in detail after their involvement is completed.

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4.5. After the experimental session

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4.5.1. Wash the sponges with running water and soap so that the saline solution is fully washed away. Let the sponges to dry completely before putting them away.

4.5.2. Use warm water and alcohol to clean all reusable materials including comb, silicone cap and measuring tape.

4.5.3. Make notes on all unusual, unexpected, or unplanned events that might have happened during the session – including any equipment malfunctions, relevant comments made by the participant, interruptions, etc.

REPRESENTATIVE RESULTS:

The described protocol has been successfully used to enhance memory performance in several studies in our laboratory. However, similar protocols have been used in other research laboratories as well (e.g., see ^{36,37}).

When it comes to working memory, our results have shown that 20-minutes of right frontal tDCS (F4 location; constant current of 1.8 mA) enhanced verbal WM, while the same stimulation protocol applied over left parietal cortex (P3 location) resulted in better spatial WM performance. In contrast, no significant effects were found when the same stimulation protocol was applied over the left frontal (F3) and right parietal (P4) cortices. **Figure 4** shows the representative results of modeling of the electric field generated by tDCS as well as the performance measures following active and sham tDCS based on the data reported in Živanović et al., 2021³⁸.

[Place **Figure 4** here].

The effects of parietal tDCS on associative memory have been consistent and robust. That is, in the series of within-subject experiments we have shown that 20 minutes of tDCS over left PPC (F3 location; constant current of 1.5 mA) improves memory for face-word associations ^{27,39,40}. **Figure 5** shows representative task and results. In addition, comparable effects were observed on AM task assessing the object-location associations when right PPC (P4 location) is stimulated using the same constant tDCS protocol ⁴⁰.

[Place Figure 5 here].

The more advanced protocols such as theta-oscillatory tDCS have been less extensively studied, however the study by Lang and colleagues ²⁶ as well as recent study conducted in our laboratory ²⁷ showed improvement in face-word AM following theta-oscillatory tDCS protocol in comparison to sham. The animated figure shows simulation of the electric field induced by theta oscillatory tDCS over left PPC.

[Place **Video 1** here].

FIGURE AND TABLE LEGENDS:

Figure 1: Materials for tDCS experiment (see text for details). 1) tDCS device; 2) electrodes; 3) sponges; 4) saline solution; 5) alcohol; 6) silicone cap; 7) measuring tape; 8) skin pencil; 9) cotton pads; 10) combs and silicon hairbands; 11) syringe

Figure 2: tDCS protocols: (A) Constant anodal tDCS; (B) Theta oscillatory tDCS; (3) Sham tDCS.
Fade in period is marked orange; fade out period is marked green.

Figure 3: Electrode placement scheme.

Figure 4: (A) Effects of constant anodal tDCS of left PPC (P3-contralateral cheek montage) on spatial working memory performance (spatial 3-back task); (B) Effects of constant anodal tDCS of right DLPFC (F4-contralateral cheek montage) on verbal WM performance (verbal 3-back task). The figure shows simulation of electric fields induced by tDCS, outline of the task trials, and the within-subjects performance across active and sham condition (the values are centered to the order of the session to account for counterbalancing i.e., positive values indicate above-average performance, while negative values indicate below average performance at session). The simulation of local electric fields generated by the electrode set up is performed using COMETS2 MATLAB toolbox ⁴¹.

Figure 5: Effects of constant anodal tDCS of left PPC (P3-contralateral cheek montage) on associative memory performance (A) Face-word pairs task; (B) Effects of constant anodal tDCS of left PPC (P3-contralateral cheek montage) on associative memory performance (proportion of correctly recalled words on cue).

DISCUSSION:

The outcome of the tDCS study on memory depends on number of factors, and some of which e.g., homogeneity/heterogeneity of the sample, sufficient statistical power, the difficulty of the memory tasks and motivation of the participants have been previously discussed (see Berryhill, 2014). Several excellent papers on tDCS method, as well as more general tutorials on the application of tDCS to study cognitive functions are available and can be well applied to the memory research too (see^{17,43-47}). Here we will focus on the aspects of the protocol which, based on our experience, are relevant but often overlooked or not discussed in sufficient detail elsewhere.

