BSTIM Protocol – Version 4 (December 13, 2023)

Originally proposed DSMB protocol, with statistical analysis plan moved to Statistical Analysis File.

Effect of Noninvasive Electrical Brain Stimulation on Memory Performance at Different Times of Day in Younger and Older Adults

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BSTIM:
Effect of Noninvasive Electrical Brain Stimulation on Memory Performance at Different Times of Day in Younger and Older Adults

Objectives

We have recently found that tDCS has a larger impact on episodic memory in the morning, but given the mixed literature on tDCS, we aim here to (1) complete a reliable replication attempt of these published tDCS findings, (2) extend this time-of-day tDCS research in new directions, including aging, and (3) explore interesting, yet secondary, aging and memory questions within our non-tDCS (baseline data) data.

Aim 1: Determine the extent to which tDCS to left dorsolateral prefrontal cortex (or dlPFC) boosts recollection accuracy and working memory performance as a function of time-of-day in younger and older adults.

Secondary: Assess the relationship between objectively measured sleep patterns and self-reported morning-evening preferences on memory performance, and possible interactions with tDCS.

Aim 2: Determine the extent that tDCS to dlPFC boosts information-specific processes compared to information-general cognitive control processes in younger and older adults. The cognitive tasks will experimentally pit information-specific (words, pictures) and cognitive control (easy, hard) to test dlPFC tDCS effects in each age group.

Secondary: Determine the regional specificity of tDCS on task performance in younger adults, by comparing the impact of tDCS to left dlPFC and left parietal cortex (an active stimulation control site, as in Gray et al., 2015).

Aim 3: Determine the extent to which a pre-post between-subjects tDCS design can improve the detection of stimulation effects, while also preserving the validity of the sham condition.

Design and Outcomes

50 Younger Adults will be randomly assigned to three between-subjects tDCS conditions (left prefrontal, sham, left parietal control, total YA n=150) and 50 cognitively normal older adults will be randomly assigned to two between-subject tDCS conditions (left prefrontal, sham, total OA n=100). Each participant will complete 3 sessions: orientation/baseline (no tDCS, time varies), AM tDCS, PM tDCS (counterbalancing the order of the AM and PM sessions). Primary outcome measures will be cognitive task performance (episodic recollection accuracy primary, working memory accuracy secondary) as a function of 3 key factors (age group, tDCS

condition, and time-of-day) and their interaction, and also as a function of secondary factors obtained from actigraphy sleep data, sleep diary/log data, and the Morningness-Eveningness Questionnaire (aka the Owl-Lark assessment).

Interventions and Duration

Morning testing sessions will be scheduled at 8 or 9 AM, afternoon testing sessions will be scheduled at 3 or 4 PM. These times were chosen to be similar to those we used in our preliminary work that demonstrated time-of-day effects on tDCS in younger adults (9 am vs 1 pm), except we have shifted the PM session to 3 or 4 PM to be more consistent with aging studies that have found AM and PM effects on episodic memory (May & Hasher, 2017; May et al., 1993; Intons-Peterson et al., 1998)

Active tDCS will involve 20 minutes of continuous electrical stimulation (2.0 milliamperes, mA). Sham tDCS will involve 1 minute of stimulation at the start of the 20 minute period (i.e., 30 s to gradually ramp up to 2.0 mA, and then 30 s to ramp down), and also 1 minute of stimulation at the end of the 20-minute period. As in our prior work, during stimulation participants are instructed to sit quietly while remaining awake in preparation for the upcoming memory test, but no further instructions are given.

Standard Anodal Procedure. The tDCS technique that we use, modeled after the most effective techniques found in the literature (e.g., Nitsche et al., 2008), and aimed at targeting left
prefrontal cortex (for recent electrical models of tDCS, see Opitz, Paulus, Will et al. NeuroImage 2015) involves 3 basic steps: (1) the anodal electrode (35 cm^2) is placed on the scalp over left dlPFC (F3 using the 10-20 EEG system, which typically centers over posterior middle frontal gyrus BA 8/9, Herwig et al, 2003), and the cathodal electrode is placed over the contralateral supraorbital site, thereby maximizing anodal stimulation of dlPFC and surrounding areas; (2) while sitting quietly, the brain is stimulated with mild electrical current (2 milliamperes, mA, using gradual ramping) for a total time of 20 minutes (stimulation condition), or a few seconds of mild current with no stimulation during most of 20 minutes (sham condition); and (3) post-stimulation cognitive task are administered, measuring performance enhancements (stimulation > sham). We have found this procedure reliably boosts recollection accuracy for at least 25 minutes post-stimulation, and in the literature, dlPFC effects on working memory have lasted at least 30 minutes post-stimulation (Ohn et al., 2008). Note that the cathodal tDCS electrode has been used to interfere with cognition, but the current project focuses on the anodal-based cognitive enhancement technique.

For tDCS, we will use a standard 1x1 tDCS Clinical Trials device (Soterix Medica, NY), specialized for double-blinding. Active stimulation will deliver 2 mA of current using 2

electrodes in 5 x 7 cm saline-dampened sponges. The anodal electrode is placed over areas F3 (left dlPFC) or P5 (left parietal) according to the 10-20 EEG-system, with the cathodal electrode on the contralateral supraorbital region. To balance the design for blinding, each of the 2 different electrode placements in the active stimulation conditions will be evenly represented in the sham condition (younger adults only). To rule out general arousal effects, participants are prompted to make an arousal rating immediately prior to tDCS and immediately following tDCS. (Our prior work found that tDCS did not affect arousal, compared to sham.)

