



Potential role for peripheral nerve stimulation on learning and long-term memory: A comparison of alternating and direct current stimulations

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ARTICLE INFO

Article history:

Received 5 October 2021

Received in revised form

22 February 2022

Accepted 4 March 2022

Available online 20 March 2022

Keywords:

Alternate current stimulation

Attention

Direct current stimulation

Consolidation

Locus coeruleus

Occipital nerve stimulation

ABSTRACT

Background: In the past decade, a rising interest in transcranial electrical stimulation has emerged owing to its advantageous capacity to facilitate the extraction of casual links between neuromodulation and the obtained behavioral effects in cognitive performance. However, an insufficient number of direct comparative studies between transcranial alternating current stimulation (tACS) and transcranial direct current stimulation (tDCS) effects on associative memory have caused optimal parameters and procedural application to remain undefined.

Objective: The current study aimed to comparatively investigate the effects of tDCS and tACS applied to the occipital nerve (ON), targeting the locus coeruleus, on associative memory performance.

Methods: We employed a randomized, double-blind, two-visit, active-controlled study design. 85 cognitively normal adults were assigned to receive either active ON-tDCS, 40 Hz ON-tACS, sham ON-tDCS, or 1 Hz ON-tACS during encoding of a 50-word Swahili-English associative memory recall task. To evaluate the effects of electrical stimulation, we measured the cumulative rate of learning on Day 1 and to assess possible long-term effects, we measured the number of words recalled on Day 7.

Results: Results presented two notable findings: (1) participants who received 40 Hz ON-tACS learned significantly more words on Day 1 ($F_{3,81} = 4.37$, $p = .007$, $\eta^2 = 0.14$), and (2) participants who received 40 Hz ON-tACS or active ON-tDCS recalled significantly more words on Day 7 ($F_{3,81} = 11.08$, $p < .001$, $\eta^2 = 0.29$).

Conclusions: The evidence from this study alludes to 40 Hz ON-tACS and active ON-tDCS inducing distinct behavioral effects, whereby 40 Hz ON-tACS generated an effect during memory encoding via enhanced attention, however, active ON-tDCS elicited an offline effect transpiring during consolidation. Further neuroimaging studies are needed to validate these findings and proposed mechanisms of action.

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1. Introduction

Associative learning and memory refer to the ability to associate two items, thus establishing a link which in turn constructs a declarative memory – a long-term memory that associates experiences, objects, and words through spatial, temporal, or other

context relationships [1,2]. Daily life is highly dependent upon associative memory, such that it enables remembering a name to a face or a word to its meaning. Therefore, deficits in associative memory, such as those seen in patients of Alzheimer's disease (AD), can have critical implications that substantially affect routine, normal life tasks [3]. In an effort to delay or prevent such deficits, experimental and clinical fields have reintroduced transcranial direct current stimulation (tDCS) and transcranial alternating current stimulation (tACS) as a tool to modify neuroplasticity to induce neurocognitive enrichment [4–7].

tDCS and tACS have risen to the forefront of neuromodulatory interventions given their advantageous characteristics, such as

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their noninvasive, highly tolerable approach, convenience, low cost, detection of cognitive enhancement, and lack of serious adverse events [8,9]. The current understanding of tDCS' and tACS' mechanisms of action suggest that technical differences exist in their delivery and modulation of the brain (for mechanistic overview, see [10,11]), however, they both facilitate the extraction of causal links between brain modulation via transcranial electrical stimulation (tES) and the obtained behavioral effects [12]. However, a lack of direct comparison studies of the two techniques has hindered our understanding of which tES technique is most effective in generating beneficial improvement for associative memory.

Declarative (associative) memory is measured in experimental settings via tasks assessing recognition/familiarity or recall stimuli such as faces, locations, and words. Only one prior study has directly compared the effects of high definition (HD)-tDCS and theta band HD-tACS applied to the right fusiform cortex in healthy individuals during a visual memory task to assess which technique best enhanced associative memory performance [13]. Theta band HD-tACS generated improved associative memory performance in identifying correct picture-pairs immediately after learning, yet prolonged effects of stimulation were not seen in either condition 24-h post testing [13]. However, it should be noted that the study explicitly included HD-tACS to more focally target the fusiform cortex due to its well-recognized function in facial encoding [14], as well as theta band frequency for its known improvement in visual memory task performance [15], suggesting the effects may be specific to facial associative memory.