Placement of the return electrode. It is important to keep in mind that the return electrode is not passive but negative-polarity terminal (i.e., cathode). Therefore, it can induce physiological effects that are opposite to the target electrode. Furthermore, the current flow, depends on the positioning of the return as much as it depends on the target electrode. Moreover, since the current flows along the path of the least resistance, if the anode and cathode are located too close to each other, the current may flow only over the skin surface and/or through the cerebrospinal fluid between the electrodes, thus leaving the cortical tissue unaffected. For these reasons, the careful choice of the return electrode is as relevant as the position of the target electrode. There is meta-analytic evidence to suggest that extracranial cathodes are more likely to produce significant effects ⁴⁸. Positioning of the return electrode on contralateral cheek for memory enhancement was based on current flow modeling and selected to avoid potential confounding effects of generating negative polarity over function-irrelevant brain areas. The positioning of return electrode on the contralateral cheek has successfully been used

in previous WM studies (see ^{36–38,49}, as well as in AM studies ^{27,39,40}), and has been highlighted as a good choice for tDCS montages aiming to modulate other cognitive functions as well ⁴⁵.

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Blinding. In single blind experiments, to ensure blinding of the participant, the position of the stimulator and/or monitoring display should be out of participant's sight. This is especially important when using stimulators that have lights indicating when the unit is on and/or delivering current. For double-blind designs (when both participant and experimenter are unaware of the protocol which is administered), one should use the double-blind option, or similar option that is available for a given device. If such option is not available, the good practice is to have two-experimenter procedure. That is, one experimenter comes in only to run the stimulation protocol, while the other experimenter who runs the participant through the experiment, including the subsequent memory task and analyzes the data, leaves the room just before and during the stimulation. By methodological standards, double-blind experiments are preferred to the single-blind designs because they reduce the bias or the "experimenter" effects. This is highly relevant when conducting clinical trials and/or using the interview-based assessments of cognitive functions. However, blinding of the experimenter is less of an issue when participants are highly motivated to maximize their performance (which is mostly the case in memory assessment or cognitive enhancement in general), and when the task is administrated as well as scored automatically (i.e., when experimenter has little to no intervention in the assessment phase).

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Activity during tDCS. Authors of tDCS papers rarely report on what were the participants doing during stimulation. When the activity is not reported it is usually implied that the participants were instructed to sit comfortably and relax. However, the absence of structured activity represents source of the uncontrollable "noise" in the experiments. Namely, 20 minutes is rather long time, so some participants may use the time to relax (with possibility to even fall asleep) while others may focus on tDCS sensations or start ruminating or excessively thinking about some tDCS unrelated topics. There is evidence to suggest that function-relevant but not tiring activity performed during tDCS has the potential to promote tDCS effects 50. For these reasons, in our experiments, participants perform either practice trials of the memory tasks to be used as outcome measures or similar memory tasks. Practice trials are good choice because they engage the same neural networks as the target function but are easier and therefore not frustrating or tiring for the participants. Besides that, performing practice trials during stimulation is economical in a sense that it cuts down the testing time following tDCS, which comes as a benefit especially when the study design includes multiple tasks to be completed post-tDCS. However, the practice trials are usually much shorter than 20 minutes, thus alternative activity needs to be presented too. For this purpose, we have used common memory games ⁴⁰, that keep the participants focused, help them pass the time and keep the mind off the tDCS induced sensations and make them overall more comfortable in the testing setting. A few things to keep in mind when choosing the memory task to be performed during tDCS are that the task should not be difficult but also not boring (adaptive tasks set at 80% success rate are good in this context); the task should not have the material that might interfere with subsequent memory assessment (e.g., when assessing memory for faces and words, one can use abstract images/shapes pairs). Another important issue is the duration of the "habituation period" i.e., how long after the beginning of stimulation should participants start to perform the "distraction activity". There are individual differences in the intensity of the sensation and habituation times, but majority of participants will be ready to start the activity after 3-5 minutes of stimulation.

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Cutaneous sensations. Some participants may be more sensitive to cutaneous tDCS effects, thus reporting elevated levels of discomfort, although this does not happen very often. It is important to inform participants about potential sensations they might experience prior the experiment. If someone is afraid of the procedure, we often let participants "feel" the current on their hand before putting the sponges on their head. The participants should be continuously monitored and asked to provide feedback on their level of comfort and sensations at regular intervals. If the participant reports increased level of discomfort, always offer to abort the experiment. It is essential that the participants are aware that the stimulation can be stopped at any time if they ask. If participant decides to stop the stimulation, the current should be slowly turned down (abrupt cancelation of the stimulation protocol may induce even stronger sensations). It is often recommended that in the case of unpleasant sensations the current intensity is temporarily lowered to the highest comfortable level, until participant adjusts, and then gradually returned to the target intensity. This seems like an appropriate alternative to stopping the stimulation protocol, especially if tDCS is used in clinical setting. However, when tDCS is used for the research purposes, and especially in relatively small samples, it is essential that all participants undergo the same procedure. Therefore, stopping the experiment is preferred to lowering the intensity of the stimulation for some participants for some time.

