Protocol

Cognitive Outcomes of Brain Stimulation As a Later in Life Treatment (COBALT): A Phase II Randomized Controlled Trial

Document History:

Version	Date	Author	Notes
0.1	February 27,2023	Chiang & LoBue	Original draft
0.2	April 6, 2023	Chiang & LoBue	Listed University of Texas at Dallas as an external site for administration of electroencephalography and some cognitive outcomes.
0.3	March 14, 2024	A Salter	Review
0.4	March 14, 2024	Chiang & LoBue	Modified analysis plan as follows. Changed primary outcome analysis from mixed effects models to ANCOVA, with baseline performance as the covariate. Updated mixed effects models to be sensitivity analysis in plan. Added chi-square tests for evaluating significance of proportions of clinically meaningful change.
0.5	April 15, 2025	LoBue	Revised recruitment strategy to include digital tools in electronic health record to screen eligible patients who opted to be contacted for research.
1	April 15, 2025	LoBue	Location of research site at University of Texas at Dallas changed to correspond to the relocation of the lab for electroencephalography and some cognitive outcomes.

Version: Final

Signatures
Principal Investigators: Hsueh-Sheng Chiang, MD, PhD and Christian LoBue, PhD

Sponsor:	Texas Alzheimer's Research and Care Consortium
Title:	Cognitive Outcomes of Brain Stimulation As a Later in Life Treatment (COBALT)
Trial registration:	ClinicalTrials.gov; NCT05564715
Trial design:	randomized, double-blind, parallel-group sham-controlled trial
Number of arms:	2
Trial site:	**1)** University of Texas Southwestern Medical Center **2)** University of Texas at Dallas
Protocol ID:	STU-2022-0799
Participant time in trial:	Two months

Background:
The pre-supplemental motor area (preSMA) and dorsal anterior cingulate cortex (dACC) have been shown to play a role in episodic memory and word retrieval. Although the hippocampus is a brain structure most often associated with early deficits in amnestic mild cognitive impairment (aMCI), the earliest clinical threshold preceding dementia, the dACC is another node in the neural network for episodic memory that may be more accessible to neurostimulation methods. Entraining the preSMA/dACC circuits with 10 sessions of neurostimulation could be used to improve cognitive functioning evaluated by EEG-based and neuropsychological measures. High-definition transcranial direct current stimulation (HD-tDCS) is one neurostimulation approach that uses an array of small electrodes to pass a low level current through targeted brain regions (e.g., anterior cingulate cortex) to modulate neuronal activity and brain circuitry.

Purpose:
The purpose of this study is to examine the efficacy of HD-tDCS to the preSMA/dACC region and its influence on EEG and neuropsychological measures in patients with aMCI.