Sample Size and Population

Younger adults – Frontal stimulation (n=50)
Younger adults – Parietal stimulation (n=50)
Younger adults – Sham (n=50; n=25 frontal placement, n=25 parietal placement)
Older adults – Frontal stimulation (n=50)
Older adults – Sham (n=50)

STUDY TEAM ROSTER

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1 STUDY OBJECTIVES

1.1 Primary Objective

Test the extent to which tDCS to left prefrontal cortex (compared to sham) impacts different aspects of cognitive performance on different cognitive tasks as a function of age group (younger, older) and time of day (AM, PM).

1.2 Secondary Objectives

Test the extent that sleep-related measures inform the primary objectives (above), evaluate the value of including the pre-tDCS baseline cognitive measures in the analyses, evaluate the regional specificity of tDCS effects in younger adults by including a left parietal control site. Also, secondary objectives will include interesting memory and aging questions, independent of tDCS, described below.

2 BACKGROUND AND RATIONALE

2.1 Background on Condition, Disease, or Other Primary Study Focus

A better understanding of how tDCS impacts cognitive function in younger and older adults will have widespread impact. tDCS is the safest and most accessible, non-invasive brain stimulation technique available for testing causal links between different brain regions and functions, and also for attempting to improve and train cognitive abilities in older adults. By identifying key experimental factors that can improve the reliability and robustness of stimulation effects on cognitive performance in different age groups, this project should lead to the widespread adoption of these design features in future scientific and clinical applications. Moreover, we will test the extent that tDCS to dlPFC improves memory performance by boosting information-specific processes and/or cognitive control processes that operate across different types of information, thereby informing basic theories of how dlPFC contributes to memory in younger and older adults.

2.2 Study Rationale

Transcranial direct current stimulation (tDCS) to dorsolateral prefrontal cortex (dlPFC) has been shown to temporarily improve cognition in younger and older adults. Although the current spatial resolution is limited, the technique's ability to improve cognition by selectively stimulating brain regions has significant potential to advance scientific studies of human

cognition. Recently, we have discovered that time-of-day is critical for finding tDCS effects on memory in younger adults, an overlooked factor that may be responsible for mixed results in past studies. Our results also indicate that prior tDCS studies have had too few participants to reliably find the kinds of tDCS effects that we have observed (see also Minarik et al., 2016). Motivated by these discoveries, the current project uses a rigorous and well-powered approach to determine the importance of time-of-day for detecting tDCS effects on memory in younger and older adults, populations that have been targeted in many prior tDCS studies but have different optimal times-of-day. We will use the same tDCS stimulation procedures that we have used in our prior work, which were based on common tDCS parameters for cognitive studies in humans and are noninvasive, minimal risk procedures.

3 STUDY DESIGN

- Study Design: Double-blind, sham-controlled, age group x time-of-day tDCS study, with secondary analyses on sleep data, within-subjects baseline, and left parietal (younger adults only)
- Study Arms: 50 YA (active frontal), 50 YA (active parietal), 50 YA (sham: 1/2 prefrontal, ½ parietal), 50 OA (active frontal), 50 OA (sham: all frontal)
- Study Location: Gallo Memory Lab, Department of Psychology, University of Chicago, Hyde Park Campus.
- Each participant ideally enrolled for 7 days (with flexibility up to 12 days), depending on scheduling constraints
- Intervention: 20 minutes of tDCS to prefrontal or parietal scalp locations, once during the
  AM (8 or 9am) and once during the PM (3 or 4 pm)
- Randomization and double-blinding separately for each age group

4 SELECTION AND ENROLLMENT OF PARTICIPANTS

Inclusion and Exclusion Criteria

Participants must meet all the inclusion criteria to participate in this study, and candidates meeting any of the exclusion criteria will be excluded from study participation:

4.1 Inclusion Criteria:

- Right-handed (according to the Edinburgh Handedness Inventory)
- Normal or corrected vision
- Fluent in English (started learning by age 6)

- Ability to understand and provide informed consent for study procedures, and to comply - with study procedures for the entire length of the study.
- For individuals in the 'younger adults' group, must be between 18 and 30 years of age
- For individuals in the 'older adults' group, must be between 60 and 75 years of age
- For individuals in the 'older adults' group, a score of 23 or above on the Montreal Cognitive Assessment (out of 30, education-corrected) is required. This is to minimize the inclusion of suspected mild cognitive impairment (MCI) or dementia, targeting individuals that score in the normal range according to the recent meta-analysis of MoCA's ability to differentiate normal aging from MCI in Carson et al. (2018, Int. J of Geriatric Psychiatry).
- Performance above threshold on the episodic memory task during the baseline session. The threshold is defined as having a hit rate that is at least 5% greater than the false alarm rate, where hit rate is defined as the number of studied items identified as studied, divided by the total number of studied items, and false alarm rate is defined as the number of new items identified as studied, divided by the total number of new items. We don't anticipate this threshold to exclude many, if any subjects.