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Reporting tDCS methodology and monitoring for potential confounds. The tDCS research field is highly heterogeneous regarding methods and measures, thus it is important to clearly report all aspects of the tDCS procedure, including blinding procedure and assessment; the headpositioning of the target as well as the position of the return electrode; the size and shape of the electrodes; type of conducting substance used (saline or gel); the current intensity (mA) and density (mA/cm²) as well as the duration of fade-in/out period; the impedance levels if measured; the duration of the stimulation (including the fade-in/out period); the detailed account of the activities participants were engaged in during the stimulation; the timing and the duration of the cognitive tasks following the stimulation (including break-times, if any). This type of information facilitates standardization and systematic analysis of the published studies (see recent review for example 51). The aspects that are rarely reported on are the effect of potentially moderating/confounding variables such as time of the day of tDCS session, level of tiredness/mood reported by the participants, successfulness of blinding (i.e., beliefs about the type of stimulation they are receiving), the order of experimental sessions in within-subject designs, etc. Most of these variables have been reported to modulate the effects of tDCS, but their effect remains understudied and inconsistently reported. Therefore, tDCS studies should ensure to collect and report on any potentially confounding variables; for details on good practices see **Tables 10A, 10B, 11** by Antal and colleagues ³⁴.

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Application of the described protocol for anodal tDCS either in its standard or, even more, in its

advanced form (i.e., oscillatory-modulated tDCS) provides a mean not only for enhancement of memory functions (and prospective use in clinical populations), but also allows for investigation of the neurobiology of the functional neural networks behind these functions.

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DISCLOSURES:

Authors have no conflicting interests to disclose

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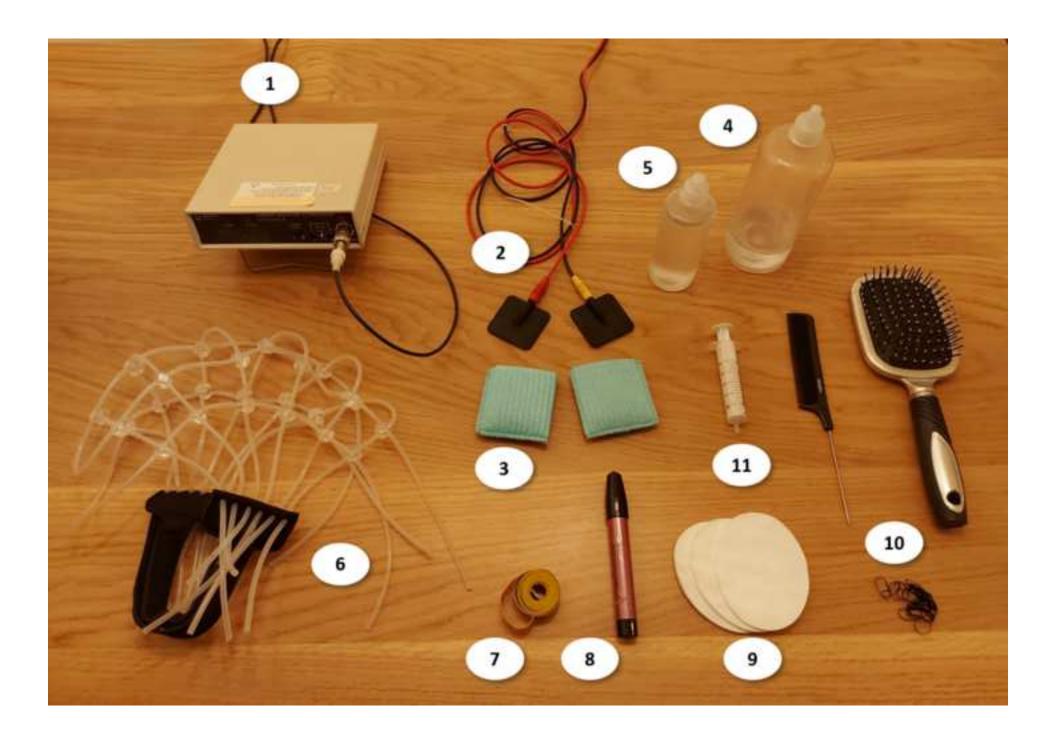
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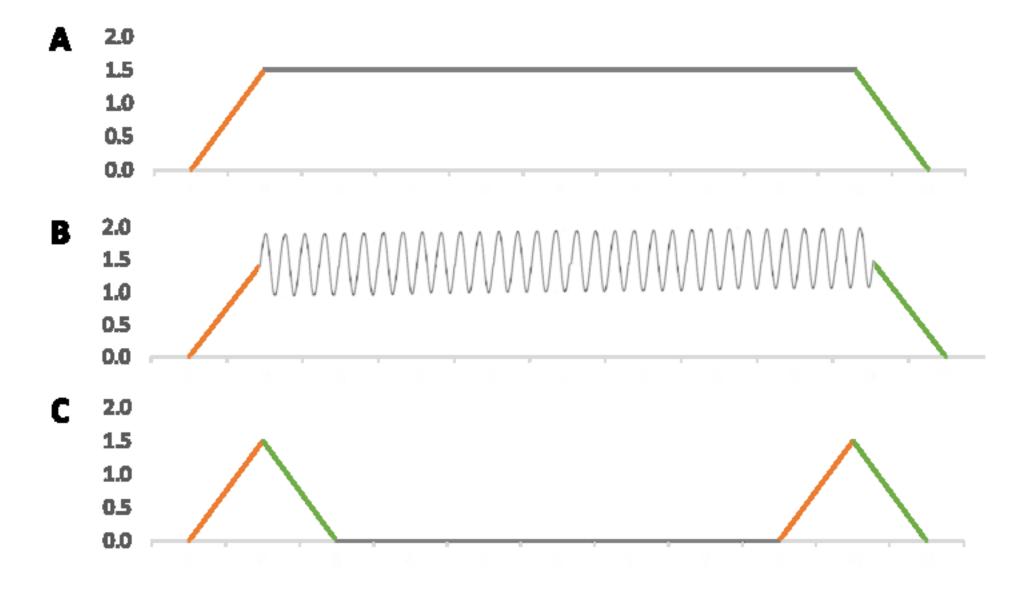
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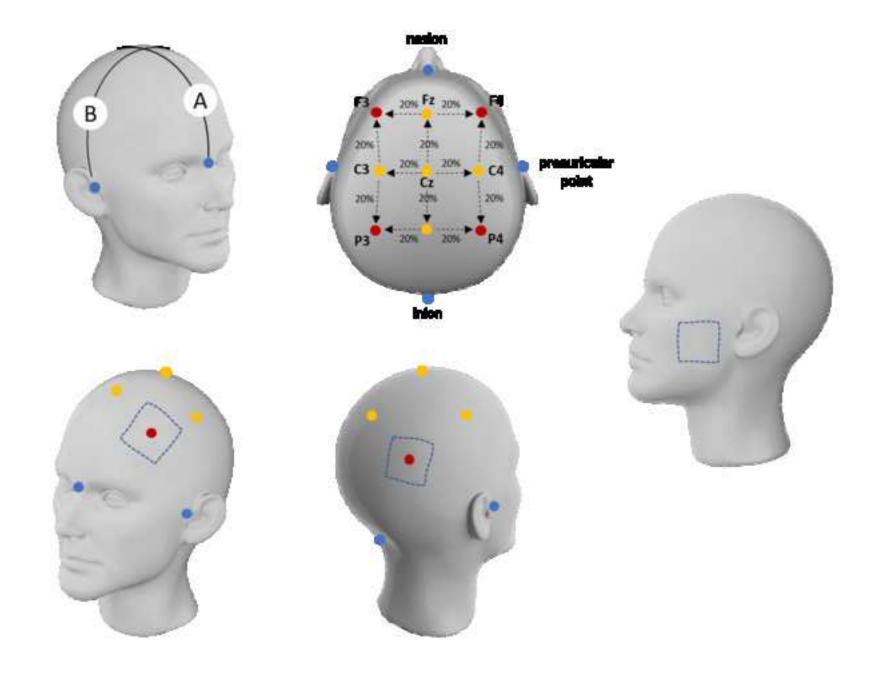