Alternatively, the present study incorporated a highly divergent study design regarding stimulation protocol, electrode location, and task type, to directly compare the behavioral effects generated by tDCS and tACS (an active and control for each: active tDCS, sham tDCS, 40 Hz tACS, 1 Hz tACS) when applied to the greater occipital nerve (ON) targeting the locus coeruleus (LC) while learning an associative memory task – replicating previous laboratory parameters strategic placement of electrodes on the left and right C2 nerve dermatomes [16,17] in an effort to polarize the regions [18]. In a series of experiments, we demonstrated ON-tDCS' viability to upregulate the locus coeruleus noradrenaline (LC-NA) pathway to increase LC and hippocampus connectivity and improve associative memory recall in young [17] and older [17] healthy participants. These experiments highlight our proposal of how specific afferent activity can modulate noradrenergic (NAc) neurons via direct projections from the ON to the nucleus tractus solitarius (NTS) [19]. In response, NTS neurons influence central NAc activity through direct synapses on neurons in the LC [19]. Additionally, the LC-NA system is known to be a significant contributor to the signal transduction and synaptic plasticity required for the LC's dual involvement in behaviors and cognition, primarily attention, learning, and memory [20]. NA release throughout the cortex, in addition to increased levels of dopamine in both the prefrontal cortex and hippocampus [21,22], further suggests a strong modulatory influence on synaptic plasticity [23,24], thereby playing a crucial role in memory formation [25].

Our present research stimulated the ON, a lesser-known peripheral pathway that is capable of having similar effects to those of the vagus nerve [26], such as influencing bottom-up regulations of cortical gain, arousal, and neurobiological responses to environmental stimuli via the LC-NA system [20,27]. Despite vagus nerve stimulation's capability to induce task-specific plasticity [28,29], the ON is a more optimized stimulation target for our research due to its numerous branches innervating the posterior occiput up to the vertex of the scalp [30], thus facilitating easier accessibility via non-invasive stimulation [31].

Although electrical stimulation of the scalp modulating the excitability of neurons directly is currently debated, there is a

growing body of evidence that recognizes the potential of neuro-physiological effects of tES primarily being caused by transcutaneous stimulation of peripheral nerves [32–34]. Moreover, a substantial finding indicated that only 25 (up to 50) percent of applied current reaches the brain due to the skull's high electrical resistance [34], thus suggesting tES affecting neural circuits indirectly via peripheral nerves holds merit and should be further explored in experimental designs.

Modulation of associative memory may benefit from an alternative stimulation from tDCS, such that current tACS paradigms hypothesize that tACS' frequency may be applied to amplify power and entrain endogenous brain rhythms to improve communication and process incoming information and thus elicit advantageous effects [11,35]. More specifically, gamma band tACS is most commonly employed due to its involvement in attention and long-term memory processes [36,37], as well as it being the frequency band that corresponds to AD progression [38]. This rationale is broadly supported by recent research exhibiting significant effects of tACS when gamma tACS (>40 Hz) applied to the left prefrontal cortex during both encoding and recognition/retrieval enhanced declarative memory performance of a word learning task [39,40], analogous to our previous work demonstrating ON-tDCS improving memory of an associative word learning task.

Given that tACS and tDCS protocols have demonstrated successful improvements of associative memory, and both techniques have the potential to modulate LC-NAc activity, a key contributor of neuroplasticity and memory processes [41,42], via the ON, we directly compared which stimulation technique generated greater associative memory performance enhancements. To investigate the behavioral effects of ON-tACS and ON-tDCS, we measured the cumulative rate of learning on Day 1 and, to assess possible long-term effects, we measured the number of words recalled on Day 7, thus allowing us to observe if behavioral outcome differences exist between these two techniques. The present study hypothesizes 40 Hz ON-tACS and active ON-tDCS will enhance memory recall in comparison to the control stimulations on Day 7. Moreover, we hypothesize 40 Hz ON-tACS will have an online effect during the encoding of new information while active ON-tDCS will have an offline effect during the consolidation process. This study provides valuable evidence for subsequent paradigm construction to enhance learning and memory.

2. Methods

2.1. Design

The study was designed as a randomized, double-blinded, two-visit, active-controlled study aimed to directly compare the effects of ON-tACS and ON-tDCS on associative memory recall performance. This study was in accordance with the ethical standards of the Helsinki declaration (1964) and was approved by the Institutional Review Board of the University of Texas at Dallas.