4.2 Exclusion Criteria:

- Neuropsychological conditions associated with cognitive decline or seizure
- Cochlear implants or metal in the brain/skull (except titanium)
- Psychoactive medications or psychotic diagnoses
- History of excessive use (clinically treated) alcohol or narcotics
- Hospitalization for head trauma (e.g. concussions) in the past 5 years
- Individuals above a threshold score on an assessment of depression, specifically, a score of 10 or above on the PHQ-9 (Manea et al., 2012)
- Risk of pregnancy
- Low tolerance of skin irritation
- Prior brain stimulation experience (self-report)

4.3 Study Enrollment Procedures

- Younger adults will be recruited from the University of Chicago (primarily students) and surrounding community, and older adults will be recruited from a database maintained by the Gallo lab, as well as newly recruited participants from the Chicago area using word-of-mouth and advertisements (e.g., Chicago Tribune).

- Study coordinator will maintain a Screening Log including reasons for ineligibility and for non-participation of eligible candidates.
- The Study Coordinator will recruit participants, and if they are eligible and wish to participate, use AM/PM Randomization REDCap database to assign them to have their AM session first or their PM session first. The participant will then be scheduled for all 3 sessions.
- Participants will give informed consent for all 3 sessions during the baseline/study orientation day. All participants must consent for themselves. At this time they are considered enrolled.

5 STUDY INTERVENTIONS

5.1 Interventions, Administration, and Duration

Standard tDCS will be used, with 20mA of electricity for 20 minutes during the active sessions. This is a minimal risk technique, with potential adverse effects including irritation, itching, pain or discomfort under the scalp electrodes.

For tDCS administration we will use a 1x1 Transcranial Electrical Stimulation (tES) device for Clinical Trials purchased from Soterix Medical Inc. (NY, NY). The two stimulation parameters (stimulation, and sham) will be pre-programmed into the device by Soterix, using a series of codes that are provided to the PI. The PI will use these codes to develop the participant randomization scheme for each arm of the study, to achieve double-blinding (see **Section 5.2**, below). Prior to the start of the study, a random sampling of 20 of the factory-provided codes will be double-checked by the lab using a digital multimeter (500 codes will be provided by Soterix, the study will use 250).

The University of Chicago IRB (Cheri Pettey, Director, SBS IRB) and the PI have determined that this NIH clinical trial is classified as a basic research study under FDA guidelines, and thus this study does not require FDA approval. The manufacturer of the tDCS device, Soterix, has an Abbreviated IDE for the device, but the current study will use the device for research purposes only and with minimal (nonsignificant) risk procedures that do not require FDA approval.

5.2 Handling of Study Interventions

The Study Coordinator will be responsible for collecting a large portion of the data, and they will not be privy to the stimulation/sham assignments of each participant. Instead, they will only be given access to a soterix stimulation code determined for each participant ahead of time to be inputted into the tDCS device at the time of testing. This code will be linked to

either a sham or stimulation testing session, ensuring complete double-blinding regardless of the experimenter.

Double-blinding. All participants are informed that they will receive tDCS brain stimulation during the 20 m session, with different amounts of electricity and timing depending on the experimental condition, and also that the typical sensation of tingling or itchiness might habituate. This accurately describes the sensations in the sham condition, because stimulation is briefly presented, as well as the sensations in the stimulation condition, as participants do often habituate.

Soterix Medical Inc. will create codes in advance for the tDCS device, which, when entered into the device, will deliver either active or sham stimulation. A subject will be randomly assigned to a code just before the first stimulation session, using REDCap's randomization feature. None of the the study personnel will have access to the spreadsheet that links the codes to the active or sham condition. Philip Schumm and Diane Lauderdale will have access to this spreadsheet, and will complete the randomization setup in the REDCap databases.

5.3 Concomitant Interventions

See inclusion/exclusion criteria.

5.4 Adherence Assessment

Adherence to the study regimen is defined as participants completing all 3 testing sessions and returning the actigraphy watch. Primary analyses will include data only from participants completing all 3 sessions.

6 STUDY PROCEDURES

6.1 Schedule of Evaluations

Assessment	Screening: ONLINE/ PHONE	Baseline, Enrollment, Randomization: Visit 1 (Day 0)	AM Visit	PM Visit
Safety Inclusion/Exclusion Criteria Screening Survey	X			
Depression Inventory (PHQ-9)	X			
Edinburgh Handedness Inventory	X			
Morningness-Eveningness Questionnaire		X		
Subject Information Form (Demographics, Medical History)		X		
Arousal Rating		X	X	X
Substance Use Survey		X	X	X
Shipley Assessment (verbal only)		X		
Sleep Diary		X	X	X
Post-tDCS Application Survey			X	X
Baseline Post-Rest Survey		X		
Post-Experiment Survey			X (if final session)	X (if final session)
Consent Form		X		
Montreal Cognitive Assessment		X		
Debrief Form			X (if final session)	X (if final session)
Cognitive Tasks		X	X	X
Actigraphy		X	X	X
tDCS Stimulation (active or sham)			X	X

Adverse Events Questioning			**X**	**X**

6.2 Description of Evaluations

Cognitive Tasks:

Episodic Memory Task. We will cross stimulus type (words vs. pictures) with a recollection difficulty manipulation (items studied once or twice), all within-subjects. For the picture study list, participants will study words followed by a corresponding picture of the object (half colored pictures, half line drawings). Participants will decide if each picture is high or low detail, thereby drawing attention to perceptual features. For the word study list, participants will see the names of common objects and will be prompted to make one of two semantic judgments (Made in a factory? Found in house?), thereby drawing attention to conceptual features. We will give two recollection tests alternating across mini-blocks to avoid order effects. On the picture test blocks, verbal labels for the studied pictures will be intermixed with non-studied items, and participants will make one of three response options (studied as color, line, or new) followed by a confidence judgment. The structure of the word test blocks will be similar, whereby participants will make one of three response options (studied with a factory judgment, a house judgment, or new) in addition to the confidence judgment. Within each test, the items studied in the two different picture formats (or semantic judgments) will be matched on familiarity, so that participants will need to use recollection to make source judgments. Although pictures are typically better recollected than words, we have found that discriminating between these two different picture formats (namely, color or line judgments during the picture test) and these two different semantic judgments (namely, factory or house judgments during the word test) can be matched on difficulty with the repetition manipulation (i.e., comparing once-presented pictures to repeated words, Sarfan et al., 2014), allowing us to disentangle material-specific effects from recollection difficulty. Our primary dependent variable (DV) will be source recollection accuracy (the proportion of studied items attributed to the correct source, minus the proportion of non-studied items incorrectly attributed to that source). During each experimental session, subjects will perform the episodic memory task followed by the working memory task (described below).

Working Memory Task. Similar to the episodic memory task, the working memory task will involve a stimulus manipulation (verbal vs. visuospatial working memory) as well as a difficulty manipulation, all manipulated within-subjects and across mini-blocks to control for order effects. Specifically, we will use the N-back task with a verbal version (i.e., presenting the numbers 1-9 in a varied sequence) and a matched visuospatial version (i.e., presenting a colored square in one of 9 locations on a 3x3 grid in a varied sequence). Based on neuroimaging work, both tasks rely on bilateral PFC regions, but the former relies more heavily on left PFC, and the latter more heavily on the right PFC (for meta-analysis, see Owen et al., 2005). Difficulty will be manipulated by varying the N in the N-back task into "easy" and "difficult" versions for both younger and older adults (1 and 2 back for older adults, 2 and 3 back for younger adults). Our primary DV will be proportion of targets correctly identified minus the proportion of lures incorrectly endorsed.

6.2.1 Screening Evaluation

These evaluations occur to determine if the candidate is eligible for the study. Screening evaluations to determine eligibility must be completed within 3 months of study participation.

Consenting Procedure

The Study Coordinator will oversee the recruitment and screening procedures. Participants will have the option to complete the screening procedure via phone interview or an online questionnaire, as listed in the study advertisements. The screening procedure will start by obtaining informed consent for the screening procedures (verbal consent for phone, electronic for online), and then ask questions to verify that inclusion/exclusion factors are met, as well as describe the overall procedures of the study to determine eligibility and willingness.

Signed informed consent for the 3-session experiment will be obtained in person, during the first session in the lab (orientation/baseline day). Signed consent forms will be kept under lock and key in the Gallo lab.

6.2.2 Enrollment, Baseline, and/or Randomization

Enrollment & Randomization

Enrollment begins when the participant signs the informed consent form for the 3-session experiment, at the start of the first lab session (i.e., baseline/orientation day).

After successful screening, participants will be randomized to either the AM-session-first condition or to the PM-session-first condition using the AM/PM Randomization REDCap database. Just prior to the baseline session, the participant will be assigned to a cognitive task condition using the Task Condition Randomization REDCap database. Finally, just before the first tDCS session, the participant will be assigned to a Soterix stimulation code, and to an electrode placement (either PFC or parietal), using the Participation REDCap database.

Baseline Assessments

During baseline assessment participants will complete cognitive tasks, demographic information, and other forms (Table 6.1).

Morningness-Eveningness Questionnaire (MEQ) *online survey* - Participants will take the MEQ, assessing AM and PM testing preferences, which is correlated with physiological measures of circadian rhythms and predicts time-of-day effects on cognitive tasks in younger and older adults (Horne & Ostberg, 1976; see May & Hasher, 2017). Younger adults are expected to express at least a mild preference for PM testing (approximately 70% of younger adults in our prior sample), and older adults at least a mild preference for AM testing (typically 75% of older adults, see Yoon et al., 1999). (See Appendix).

Actigraphy and Sleep Diary *online survey* - During the initial orientation session participants will be equipped with an actigraphy watch and instructions for this and the sleep diary, and these data will be collected over a minimum 7-day period. During this period, participants will return to the lab for 2 testing sessions (AM/PM, nonconsecutively), returning the actigraphy watch and sleep diary on the final testing session. These measures will be used to help interpret the differential time of day effects (AM vs PM) on cognitive functions that we predict in younger and older adults, as well as the predicted interaction with tDCS brain stimulation.

Cognitive Tasks *computerized tasks* - During the orientation session participants will take baseline cognitive tasks (without tDCS), thereby providing baseline measures for some of our analyses and also providing task-initiation practice during this baseline stage of the task, thereby minimizing the likelihood that such task-initiation effects would impact the subsequent AM/PM testing sessions (i.e., creating practice in the first part of the experiment in order to reduce such practice effects between the subsequent AM and PM testing sessions). See description above for more information.

Montreal Cognitive Assessment. *paper/pencil test* - We administer the Montreal Cognitive Assessment in order to exclude individuals that score outside the range for normal aging according to the recent meta-analysis of MoCA's ability to differentiate normal aging from MCI in Carson et al. (2018, Int. J of Geriatric Psychiatry).