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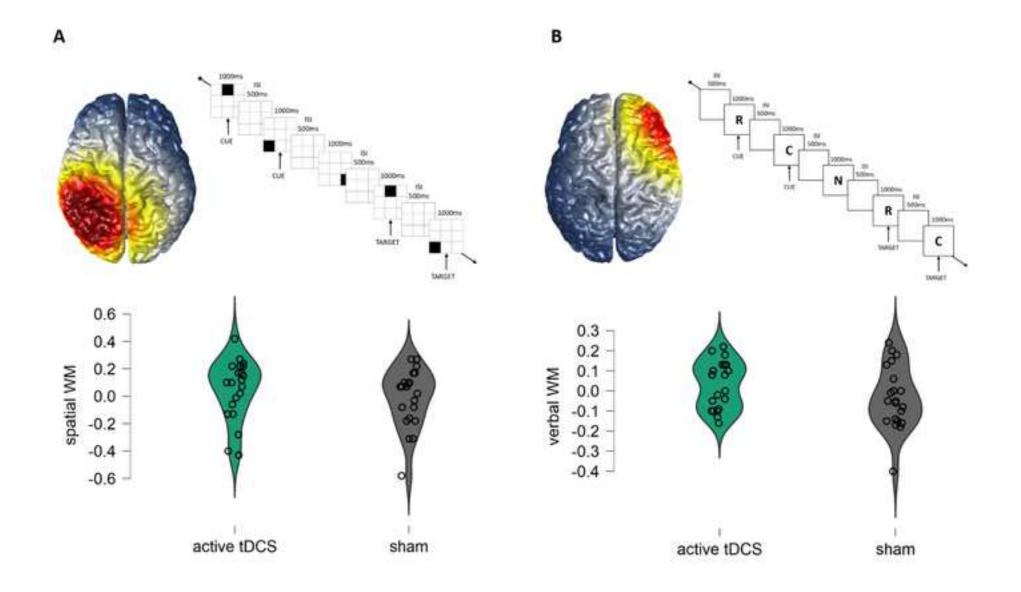
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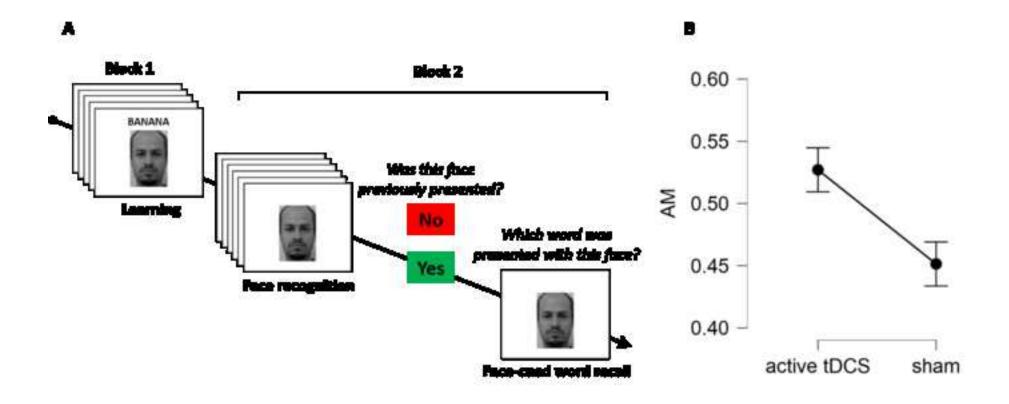
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Dear Editor and Production team,