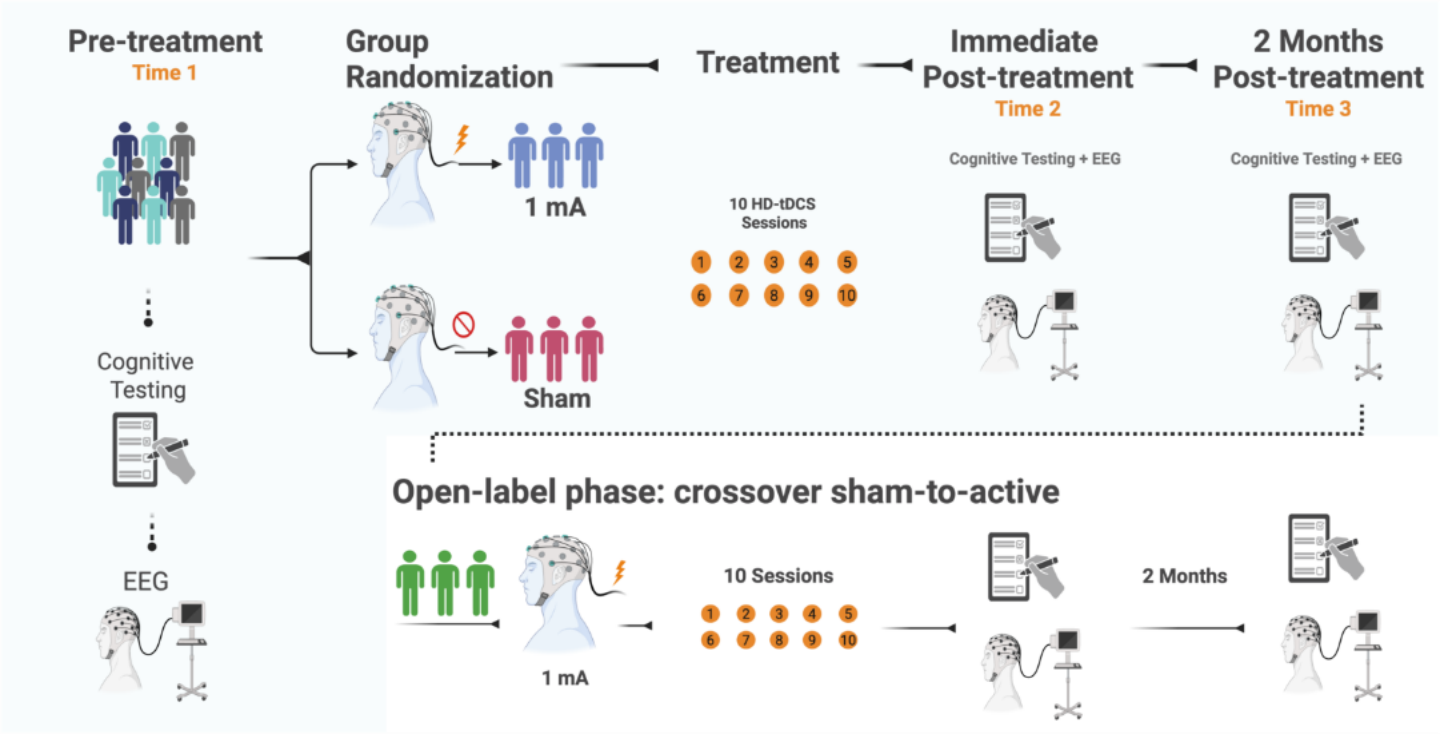
Primary Objective:
- Determine if HD-tDCS enhances verbal episodic memory functioning in aMCI

Secondary Objective:

- Determine if HD-tDCS enhances word retrieval functioning (i.e., language) in aMCI
- Evaluate if cognitive changes produced from HD-tDCS persist for 2 months
- Evaluate if HD-tDCS modulates EEG-derived neural synchronizations associated with memory functions to inform potential target engagement

Design:

Participants with a clinical diagnosis of aMCI will be enrolled and randomized to receive 10 sessions of either 1mA HD-tDCS or sham stimulation. All participants will be stable on oral medications for a minimum of 1 month before initiation of study procedures. Each HD-tDCS session will be 20 minutes in duration and have electrodes configured to target the preSMA/dACC, consisting of a single anode at Fz (1 mA) and 4 cathodes (all at 0.25 mA) at FP1, FP2, F7, F8. EEG and neuropsychological tasks will be completed at baseline, immediate after session 10, and a 2-month follow-up. Participants randomized into the sham group will have the opportunity to return after 2 months for an open-label phase to receive active treatment while being unblinded to their condition group. For participants crossing over from sham-to-active treatment in the open-label phase, EEG and neuropsychological tests will be obtained again immediately following the 10 HD-tDCS sessions and at a 2-month follow-up. With this design, we expect a 15% attrition rate and plan on enrolling ~30 adults to achieve our completed sample of 24.



Below is a table summarizing the study visits. In Phase 1, participants will be randomized to active or sham stimulation and complete 12 visits. In Phase 2, participants in the sham group will be invited to receive the active condition as an open-label phase, which would involve 11 additional visits in the study.