Shipley Assessment (verbal only) *online survey* - During the second visit only, participants are given the verbal portion of the Shipley to test cognitive ability.

Substance Use Survey *online survey* - Subjects will be asked not to consume more than twice the amount of caffeine and nicotine they normally consume 4 hours prior to each session, and not to consume more than twice the amount of alcohol they normally consume 24 hours prior to each session, and we have them record their substance use in this survey (See Appendix).

Subject Information Form *online survey* - Questions regarding demographic information and medical history.

Baseline Post-Rest Survey *online survey* - Participants are asked about what was on their mind during a rest period, to determine if some participants intentionally review studied items prior to the memory test.

Arousal Rating *online survey* - Participants are asked about their alertness level before the encoding portion of the cognitive task and after a rest period.

6.2.3 Follow-up Visits (AM and PM Visits)

All participants will be enrolled in the baseline/orientation session, and they then will be assigned to active (or sham) tDCS for two follow-up experimental sessions, one in the AM and one in the PM.

- The time between each session will be at least 48 hours (i.e., at least 1 day intervening), thereby ensuring at least 1 full day of actigraphy data collected outside the lab prior to each tDCS session.
- The time between the baseline/orientation session and the final tDCS testing session should be no less than 7 days and no more than 12 days, thereby obtaining at least 7 days of actigraphy data and also providing some flexibility in the scheduling of the final session.

Follow up Assessments

Measures will include cognitive tasks, actigraphy, and the questionnaires listed in table 6.1.

Substance Use Survey See description above.

Arousal Rating *online survey* - Participants are asked about their alertness level before the encoding portion of the cognitive task adn before and after tDCS stimulation.

Post-tDCS Application Survey *online survey* - Participants report sensations during tDCS stimulation as well as what was on their mind during the stimulation.

Cognitive Tasks *computerized tasks* - See description above.

Adverse Events Questioning *verbal report* - Participants are asked if they have experience any after the baseline session. Responses are recorded in the Adverse Events Survey (see List of Administrative Logs).

6.2.4 Completion/Final Evaluation The final experimental session will be the final evaluation, and at the end of this session participants will complete a Post-Experiment Survey and finally receive a debriefing.

Post-Experiment Survey *online survey* - Participants report their sensations during the tDCS procedure, their expectations as to whether and how tDCS impacted performance, and also whether and how they believed they performed during the AM and the PM testing sessions.

Debrief Sheet *paper info sheet* - Participants are given an information sheet about the purpose of the study, which includes information about the stimulation and sham conditions (but not their own assignment), as well as hypotheses for AM and PM testing.

The debriefing sheet also will include citations for further reading. (See Appendix).
About Early Termination: Participants have the right to end their participation at any time. In addition, the PI may terminate participation if a participant is in obvious non-compliance with the study procedures (e.g., responding randomly or sleeping during the cognitive task) or shows signs of moderate or severe adverse events associated with the study procedures (e.g., excessive discomfort with the tDCS electrodes).

7 SAFETY ASSESSMENTS

Participant safety will be monitored once an individual is enrolled in the study. The most typical negative experiences associated with tDCS include discomfort, tingling, itching, or mild burning sensations associated with the electrode application to the skin and/or electrical current, as well as headache, fatigue, or transient skin redness. During tDCS participants will be asked to report any sensations that become intolerable, so that participation can cease, and the Post-tDCS Application Survey administered after each tDCS session will include questions on the severity of any uncomfortable experiences during the procedure.

Participants might experience boredom, anxiety, or frustration during the cognitive tests. In these cases the researcher will attempt to minimize these risks by encouraging the participant and indicating that the tasks are designed to be difficult.

7.1 Specification of Safety Parameters

In over a decade of studies using tDCS, in people of different ages and psychological conditions, no significant adverse effects have been identified.

In 2011, a review of the adverse effects of tDCS (Brunoni et al., 2011, International Journal of Neuropsychopharmacology) identified 209 studies assessing a wide variety of participants, including persons from potentially vulnerable populations. Of these 209, only 74 studies reported at least one adverse effect (35%), with 43 studies explicitly reporting no adverse effects (21%) and 92 studies not reporting the presence or absence of an adverse effect (44%). Considering only the 117 studies that explicitly reported the presence or absence of an adverse effect, the most common adverse effects reported in the active stimulation groups were itching (39%), tingling (22%), headache (14.8%), burning sensation (8.7%) and discomfort (10.4%), with no reports of serious adverse effects. Importantly, similar frequencies of each of these effects were reported in the non-stimulation sham (placebo) control group (differing by 6% at most, for itching), which also requires the presence of an electrode on the skin but no sustained electrical stimulation (or only brief stimulation for the first few seconds). Overall, there were no unexpected or severe adverse events in over 40 studies with more than 600 older adults regardless of cognitive or disease status, and we are unaware of any evidence for increased risk of serious adverse effects with aging subjects. A recent meta-analysis indicates that repeated sessions of tDCS, separated by 48 hours, should pose no greater risks than those associated with a single-session (Nikolin et al., 2018). For further details on the safety of tDCS in human trials, see Bikson et al, 2016, Brain Stimulation.
The Actigraph device captures and records continuous mobility data related to real-world activity, mobility and sleep outcomes. There are no risks associated with the Actigraph device given it is considered completely noninvasive and comfortable. The experience on behalf of

the participant can be compared to that of a digital wristwatch. The actigraph watch captures and records continuous data related to mobility and sleep patterns. For more information on its uses and safety, refer to https://www.actigraphcorp.com/.