We would like to thank you and both reviewers on valuable comments and suggestions, as well as overall positive feedback on our manuscript and video *Transcranial direct current stimulation (tDCS)* for memory enhancement (62681). We are now submitting the revised manuscript (all changes are marked in red font) and the point-by-point response to reviews and production team comments. The revised video is submitted via the link. We have addressed all concerns raised by reviewers, and we hope that the manuscript in its current form is suitable for publishing in JoVE.

Editorial and production assessments		
Editorial and production comments		
Please take this opportunity to thoroughly	Thank you – we have proofread the manuscript	
proofread the manuscript to ensure that there	thoroughly.	
are no spelling or grammar issues		
For in-text formatting, corresponding reference	In-text references are now formatted to be	
numbers should appear as numbered	numbered superscripts, and we followed JoVe	
superscripts after the appropriate statement(s).	guidelines for end of text references as well.	
Please do not use any footnotes.	Corrected – the two footnotes have been moved	
	to the main text (L 85-89).	
Please include an ethics statement before the	Corrected - the ethics statement sentence has	
numbered protocol steps, indicating that the	been added (L 386-389).	
protocol follows the guidelines of your		
institution's human research ethics committee.		
Please ensure that all text in the protocol section	Corrected – we checked and ensured that all text	
is written in the imperative tense as if telling	in the protocol section is written in imperative	
someone how to do the technique (e.g., "Do	tense, with only a few exceptions (when the step	
this," "Ensure that," etc.). The actions should be	is optional or could depend on the type of study	
described in the imperative tense in complete	design or equipment used). All the safety	
sentences wherever possible. Avoid usage of	procedures have been covered in the protocol.	
phrases such as "could be," "should be," and		
"would be" throughout the Protocol. Any text		
that cannot be written in the imperative tense		
may be added as a "Note." However, notes		
should be concise and used sparingly. Please		
include all safety procedures and use of hoods,		
etc.		
The Protocol should contain only action items	Corrected – The protocol contains only action	
that direct the reader to do something. Please	items - with a few words justifying or explain the	
move the discussion about the protocol to the	rationale behind each step, or providing	
Discussion.	additional information about how the step	
Discussion.	should be performed e.g., Write down the	
	measures in Protocol sheet for each participant.	
	These can be used to check against when taking	
	measurements in subsequent sessions.) These "additional information" is very short, and we	
	believe add to the better understanding of how	
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	and why some steps should be performed, and do not require extensive discussion.		
Please add more details to your protocol steps.	We tried to add more details and ensure that the		
Please ensure you answer the "how" question,	"how" question is covered throughout the		
i.e., how is the step performed?	protocol.		
Step 1 of the protocol should be moved to the	We are aware that this may not be typical for		
introduction.	JoVe papers. However, in this research field		
	Study design is one of the most important		
	aspects of the method, which is why we see it as		
	the first steps of the protocol (please note that		
	the Reviewers also complimented this part of the		
	paper). Our main rationale behind having it in		
	the Protocol rather than in the Introduction is		
	that the decisions which are made in this regard		
	are: a) methodological b) affect many of the		
	steps to follow, c) we provide "how to" design a		
	study and the rationale behind that. Therefore,		
	we would highly appreciate if it is possible to		
	leave this section as a part of the protocol.		
Step 2 of the protocol can be moved to the Table	As many different materials/devices/software's		
of Materials. Please revise the table of the	can be used to perform the described protocol –		
essential supplies, reagents, and equipment. The	we believe that it is important to describe the		
table should include the name, company, and	properties/characteristics/utilities of the		
catalog number of all relevant materials in	materials/devices and use generic terms of the		
separate columns in an xls/xlsx file. Please sort	products that are needed rather than providing		
the Materials Table alphabetically by the name of	names of manufacturers. We understand how		
the material.	catalogue numbers and manufacturers may be		
	important for some protocols to ensure		
	reproducibility of the results, However, in case of		
	our protocol, many of the materials can be self-		
	made (e.g., table with precalculated measures),		
	or not strictly scientific items (e.g., comb,		
	measuring tape, or a makeup product used to		
	mark the skin), or even items that are very widely		
	used (e.g., alcohol, syringe) thus many of the		
	materials don't have "catalogue numbers".		
	Furthermore, this paper is not industry-funded,		
	thus we would like to avoid putting forward one		
	manufacturer and omitting the names of others		
	that are equal in terms of quality. This is why we		
	give some examples in the text, but discuss how		
	the same results can be achieved with different		
	equipment/software.		

Reviewer #1: Manuscript Summary: The protocol is very interested and well written. All the needed information is clearly presented. Major Concerns: No Minor Concerns: The authors could add a control frequency for the specificity of tACS. For instance gamma seems very important for aging. Thank you for the positive feedback. Thank you for this suggestion – we have added this suggestion (L 151-154)

Reviewer #2:

Manuscript Summary: The paper is excellent. The authors provide a detailed account of a tDCS protocol for memory enhancement that has proven its efficacy in multiple studies. The explanations are thorough. It will be a helpful resource for anyone looking to conduct a tDCS study about memory enhancement and who does not know where to start or does not know which tDCS protocol to use.

Thank you for taking the time to review our manuscript. We highly appreciate positive feedback any suggestions for improving the paper.