2.2. Participants

Participants were 85 healthy, native-English speaking adults (49 females; mean age of 21.56 years, $Sd = 2.11$ years) with a similar undergraduate background in education who all had the maximum score on the Mini Mental State Examination [43] as well as normal or corrected-to-normal vision. Participants were screened over the phone (e.g., handedness, tES contraindications, neurological impairments, not participated in a tES study) prior to enrolling into the study. None of the participants had a history of major psychiatric or neurological disorders, or any tES contraindications, including previous history of brain injuries or epileptic insults,

cardiovascular abnormalities, implanted devices, taking neuropsychiatric medications, or prescribed stimulants use, or chronic use of illicit drugs.

If screening discovered that participants were familiar with Swahili/Arabic language or Swahili culture, then the participant was excluded from the study due to the nature of the stimuli. Furthermore, study instructions were emailed to the participants to ensure that they abstain from alcoholic beverages, and energy or caffeinated beverages for 24-h prior to the scheduled study session. Additionally, participants were asked to withhold from using any hair styling products (e.g., hair gel, hair spray ...) the day of the study. To assure the highest levels of accuracy for saliva collection, participants were asked to refrain from the following products or activities for the associated time window prior to saliva collection: dental work for at least 48-h, major meals for 60-min, brushing their teeth for 45 min, as well as water or rinsing their mouth for 10-min in order to avoid any risk of lowering pH levels and influencing bacterial growth. Participants were also asked to refrain from taking any nonapproved prescription drugs, steroidal/anti-inflammatory drugs and were also asked to avoid foods high in sugar content or acidity, and nicotine consumption. Lastly, if participants were scheduled for a study in the afternoon, they were requested to avoid taking a nap during the day. Participants provided written, informed consent on the day of the study session and received course credit for their contribution to the study.

2.3. Word association task

All participants underwent a computerized, word association task consisting of Swahili-English vocabulary learning that was adapted from a well-established study design of Karpicke and Roediger [44]. The 50 Swahili-English word pairs were taken from the Nelson and Dunlosky study [45], excluding the word pair rafiki-friend, as this word is also the name of a character in *The Lion King* and therefore could have been greatly familiar to participants. The task was programmed in Visual Studio software using C# and shown on a computer with a ~68.58 cm screen positioned at eye level.

Participants had six alternating study and test periods to learn the list of 50 Swahili-English (e.g., Swahili: tumbili, English: monkey) vocabulary word pairs made up of common day-to-day words. The verbal paired-association memory task was divided into 3 blocks with each block consisting of a study period (S), followed by a 30-s rest period, and a test period (T).

Participants studied and were tested on the exhaustive list of 50-word pairs in block 1. Whereas in blocks 2 and 3, the comprehensive list of 50 words were studied in each study period, but only items that had not yet been recalled were tested in the test periods (denoted SDT_N, where T_N indicates that only the non-recalled pairs were repeatedly tested). Therefore, in blocks 2 and 3, the number of word pairs tested diminished across the blocks and varied according to the test condition. This paradigm was used to ensure that all participants would avoid a ceiling effect [44].

During the study phase, each word pair (black words on white background) was presented one below the other in the middle of the screen for 5 s to provide adequate time for encoding. Participants were instructed to learn as many word pairs as they could, so they may recall the English word when given the Swahili word. During the test phase, participants were instructed to type in the correct English translation of the Swahili word that was presented for 8 s using a computer keyboard. Once the 8 s expired, the computer program would automatically advance to the next Swahili word regardless of whether the participant had entered a

response. Participants' responses, as well as their reaction times, were recorded by the computer program. The word pairs sequence was randomized between blocks, conditions, and participants. See Fig. 1 for design overview