Other risks are those associated with basic computer or paper tasks, including boredom, mild fatigue, test taking anxiety, or breach of confidentiality. The expected frequency of breach of confidentiality is never.

7.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

This study will only use one stimulation parameter that already is established as minimal risk, and highly tolerable for both younger and older adults. After the tDCS sessions, participants will be asked to report their sensations and rate their unease during the stimulation.

7.3 Adverse Events and Serious Adverse Events

Definitions: An **adverse event (AE)** is generally defined as any unfavorable and unintended diagnosis, symptom, sign (including an abnormal laboratory finding), syndrome or disease which either occurs during the study, having been absent at baseline, or if present at baseline, appears to worsen. Adverse events are to be recorded regardless of their relationship to the study intervention. A **serious adverse event (SAE)** is generally defined as any untoward medical occurrence that results in death, is life threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly.

During the second and third testing sessions, after the cognitive tasks, the participants will be asked to report any adverse events that have begun or worsened since the baseline session. They will also be asked explicitly describe sensations occurring during the tDCS stimulation (see Post-tDCS Application Survey in the appendix), some of which may qualify as adverse events. The Study Coordinator will record adverse events reported by participants, and the extent that they appear to be related (or unrelated) to the study procedures in the Adverse Events Survey.

7.4 Reporting Procedures

All adverse experiences will be recorded in the Adverse Events Survey . If the event is classified as a Serious Adverse Event, the PI will be notified immediately. The PI will then notify the NIA, the DSMB chair, as well as the IRB with in 24 hours, at which time they may ask for additional information.

AEs or SAEs that may occur during the study (regardless of their relationship to the study procedures) will submitted in routine reports to the DSMB, prior to DSMB conference calls.

7.5 Follow-up for Adverse Events

Any unresolved AE that is possibly related to the study procedures will result in halting the subject's participation in the study. The study is minimal risk and no long-lasting adverse events are expected. Any AE that renders the participant ineligible to be in the study (as per the inclusion/exclusion criteria) will also be resolved by halting participation in the study.

7.6 Safety Monitoring

An NIA-appointed Data and Safety Monitoring Board will review the project, and adherence to the Data Safety and Monitoring Plan.

8 INTERVENTION DISCONTINUATION

Participates may withdraw voluntarily from participation in the study at any time and for any reason. Because the adverse events associated with tDCS are mild and transient, we will not continue to follow participants that withdraw from the study. Participants reporting headache will be given the option to rest in the lab until the situation resolves.

Data from participants that discontinue without completing both experimental testing sessions will be replaced with a new participant assigned to the same condition.

In the event that participation in the study is halted during or following tDCS, the participant will be asked to complete the Post-tDCS Application Survey and also will receive debriefing.

9 STATISTICAL CONSIDERATIONS

9.1 General Design Issues

Overall design (from Precis section): 50 Younger Adults will be randomly assigned to three between-subjects tDCS conditions (left prefrontal, sham, left parietal control, total YA n=150) and 50 cognitively normal older adults will be randomly assigned to two between-subject tDCS conditions (left prefrontal, sham, total OA n=100). Each participant will complete 3 sessions: orientation/baseline (no tDCS, time varies), AM tDCS, PM tDCS (counterbalancing the order of the AM and PM sessions). Primary outcome measures will be cognitive task performance (episodic recollection accuracy primary, working memory accuracy secondary) as a function of 3 key factors (age group, tDCS condition, and time-of-day) and their interaction,

and also as a function of secondary factors obtained from actigraphy sleep data, sleep diary/log data, and the Morningness-Eveningness Questionnaire (aka the Owl-Lark assessment).

Rationale for within-subjects AM and PM testing: Each participant will take both an AM and a PM testing session. Within-subjects designs are generally preferred over between-subject designs, because they reduce the impact of individual variability on performance that is unrelated to the AM/PM manipulation, thereby increasing the likelihood of detecting AM/PM effects. However, within-subject designs introduce the possibility of practice effects across sessions, but we will control for such order effects by counterbalancing the order of the AM and PM sessions across participants in each arm. Moreover, collecting a no-tDCS cognitive baseline for all participants (during the first session) will give all participants familiarity and practice with the cognitive tasks, thereby reducing the impact of such practice effects on the subsequent AM and PM tDCS/sham testing sessions.

Rationale for between-subjects tDCS/Sham design: Our previous tDCS work suggests that, on average, participants in stimulation and sham conditions do report different kinds of sensations. To avoid cross-session learning effects, which would invalidate the sham condition, the experiments we propose here will manipulate tDCS between-subjects: Each participant will participate in an AM (8 or 9 AM) and PM (3 or 4 PM) testing session (within-subjects), and will be randomly assigned to the same tDCS condition in each session (left dlPFC or sham in older adults; left dlPFC, left parietal, or sham in younger adults), thereby avoiding any cross-session learning effects from experiencing different tDCS conditions.