SUMMARY OF VISITS		
Phase 1	**PROCEDURES**	**TIME**
Visit 1	Pre-testing neuropsychological assessment/semantic EEG	3 hours
Visits 2-10	HD-tDCS active or sham stimulation	30 minutes
Visit 11	HD-tDCS active or sham stimulation and post-testing neuropsychological assessment/semantic EEG	3 hours

Visit 12	8-week follow-up assessments/semantic EEG	3 hours
Phase 2	**Open-label Phase**	
Visits 13-21	HD-tDCS active stimulation (for Phase 1 sham group participants only)	30 minutes
Visit 22	HD-tDCS active stimulation and post-testing neuropsychological assessment/ semantic EEG	3 hours
Visit 23	8-week follow-up assessments/semantic EEG	3 hours

Inclusion criteria:

1. Age 55 and older
2. Fluent in English
3. Active diagnosis of aMCI
4. Deficit in at least one measure of language functioning, defined as performing >1 standard deviation below the standardized score mean on either the Boston Naming Test, phonemic fluency, or semantic fluency measures

Exclusion criteria:

1. Substance use disorder within past year
2. Has metal fragments or implants in skull/head
3. Profound vision or hearing impairment that interferes with testing
4. Major neurologic conditions (stroke, epilepsy, brain tumor, concussion in past year)
5. Current medication use known to alter HD-tDCS reactivity: amphetamines, L-dopa, carbamazepine, sulpiride, pergolide, lorazepam, dextromethorphan, D-cycloserine, flunarizine, or ropinirole

If a medical condition becomes apparent during the evaluation or follow-up that indicates an increased risk, then the subject's involvement in the study will be discontinued.

Procedures:

HD-tDCS arms of intervention: The study has 2 arms of intervention. The HD-tDCS treatment uses a cap with arrays of electrodes, combinations of which can be optimized for targeting delivery of electrical current to specific brain regions. HD-tDCS is delivered with sintered 12 mm diameter Ag/AgCl disc electrodes. The EEG cap is gently secured on the head of a subject and positioned with Cz at the vertex, as measured using surface anatomical landmarks, and defined as the intersection of the nasion–inion and interaural lines. Electrical stimulation will be performed while awake and at rest using a constant current controlled device manufactured by Neuroeletrics (Starstim system). Electrodes will be filled with a conductive gel. Contact quality and impedance levels (< 10 kOhms) will be verified for each electrode before each stimulation session begins. Daily HD-tDCS sessions lasting for 20 minutes (total of about 30-40 minutes including preparation) will be administered over 2 weeks (5 daily sessions each week), After each session, patients will be administered a standard post-treatment symptom questionnaire (e.g., assessing for skin irritation, fatigue).

Arm 1: For the 1 mA active HD-tDCS condition, administration parameters will consist of the stimulation being ramped up over 60 seconds until it reaches 1 mA and then maintained for 20 minutes. The anode will be placed over Fz according to the International 10-20 EEG system, corresponding to the approximate location of the dACC. Four return cathodal electrodes will be placed at FP1, FP2, F7, F8.

Arm 2: For the sham condition, parameters will consist of the current being ramped up over 60 seconds until it reaches 1 mA and then stopped. This allows for the same scalp sensation for both the sham and active condition so that subject and experimenter will remain blinded. This ramp up for one minute and then stopped and off for 20 minutes will be given each 20-minute sham session to keep the timing and subjective experience similar to the active condition.

All HD-tDCS protocols for participants will be pre-programmed into the Neuroelectrics software. HD-tDCS protocols will be set by a PI (C.L.) for each participant using an encrypted approach, labeling with the participant research identifier to conceal condition assignment. Aside from the PI, other study team members will be unaware of the HD-tDCS condition allocation to preserve blinding.