2.4. ON-tDCS and ON-tACS

Alternate current (AC) and direct current (DC) were transmitted via a saline-soaked (1.3% saline) pair of synthetic sponges (5 cm × 7 cm) and were delivered by a specially developed, battery-driven, constant or alternating current stimulator with a maximum output of 10 mA (Eldith®; <http://www.neuroconn.de>). A fully automated Realistic Volumetric-Approach to Simulate Transcranial (ROAST <https://www.parralab.org/resources.html>) [46] electric simulation pipeline was used to depict the electrode placement and computational modeling of the resultant electrical field of the stimulation targeting the LC via the ON. ROAST is a Matlab based toolbox that combines the segmentation algorithm of SPM12 and automatic electrode placement, the finite element mesh iso2-mesh and the solver getDP [46]. Based upon previous laboratory evidence, whereby the target electrode on the left and right facilitated active stimulation, respectively, exhibited no significant difference on memory recollection between active positioning [17]. Therefore, in the present study, we elected each participant receiving ON-tDCS to have the anodal electrode, acting as the target stimulation, placed over the left C2 nerve dermatome and the cathodal electrode, acting as the reference, placed over the right C2 nerve dermatome. The electric fields and voltage maps for stimulation are displayed in Fig. 2 (model based on tDCS). The electric field describes the direction of the direct current and areas it passes through, whereas the voltage map describes the polarity of the stimulation, and the electrode montage describes the location of the electrodes of the stimulation on a human head model. The same electrode placement was used for participants receiving ON-tACS, however, due to its nature of stimulation each electrode functioned as the anode/cathode electrode respectively [47]. In order to maintain consistency across all participants, research assistants were trained to map out the placement according to the length of the participant's head. To minimize skin sensations and to acclimate participants to the stimulation types, the current intensity was ramped-up (gradually increasing) until it reached its programmed maximum output for the duration of each study period, followed by a ramp-down (gradually decreasing) denoting the end of the study period. The impedance under each electrode was maintained under 10 kΩ.

Active ON-tDCS was applied to the left C2 dermatome at a constant current of 1.5 mA (current density 42.28 $\mu\text{A}/\text{cm}^2$) during each of the 3 study blocks, resulting in a total stimulation time of 12.5 min (i.e., 250 sec × 3 blocks). Sham ON-tDCS was applied to the left C2 dermatome at a constant current of 1.5 mA (current density 42.28 $\mu\text{A}/\text{cm}^2$) during each of the 3 study blocks, resulting in a total stimulation time of 45 s (i.e., 15 sec × 3 blocks). The rationale behind the sham procedure was to mimic the transient skin sensation at the beginning of active ON-tDCS without producing any conditioning effects on the brain. ON-tACS was applied using a current of ± 1.5 mA peak intensity during each of the 3 study blocks (3 mA peak to peak; current density 21.43 $\mu\text{A}/\text{cm}^2$). The two groups receiving AC stimulation differed in the frequency (Hz) of tACS. The stimulation was phasic with a sinusoidal waveform. One group received stimulation delivered at a frequency of 40 Hz with 0° phase difference. The second group, active-control, received stimulation delivered at a frequency of 1 Hz with 0° phase