Rationale for cognitive baseline measures: All participants will take a baseline version of the cognitive tasks (without tDCS) during the orientation session, serving as a no-tDCS baseline (i.e., the "pre" condition in a pre-post design). These cognitive baseline measures are included to assess Aim 3: Determine the extent that a pre-post between-subjects tDCS design can improve the detection of stimulation effects, while also preserving the validity of the sham condition. Our initial analyses will focus on the between-subjects comparisons described above, but the statistical power of the between-subjects design to detect group differences (stimulation vs sham) is likely limited by individual variability in task performance (unrelated to stimulation). In an attempt to reduce the contribution of individual variability that is unrelated to stimulation, and thereby provide a more sensitive test of tDCS effects, we will conduct additional analyses that include the baseline performance for all participants as a covariate. The primary purpose of this measure is to assess the value of a pre-tDCS baseline in detecting tDCS effects, independent from potential time of day effects explored in the other aims. Thus, to minimize time constraints on the completion of the project, these orientation

session testing times (and baseline cognitive assessment) will be scheduled at the convenience of the participant and experimenter (i.e., baseline sessions can occur outside the AM and PM testing windows that are reserved for the tDCS/sham sessions).

2 Sample Size and Randomization

Power analysis: Our power analysis was based on our most recent and largest tDCS experiment with younger adults (Wong et al., in press, n = 30 per each between-subjects condition), which yielded a 12% difference in word test accuracy between sham (.23, SD=.21) and active tDCS (.35, SD = .21) in the 9 AM group, predicting that we would need 50 participants in each stimulation group in order to obtain a similar effect size (Cohen's d = .57, or a medium effect size) with 80% power at $p<.05$ (2 tailed, calculated with G-Power, v. 3.1.9.3). We therefore plan to test 50 younger adults and 50 older adults in each stimulation condition in order to detect effects of this magnitude. These sample sizes are considerably larger than most (if not all) of the between-subjects tDCS experiments on episodic memory published as of 2017. A sample size of 50 per condition also should yield sufficient power to detect the 2x2 interaction between brain stimulation (between-subjects) and time-of-day (within-subjects) in each age group, if we assume a similar effect size (partial eta squared = .06) for the interaction between time-of-day and stimulation we had obtained in younger adults in Wong et al. (in press), and also a .25 correlation between the repeated measures during AM and PM (80% power, alpha = .05).

9.2.1 Treatment Assignment Procedures

Younger adults will be randomly assigned to one of the three stimulation conditions (active frontal, active parietal, or sham: ½ frontal, ½ parietal), and older adults will be randomly assigned to one of the two stimulation conditions (active frontal, sham frontal).

Phil will keep the stimulation codes in a password protected Excel file, which will be shared with the DSMB. No other member of the research team will have access to the file.

Planned breaking of the stimulation codes will occur when the 50 planned participants have been tested in each arm of the study, or until the study is terminated in consultation with the DSMB.

The research team and DSMB will have full access to all other data collected throughout the study, in order to monitor the quality of the data and allow preliminary analyses of time-of-day effects, age group, and other variables (actigraphy, MEQ) on cognitive task performance, as well as the exploratory metamemory analysis of baseline cognitive task data (no tDCS).

9.3 Interim analyses and Stopping Rules

No interim analysis of tDCS/sham differences is planned. We plan a mid-point analysis of the baseline cognitive task data (see Exploratory Metamemory Analysis), but this will not involve breaking the blinding codes (baseline data are not associated with tDCS). The PI, in consultation with the DSMB, will temporarily suspend enrollment in the tDCS portions of the study if serious questions arise about efficacy, safety, or poor study performance (e.g., slow accrual, high losses-to-follow-up, and poor quality control). In this event, study enrollment will only recommence if DSMB and the PI agree that the issue(s) has been resolved, and make a designation that the study should (a) continue per protocol, (b) proceed with caution, (c) be further investigated, (d) be discontinued, or (e) be modified and then proceed.

Examples of findings that might trigger a safety review are the number of SAEs overall, the number of occurrences of a particular type of SAE, severe AEs/reactions, or increased frequency of events. Such findings are presented to the DSMB statistician to review the events by group to determine whether there are statistical as well as clinical concerns. The statistician reports their findings to a closed session of the DSMB or to the Safety Officer and/or NIA. The findings are used to determine what steps will be taken.

9.4 Outcomes

9.4.1 Primary outcome

Our primary dependent variable (DV) will be accuracy on the episodic memory and working memory tasks. These will be assessed in each of the 3 sessions (baseline no-tDCS, AM tDCS, PM tDCS). See Section 6: Study Procedures.

10 DATA COLLECTION AND QUALITY ASSURANCE

10.1 Data Collection Forms

Information will be collected for each participant by the Study Coordinator, or a research assistant under their immediate supervision. For double-blinding, no member of the PI's research team that will interact with participants will know the stimulation codes that differentiate active vs. sham tDCS. The PI will generate this code list before the study is initiated, and then share it with the DSMB. The PI will not refer back to this code list until data collection is ended, and the PI will not administer study procedures to study participants. The research team will share de-identified data with the PI during the study, to troubleshoot or as needed, but no analysis of tDCS vs sham will be conducted until the end of the study. Maintaining confidentiality of participant records will be achieved by storing these records in

password protected excel files on a lab computer, backed-up on the University's protected server, and also on external hard-drive in the lab by the Study Coordinator.