Randomization: Participants will be randomized at a ratio of 1:1 to receive active 1 mA and sham conditions. Randomization of HD-tDCS condition assignments will be generated by a digital program, and the assignments (Protocol 1 = active or 2= sham) recorded in an encrypted spreadsheet that only the PI (C.L) has access to.

Blinding: Participants will be informed that sham and active HD-tDCS will be used in the study, but they will be blinded to condition. Aside from the PI (C.L), other study team members who deliver HD-tDCS and perform assessments will be blinded to HD-tDCS condition. Both the subject and study personnel will complete a questionnaire to check if blinding was successful.

Sample size calculation: Pre-study power analyses were conducted for the most power intensive phase (Phase 1 - randomized controlled trial) using a clinically relevant effect size derived from our prior studies. A sample size of 20 provides 80% power to detect a f =.27 effect size for the analysis of the primary outcome using an analysis of covariance model, assuming the baseline covariate of performance explains R2 =.2 and α =.05. Given that both our and other's research without intervention suggests that effect sizes tend to be larger in EEG compared to cognitive measures, we expect the effect size of EEG outcomes to be at least equivalent to cognitive outcomes.

Recruitment strategy: Participants will be recruited from memory specialty clinics in Dallas, Texas, referred by clinicians in neurology, psychiatry, and neuropsychology. Recruitment will occur at clinics situated at UT Southwestern comprised of a Neuropsychology Clinic and a separate Neurology Memory Clinic, wherein each receives internal and external referrals to clinically diagnose patients with aMCI. Our catchment area will extend outside UTSW to the broader Dallas-Fort Worth Metroplex and involve Baylor Medical Center's AT&T Memory Center. Clinician referrals and self-referrals will primarily be used to identify subjects directly for screening/recruitment. Strategies for obtaining these will include 1) providing study-related information with colleagues at selected Clinics via e-newsletters and university presentations, 2) placing flyers in exam rooms and waiting areas at clinic facilities, 3) advertising on the UT Southwestern website, and 4) outreach to regional community sources. Digital tools in the electronic health record for UT Southwestern will also be leveraged to create a data registry for screening of eligible patients who opted to be contacted for research.

Screening and consenting strategy: Prospective participants will undergo an initial phone screening to ensure basic eligibility criteria are met (e.g., diagnosis of aMCI, conditions in their medical history, medications). Prospective participants meeting pre-screening inclusion/exclusion criteria will be scheduled an appointment to review the study procedures and Consent Form. Study staff will go over the consent form in detail with the prospective participant and answer any questions about the procedures.

Participants or the legal authorized representative will be asked to sign the Consent Form if they understand the study procedures, appreciate potential risks/benefits, and desire to be in the study.

Timing and procedures of follow-up: Cognitive and EEG evaluations will be completed immediately after session 10 and 2-months later. Patients will be called back for the 2-month follow-up. For individuals who received the sham condition, they will be invited back for an open-label phase of active HD-tDCS. Cognitive and EEG outcomes will be obtained immediately after session 10 and 2-months later during the open-label phase of sham crossing over to active treatment.

Cognitive Outcomes:

Primary
Episodic memory functioning will be assessed through standardized scores in Total Learning and Delayed Recall for the Rey Auditory Verbal Learning Test (verbal memory).

Secondary

- Language will be assessed through standardized scores for the Boston Naming Test-Short Form, DKEFS Phonemic Fluency, and DKEFS Category Fluency.

Additional Outcomes

- Visual memory will be assessed through standardized scores in Total Learning and Delayed Recall for the BVMT-R.
- Attention will be assessed through standardized scores for the WAIS-IV Digit Span and DKEFS Visual Scanning.
- Processing speed will be assessed through standardized scores for the SWAPS, DKEFS Color Naming, DKEFS Word Reading, DKEFS Motor Speed, DKEFS, Number Sequencing, and DKEFS Letter Sequencing.
- Executive functions will be assessed through standardized scores for DKEFS Category Fluency Switching, DKEFS Inhibition, DKEFS Inhibition/Switching, and DKEFS Number-Letter Switching.
- Visuospatial organization will be assessed through standardized scores for the WAIS-IV Visual Puzzles.