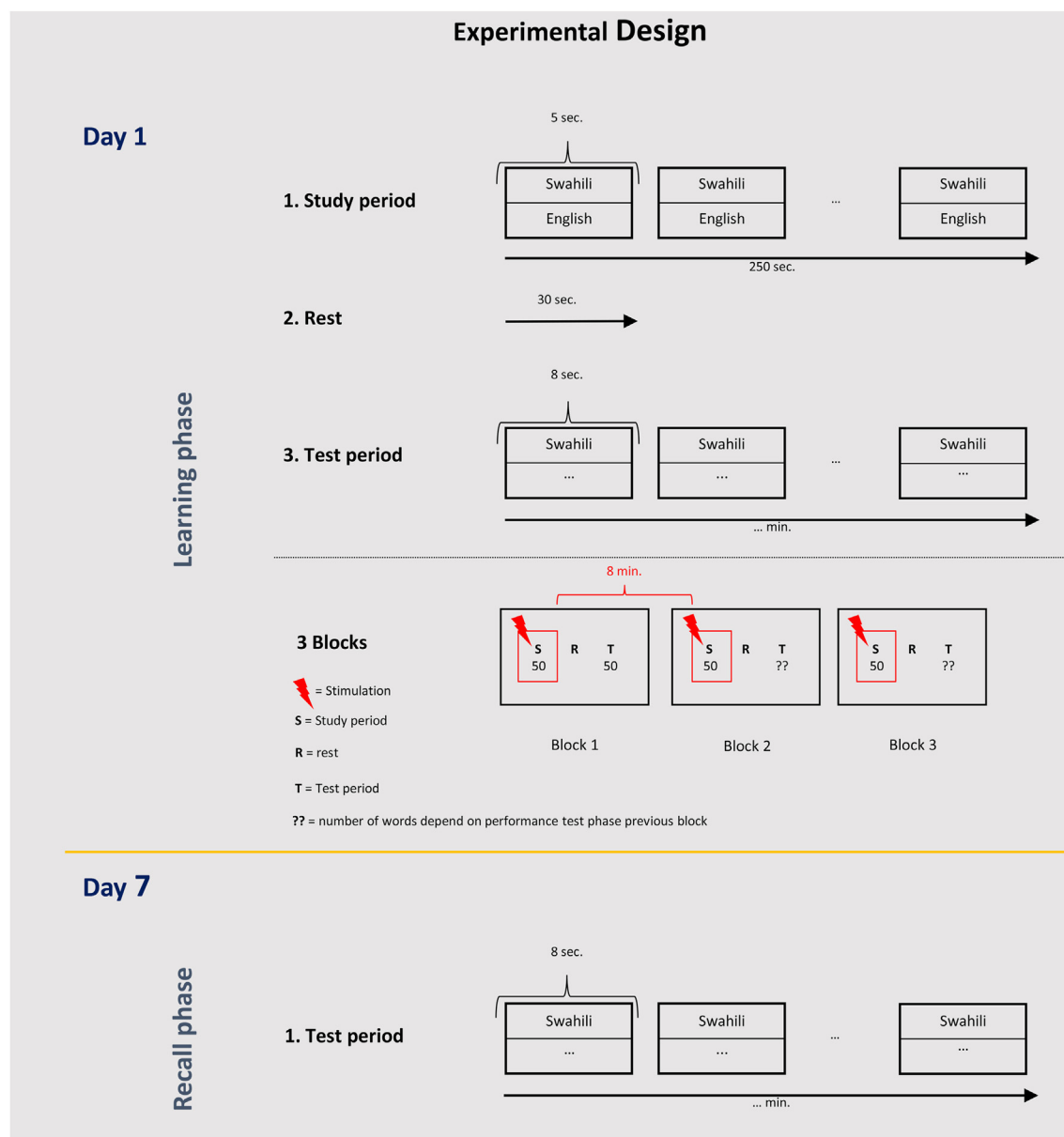


Fig. 1. Schematic of experimental design

Diagram of the word-association memory task undertaken by participants while receiving either 40 Hz ON-tACS, 1 Hz ON-tDCS, active ON-tDCS, or sham ON-tDCS during the study phase.

difference. The frequency was fixed for the duration of 12.5 min in both groups (250 sec × 3 blocks).

2.5. Saliva collection

Salivary α amylase, a marker of endogenous NA activity, was used to test the effect of ON-tES on the LC-NA pathway. Participants' saliva was collected two times during the experiment: before and after stimulation. When the participants were ready to collect saliva, they were requested to gently tip their head backwards and collect saliva on the floor of their mouth and when ready, passively drool into the mouthpiece of the tube provided by Salimetrics. The participants were requested to collect 2 ml of saliva in one straight flow and avoid breaks between drool as much as possible. The length of time to collect 2 ml of saliva was noted and the timer was

started only when participants began to passively drool into the tube. All saliva samples were stored in 2 ml cryovials and immediately stored in a -80°C laboratory freezer. Due to a technique problem, a batch was lost. Upon completion of the collection procedures, a total of 132 saliva samples from 66 participants (24 active ON-tDCS, 21 sham ON-tDCS, 10 40 Hz ON-tDCS, and 11 1 Hz ON-tACS) were packed in dry ice and sent to the Salimetrics laboratory for analysis. The Salimetrics analysis protocols and determination techniques for the targeted biomarker are described below.

The flow rate was calculated using the formula given by Salimetrics: $\text{rate}\left(\frac{\text{ml}}{\text{min}}\right) = \frac{\text{amount of saliva (ml)}}{\text{time (min)}}$. This flow rate correction was used in the calculation of concentration of salivary α -amylase. Furthermore, the tubes were also weighed; the weight of the saliva was determined as the difference between the weights of the full tube and the empty tube. The amount of saliva α amylase in the