1. Qualtrics Survey - *Adverse events*
    - Contains information about any Adverse Events (both serious and non-serious). Data from this survey will be used to generate routine updates to the DSMB regarding adverse events. The survey is modeled off of the NIA templates for adverse event and serious adverse event logs.
2. REDCap DB - *AM/PM Randomization DB*
    - Enables participant assignment to the AM-session-first or PM-session-first condition, prior to the participant's baseline session.
3. REDCap DB - *Task Condition Randomization DB*
    - Enables participant assignment to one of 12 task varieties, during the participant's baseline session.
4. REDCap DB - *Participation DB*
    - Enables participant assignment to active or sham stimulation, and, if the participant is a younger adult, to either the dlPFC or parietal electrode placement. Will also contain information about each of the participant's visits.
5. Spreadsheet - *OA Contact Log*
    - Contains information about older adults who have been contacted about participating in the study.
6. Spreadsheet - *Screening Log*
    - Contains information about participant eligibility based off the Inclusion/Exclusion Criteria Screening Survey.
7. Paper log - *Payment Log*
    - Contains the subject's name and a place to sign to indicate payment was received.
8. Spreadsheet - *Blinding Key*
    - Links a Soterix stimulation code to either the active or sham condition. The study personnel will not have access to this file until the close of the study. Phil Schumm will maintain the key, and use it to setup the randomization scheme in the REDCap databases mentioned above.

10.2 Data Management

- All **Administrative Logs** in the form of spreadsheets will be maintained in a secure Google Drive folder on UChicago's GSuite, which will be periodically backed up to the Study Coordinator's computer. *Note:* this excludes the *Blinding Key* which only Phil Schumm and Diane Lauderdale will have access to.
- Study forms in paper/pencil format (see **Description of Evaluations**) will be kept in a locked file cabinet in the Gallo Memory Lab.
- Electronic data from online surveys (see **Description of Evaluations**) will maintained on the Study Coordinator's Lab Computer for backup, which will be automatically backed-up on the University server. It will be shared with the research team and the DSMB upon request.
- Electronic cognitive task data (raw and organized in Excel), blinded to tDCS condition (active, sham), will maintained on the Study Coordinator's Lab Computer, which will be automatically backed-up on the University server. Additionally it will be uploaded to a private Open Science Framework project. It will be shared with the research team and the DSMB upon request.

10.3 Quality Assurance

10.3.1 Training

The Study Coordinator will be trained in study procedures by the PI, and in tDCS procedures by the PIs current research team, who have conducted tDCS research in the lab.

10.3.2 Quality Control Committee

The DSMB will review certain blinded data at the midpoint, as described elsewhere, and at other times they might request.

10.3.3 Metrics

Quality control metrics for outcome measures will be finding the expected effects of age-group (younger > older) on episodic memory (recollection) accuracy and working memory accuracy.

Moreover, for each participant, we expect hits to be greater than false alarms on these tasks. These and other aspects of quality and compliance (study forms, actigraphy) will be monitored by the Study Coordinator and PI.

10.3.4 Protocol Deviations

Protocol deviations will be reported to the PI by the Study Coordinator, and they will be documented by the Study Coordinator in the notes section of the Participation REDCap DB and made available for review by the DSMB.

10.3.5 Monitoring

The Study Coordinator, the PI, and the research team will work together to implement the data collection and monitoring procedures described here. They will conduct a pre-review of all appropriate materials prior to each DSMB meeting.

11 PARTICIPANT RIGHTS AND CONFIDENTIALITY

11.1 Institutional Review Board (IRB) Review

This protocol and the informed consent documents and any subsequent modifications will be reviewed and approved by the IRB or ethics committee responsible for oversight of the study.

11.2 Informed Consent Forms

A signed consent form (younger or older adult versions) will be obtained from each participant. All participants must be able to consent for themselves. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy will be given to each participant and this fact will be documented in the participant's record.

11.3 Participant Confidentiality

No medical records will be accessed or created as part of this study, and as such, no HIPAA regulated data will be collected in this study.

Any data, specimens, forms, reports, and other records that leave the site will be identified only by a Participant ID to maintain confidentiality. All paper records will be kept in a locked file cabinet. Information will not be released without written permission of the participant, except as necessary for monitoring by IRB, the FDA, the NIA, and the OHRP.

11.4 Study Discontinuation

The study may be discontinued at any time by the IRB, the NIA, the OHRP, the FDA, or other government agencies as part of their duties to ensure that research participants are protected.

12 ETHICAL CONSIDERATIONS

The guiding ethical principles being followed by the study are those required by The University of Chicago SSD IRB as well as those provided by the NIA.

13 COMMITTEES

The Data Safety and Monitoring Board will be responsible for the following:

- Review the research protocol, informed consent documents and plans for data safety and monitoring as defined in this document;
- Recommend subject recruitment be initiated after receipt of a satisfactory protocol;
- Evaluate the progress of the trial, including periodic assessments of data quality and timeliness, recruitment, accrual and retention, participant risk versus benefit, performance of the trial sites, and other factors that can affect study outcome;
- Review study performance, make recommendations and assist in the resolution of problems reported by the Principal Investigator;
- Report to NIA on the safety and progress of the trial;

14 PUBLICATION OF RESEARCH FINDINGS

Any presentation, abstract, or manuscript will be made available for review by the sponsor and the NIA prior to submission.

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