Major Concerns: Lines 392: I agree with the authors that letting the participant perform a task related to the variables studied is essential. However, this part could benefit from more explicit guidelines. Questions that are not answered but could matter: "How long should the habituation period to the tDCS stimulation last? After which duration of stimulation should the participant start to perform a task? What does the participant do during the first minutes of the stimulation?." Indeed, the authors did not discuss a habituation period. From my personal experience with using tDCS, I noticed that participants are often focused on the stimulation's sensations which hinders the completion of the task at hand if there is one. One option would be to only start the completion of the task after e.g. 5 minutes of stimulation (and during the habituation period, the researcher could do some small talk to put the participant at ease). In line with this, in many tDCS studies, there is usually a habituation period from 3 to 5 minutes.

Thank you for this comment – we have omitted to be specific regarding the habituation period and the timing of the tasks. We agree that this is an important point, and it seems that we all do it in the similar manner in our studies – however we fully agree it should be explicitly stated in the protocol. Following your suggestion, we have now added information to the protocol (L415 - 423), as well as to the discussion (L591-595)

Line 415: I was about to congratulate the authors for having included a link to computerized tasks, but when I followed the OSF link, I realized the tasks were removed from OSF in March. Are the authors planning on making them available again before publication? These tasks would be an essential addition to the paper and protocol. Additionally, I am also wondering which software the tasks were built with (e.g., only requiring

We are very sorry for this – out intention is to provide the tasks, but we were in the process of updating them during the submission of this manuscript. Now we made them available through OSF, and we hope they will be a useful resource for other researchers. All tasks are built in Open Sesame, so they are easy to adapt – we hope we will add the data on their psychometric

Open Sesame or a similar open-source and free software would make it easier for any researcher to use the tasks).

properties soon too.

Minor Concerns: Before the tDCS session: it would be worth summarizing the most common inclusion and especially exclusion criteria for tDCS studies. Additionally, the authors could provide or link to questionnaires including some or all of the following variables which could influence the effect of tDCS: substances use (nicotine, alcohol, caffeine/theine), the time of the day of the tDCS session, participants' handedness (this could be specified in the exclusion criteria), participants' medication use (this could be specified in the exclusion criteria), and the hormonal/menstrual cycle of female subjects. These variables are discussed in Antal et al. (2017), https://doi.org/10/gf9368. Perhaps the easiest option for the authors would be to refer the reader to tables 10A, 10B, and 11 in Antal et al. (2017). Moreover, the discussion or the protocol could benefit from mentioning the importance of clearly reporting the various parameters involved in a tDCS study, as it is an area in which tDCS papers are sometimes lacking. The authors could capitalize on recently published guidelines to report tDCS research (e.g., see the checklist provided in Hoebeke et al., 2021, https://doi.org/f83m).

Thank you for this suggestion, we have now added the list of most common inclusion/exclusion criteria in the Appendix.

Thank you for suggesting this, we have now referred to the Antal et al. (2017) in both protocol and the discussion (L374-375; L614-631). Finally, we added the section in the discussion to highlight the importance of reporting tDCS parameters (we have listed them and we refer to the Hoebeke et al. (2021), as well as the effects of potential confounding variables (such as those listed in Antal et al. 2017) (L614-631).

On lines 361-362: It would be helpful to give an example of mood and alertness assessment.

Thank you for this suggestion we have now provided a reference to the questionnaire that is simple and works quite well (L384-385).

Reviewer #3

Overview: This work describes experimental protocols that may be used to influence working memory (WM) as well as associative memory (WM). There is a lot to like about this manuscript. Given that an aim of this project is to standardize stimulation procedures, I think this work does a nice job of giving out essential background knowledge to inform best practices in experimental design. I particularly think the reasoning in section 1.4 Outcome measures is a particularly important point to impress upon the field. Overall, I think this work will make a good contribution to the tDCS literature, but have a few clarification points that the authors might consider.

Thank you for taking the time to review our paper - we highly appreciate positive feedback and useful suggestions on how to improve it.

Protocol:

1.1. Sham Control: For sham, some groups use a different sham procedure where very low

Thank you for this input – we have now added

constant stimulation (.1mA) is provided over the entire stimulation interval (For a brief Discussion, see Coffman, et al., 2014). The authors might mention this type of sham procedure as an alternative approach.

this type of sham procedure as an alternative under the section Sham tDCS (L311-314).