Rey Auditory Verbal Learning Test (RAVLT). A 15-item list learning and memory task where the tester reads aloud a list of nouns. The RAVLT includes 5 learning trials, an interference trial, a delayed recall trial, and a yes/no delayed recognition trial. The tester records how many items the patient remembers over several repeated trials and then again for the interference and 30-minute delayed recall trial. The RAVLT is a direct measure of verbal episodic memory and has multiple alternate forms. The scores for Total Learning and Delayed Recall are the primary outcomes of interest.

Delis-Kaplan Executive Function System (DKEFS) Phonemic Fluency. This is an assessment of word retrieval during which the patient is required to produce as many words in one minute that begin with a specific letter (e.g., *F, A,* or *S*). The outcome measure for this task is the total number of correct responses across the trials.

DKEFS Semantic Fluency. This is an assessment of word retrieval that involves 3 conditions. The patient is required to produce as many words in one minute that are members of specific categories (e.g., condition 1 = *animals*, condition 2 = *boy's names*) or switching between two different categories

(condition 3 = fruits-and-furniture items). The outcome measures for this task are the total words named for the two semantic conditions and the total words named during the switching condition.

Boston Naming Test – Short Form. This is an assessment of word retrieval during which a 30 item version of the task (odd and even item versions of the full test) is administered. The subject is shown pictures of objects and is required to correctly name them as quickly as possible. The outcome measure for this task is the total number of correct responses.

Brief Visuospatial Memory Test-Revised (BVMT-R). A three trial figural learning and memory task where the tester shows the patient an 8 x 11-inch array containing 6 simple geometric visual designs in a 2 x 3 matrix for 10 seconds. Patients are asked to reproduce as many designs as possible in the same location as they appeared on the display for each trial. A delayed recall trial is completed after 25 minutes followed by a yes/no recognition trial. The tester scores each trial (Learning and Delayed Recall) in terms of the accuracy and location of each design reproduced by the patient. The BVMT-R is a measure of visual episodic memory and has 6 alternate forms. The scores for Total Learning and Delayed Recall are the outcomes of interest.

Wechsler Adult Intelligence Scale-IV (WAIS-IV) Digit Span. This is an assessment of simple auditory attention and working memory that involves 3 conditions. An examiner reads aloud a series of numbers of increasing length, and patients are required to repeat each series across the 3 conditions, involving forwards, backwards, and in sequence. Each trial is scored as incorrect (0) or correct (1), and the total number of correct trials across the 3 conditions are summed for the task outcome measure.

DKEFS Trail Making Test. This is an assessment with 4 conditions. The visual scanning condition measures attention and requires patients to cancel all of the numbers randomly displayed on a stimulus page. The number sequencing condition measures simple attention and processing speed and requires patients to sequence all of the numbers randomly displayed. The letter sequencing condition is similar, utilizing the same cognitive components as number sequencing by requiring patients to sequence all of the letters in alphabetical order. The number-letter condition involves executive function by requiring patients to alternate sequencing of the two strategies switching between numbers and letters in order. The motor speed condition measures processing speed and requires patients to draw over a line on the page as quickly as possible. Outcome measures are the times to complete each condition.

DKEFS Color-Word Interference. This test measures processing speed in the control conditions of word reading and color naming, measures executive functions of inhibitory control in the interference condition, and cognitive flexibility in the switching condition. The interference condition is where the color word names are written in a different ink color than the name. The switching condition is where the patient must switch back-and-forth between the response patters of naming the ink color and reading the word. The outcome measure for this task is the time to complete each of the four conditions.

Southwestern Assessment of Processing Speed (SWAPS). This is an assessment of processing speed. The patient is required to transcribe numbers to their corresponding written symbol as quickly as possible within 60 seconds. The outcome measure for this task is the total number of correct responses.