1.2. Within- or between-subjects design: This section suggests at least a 7 day delay between active versus sham stimulation in within-subjects design (which I agree is good practice), but it might be worth offering citation(s) that empirically supports this approach.

We design our studies with minimum of 7 days between the sessions, mainly to ensure no aftereffects of tDCS (in one of our studies we have seen that the single session effects may persist up to 5 days). However, there are studies showing that even shorter wash-out periods do not affect the outcome, so we added those references as well. Unfortunately, we did not find any study that would provide a straightforward empirical support for "7 days" so we reframed it to "good practice". (L169 -171)

As an additional point, I'd suggest to offer guidance in this work about the duration of stimulation with respect to the AM/WM task. For example, if a researcher is interested in stimulation applied during both study/encoding and memory test in an AM task, then best practice is to ensure that stimulation cover both encoding and memory for all participants. This may mean that experimenters should use memory tasks that have precise timing, so that all participants undergo stimulation for the same segments of the task. This is yet another best practice to add precision to protocols.

We fully agree that precise timing is essential - we have now added the recommendation to use computerized tasks to control for the task duration and ensure precise timing. We have also added the recommendation on the duration of the task/task block, as well as the recommendation on the maximal duration of the cognitive assessment. (L211-227)

Again, I think this is a nice project that will promote more consistent procedures in the tDCS literature, which will benefit the field. References:

Thank you for providing us with useful suggestions.

Coffman, B. A., Clark, V. P., & Parasuraman, R. (2014). Battery powered thought: enhancement of attention, learning, and memory in healthy adults using transcranial direct current stimulation. Neuroimage, 85, 895-908

Changes to be made by the Author(s) regarding the video

Please increase the homogeneity between the video and the written manuscript. Please revise the narration to be more homogenous with the written manuscript. Ideally, the narration is a word for word reading of the written protocol.

Title Cards:

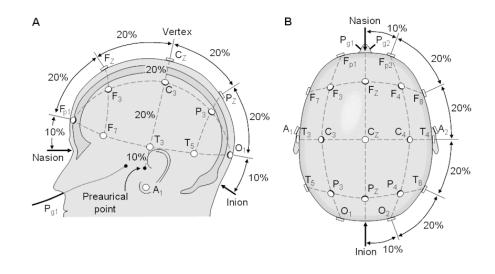
Thank you for this comment. We have reviewed the consistency of the narration in the video with the written protocol.

We have changed the title cards to have better

Please add a high-quality chapter title cards. it	resolution, and capitalized words. Ethics title
looks low resolution compared to the video	card was moved as suggested.
resolution.	
Please capitalize the first letter of every	
important word in your chapter title cards.	
• 03:39 Please remove your Ethic title card from	
this place. This title card should appear between	
the end of the introduction and the beginning of	
the protocol."	
Audio Editing and Pacing:	Thank you for this comment – we have now
Audio levels are quite low. Please ensure audio	increased the audio levels.
level peaks average around -9 dB.	

PARTICIPANT ID :	
STUDY ID:	

A/B	Cz	20%
31	15.5	6.2
32	16.0	6.4
33	16.5	6.6
34	17.0	6.8
35	17.5	7.0
36	18.0	7.2
37	18.5	7.4
38	19.0	7.6
39	19.5	7.8
40	20.0	8.0
41	20.5	8.2
42	21	8.4
43	21.5	8.6



Session 1	Session 2	Session 3	Session 4
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Experimenter:	Experimenter:	Experimenter:	Experimenter:
measures:	measures:	measures:	measures:
Sensations:	Sensations:	Sensations:	Sensations:
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Notes:	Notes:	Notes:	Notes:

Inclusion/exclusion criteria for tDCS study (suggested list for healthy participants study):

Native speakers or proficient language use (the language in which the study is conducted)

Normal or corrected to normal vision

Right-handed

Naïve to tDCS

Cognitive performance within the normal range

History of neurological/psychiatric disease; epilepsy /seizure

History of surgical procedure involving head or spinal cord

History of head trauma, followed by impairment of consciousness

Metal or electronic implants in the brain

Metal or electronic device in other sites of the body (cardiac pacemaker, traumatic metallic residual fragments)

Skin conditions (e.g. dermatitis, psoriasis, eczema) at the potential electrode placement sites; or highly sensitive skin

Fainting spells or syncope

Pregnancy

Use of medications (to be consulted with the medical doctor)

Experience of adverse effects following electric or magnetic stimulation