Wechsler Adult Intelligence Scale-IV (WAIS-IV) Visual Puzzles. This is an assessment of visuospatial skills. The patient is required to view a completed puzzle of a shape and select 3 components from 6 possible options that can be rotated and combined mentally to form the shape. Each trial is scored as

incorrect (0) or correct (1), and there is a total of 26 possible puzzles. The outcome measure is the total number of items correctly completed.

EEG Outcomes:

During the EEG session, each participant will perform a set of tasks. These include a 10-minute resting state, two Go-NoGo tasks, and two tasks designed to examine memory retrieval functions. Two-alternative forced-choice tasks: a semantic memory retrieval (Binding task) and a word meaning association task (Association task) will be the outcomes of interest.

In the resting state EEG, participants will be asked to remain seated comfortably on a chair. Five 2-minute blocks of alternating eyes close (eyes closed gently while staying awake) and eyes open (eyes fixated on a cross sign in the middle of the screen), resulting in a 10-minute long resting EEG session.

In the two Go-NoGo tasks, participants will be instructed to push a button for certain stimuli (Go) while withholding responses for others (NoGo). The single car task (SC) involves basic categorization and uses single exemplars of a car (Go) and a dog (NoGo). The object animal task (OA) involves superordinate categorization and uses multiple exemplars of objects (Go) and animals (NoGo) across trials. Each task consists of 200 trials: 160 (80%) 'Go' trials that require a response through button pressing and 40 (20%) 'NoGo' trials that require inhibition/withholding of a response.

The Binding task will always be administered before the Association task because the target object retrieved on each trial serves as the basis for the association items in the Association task. In the Binding task, a modified version of the Semantic Object Retrieval Task (Chiang et al., 2023), participants will see a pair of words presented sequentially in each trial. In the retrieval condition, the two words together strongly cue a specific object (e.g., humps–desert → camel). In the non-retrieval condition, the same pool of words will be randomly re-paired so that each pair does not reliably evoke a particular object (e.g., humps–light→ X). Retrieval and non-retrieval trials will be randomly intermixed within each block. All stimuli will be presented as centrally displayed visual word forms in white on a dark background. Each trial begins with a small fixation cross (5,000 ms), followed by the first word for 500 ms. This will befollowed by a blank screen with durations jittered between approximately 900 - 1100 ms, followed by the second word for 3000 ms, and finally a central fixation cross (+) for 2500 ms before the next trial. On every trial, participants will indicate via button press whether the pair led to retrieval of a specific object ("yes" = right index finger, "no" = right middle finger) and will be instructed to respond as quickly and accurately as possible.

The Association task will use the same base items but not require object retrieval. Instead, participants judge whether the second word is semantically associated with the first. In the Associated condition, the pair corresponds to a typical feature or associate of the target concept (e.g., desert–camel); in the not-associated condition, the second word is unrelated (e.g., desert–shoe). Trial structure and timing will be identical to the Binding task.

Each testing session includes two blocks of the Binding task and two blocks of the Association task, involving 54 trials per condition in each task (Binding task: 54 retrieval and 54 non-retrieval trials; Association task: 54 associated and 54 not-associated trials). For each task, there will be two pseudo-

randomized versions of the 108-trial list, and list versions will be alternated and counterbalanced across time points and participants so that no item list will be repeated in consecutive sessions. Trial order within each block will be pseudo-randomized with the constraint that no condition appears more than three times in a row. Each block lasts about 6 minutes (two blocks for each task, ~24 minutes total for both tasks).

EEG will be continuously recorded while subjects complete each task using a 64-electrode EEG cap (Neuroscan Quickcap) via a Neuroscan SynAmps2 amplifier using Scan 4.5 software (Compumedics Neuroscan, USA; sampling rate: 1 kHz, DC-200 Hz). The reference electrode will be placed in between Cz and CPz, and the ground electrode between FPz and Fz. EEG channels with impedance exceeding 10–20 k ohm will be discarded from further processing. Poorly functioning electrodes will be removed manually with visual inspection and spectrogram analysis. The continuous EEG data will be high-pass filtered at 0.5 Hz followed by low-pass filtered at 40 Hz using a finite impulse response filter. The filtered EEG data will then undergo independent component analysis (ICA) processing to identify artifacts (e.g., muscle, eye, heart) using EEGLab80 and those components with > 70% probability of representing artifact will be automatically removed (ICLabel; Pion-Tonachini et al., 2019). Subsequently, ICA components of each individual's data will be visually examined, and components representing artifacts not removed by the algorithm will be removed manually. EEG data will be segmented time-locked to the stimulus onset at each trial (-1000 to 2000 ms) into multiple EEG epochs. Epochs having amplitude outside -75 to 75 microV will be rejected and epochs with extreme values excluded by rejection algorithms in EEGLAB (pop_jointprob, pop_rejkurt, with 5 standard deviations). An algorithm computing the average based on spherical splines fitted to the data will be applied to interpolate EEG data to the sites of the rejected electrodes. The EEG data will be re-referenced to the average signal over the entire scalp. Trials with a response time outside the window between 300 and 3500 ms will be considered invalid, coded as incorrect, and excluded from further analysis.

Time-frequency decomposition will be performed using the newtimef function in EEGLAB. For each channel, single-trial EEG data will be transformed into event-related spectral perturbation (ERSP) values. The analysis will use the Fast Fourier transform with the following parameters: a Hanning-tapered sliding window length of 250 ms, a zero-padding ratio of 4, and 100 linearly spaced time points across the entire epoch. Frequencies between 1 and 30 Hz will be analyzed with a linear scaling at 1Hz intervals. A linear detrend will be applied to each epoch. Spectral power will be log-transformed and expressed in decibel (dB) units. Baseline correction within each 1 Hz frequency interval will be performed for each single trial by subtracting the average power between -700 and -100 ms pre-stimulus onset from each time point post-stimulus onset to calculate ERSP using a gain model. Data from the theta (4-7 Hz) and alpha (8-12 Hz) frequency bands will be averaged at each electrode-time point for statistical analysis.

Changes to outcomes: There were no changes to outcomes after the trial commenced.

Subject safety monitoring: All HD-tDCS and EEG sessions will be overseen by the PI. All safety precautions recommended by scientific literature and the UT Southwestern IRB will be carefully followed. All adverse events volunteered by the subject or elicited by the research team will be recorded in the subjects' research file. All adverse events will be reported within one week to the IRB. Any severe adverse events will be reported within 24-48 hours to the IRB.

In order to safeguard our participants from a breach in confidentiality, the data is coded with a unique semi-random subject identifier (URSI). All data after initial entry into the study is coded based on the participant's number. A digital file containing completed questionnaires and assessments from the experimental session will be maintained with the URSI number. These files will be kept in an encrypted, IRB approved database (RedCap).

Statistical analysis

General linear models (analysis of covariance) will be used to evaluate whether cognitive performance following all HD-tDCS sessions statistically differs between HD-tDCS conditions, covarying for pre-treatment scores. Mixed effects models will be used to examine for a significant interaction between HD-tDCS condition and time across the 3 outcome intervals. Participants with missing time points will not be excluded. Clinically meaningful change associated with HD-tDCS will also be assessed. Proportions of individuals with a 5+ standardized score increase (T-score) on each measure of cognitive functioning will be characterized and evaluated using chi-square analyses. Statistical significance will be set at $\alpha = .05$.

EEG behavioral data will be tested using mixed effects models to examine for a significant interaction between HD-tDCS condition and time. Group assignment (active vs sham) will be used as the between subject factor; Time Point and Condition will be two within subject factors. In the Binding task, Condition included retrieval versus non-retrieval trials, while in the Association task, Condition included associated versus not-associated trials. Statistical significance will be corrected for the multiple electrode–time tests that will be performed using the Benjamini Hochberg false discovery rate.