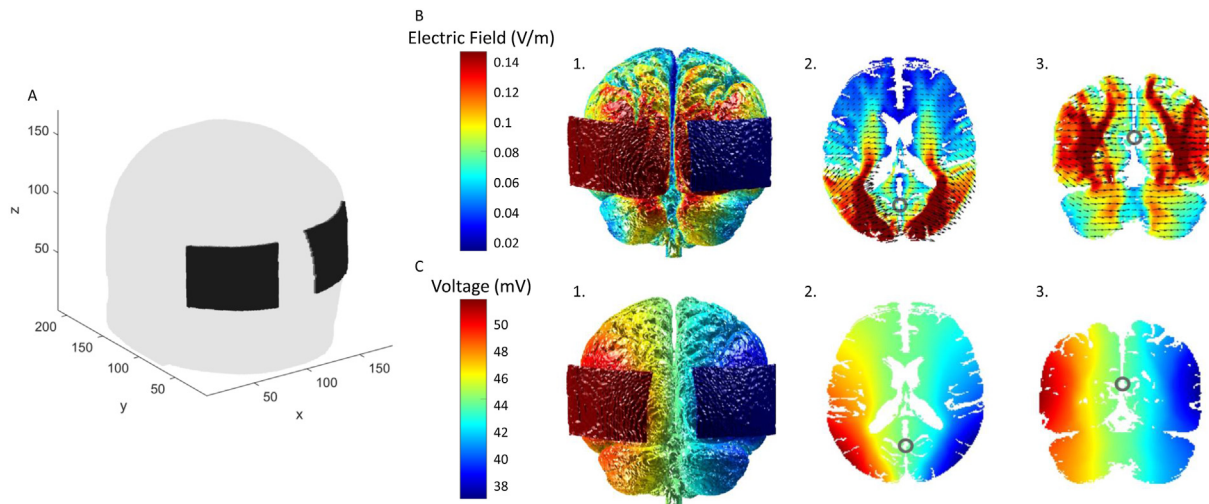


Fig. 2. Electrode montage and resultant E field

A: Simulation model from ROAST targeting the LC via the ON whereby the anodal electrode, acting as the target stimulation, was placed over the left C2 nerve dermatome and the cathodal electrode, acting as the reference, was placed over the right C2 dermatome for stimulation intensity at 1.5 mA. **B:** The upper row demonstrates the electrical field (1) distributed throughout the brain with the electrode montage represented (2) from the Axial plane (3) and from the Coronal plane. **C:** The lower row demonstrates the voltage map (1) throughout the brain with the electrode montage represented (2) from the Axial plane (3) and from the Coronal plane. This model is based on direct current stimulation.

sample was directly proportional to the increase in absorbance at 405 nm. 10 μ l of the sample was diluted and well mixed. 8 μ l of the diluted samples were then pipetted into individual wells of 96-well microtiter plate. A volume of 320 μ l of preheated chromogenic substrate solution was added to each well and the plate was rotated at 500–600 RPM at 37°C for 3 min. Optical density of the sample is determined at the 1-min mark and again at the 3-min mark.

2.6. Visual analog scale

To measure the alertness of the participant, a visual analog scale was used before and after the ON-tES procedure. The VAS measured alertness using a subjective, continuous ~24.4 cm line.

2.7. tES exit questionnaire

To assess for possible side effects of ON-tES, the participants were asked to complete the tES Exit Questionnaire [48] after the ON-tES procedure. The tES Exit Questionnaire measures symptoms: headache, neck pain, scalp pain, tingling, itching, sleepiness, trouble concentrating, and acute mood changes on a 4-point scale, ranging from 1 = 'absent' to 4 = 'severe'. If the participant had indicated any symptoms present, they were then asked to specify on a 5-point scale, ranging from 1 = 'none' to 5 = 'definitely', to determine if they thought this symptom was related to the application of tES. Lastly, to determine if the stimulation was well blinded, the participants were asked to guess if they thought they were placed in the active or control group.

2.8. Procedure

Eligible participants were scheduled for two visits to complete the study. During the study (S) period on Day 1, 25 participants received active ON-tDCS, 25 participants received sham ON-tDCS, 24 participants received 40 Hz ON-tACS, and 11 participants received 1 Hz ON-tACS. Visit 1 consisted of pre-assessments (i.e., MMSE, VAS, saliva collection) followed by the word association task paired with ON-tES. Participants were informed of the randomized allocation into one of four groups consisting of either active or

control protocols during the study period: participants either received active ON-tDCS, sham ON-tDCS, 40 Hz ON-tACS, or 1 Hz ON-tACS. The researcher who controlled the tES device was not involved in instructing the participant; this was performed by a second researcher who was blind to the stimulation protocol and not in the room during the stimulation. Visit 1 concluded with post ON-tES questionnaires (saliva collection, VAS, tES Exit Questionnaire). Participants were asked to refrain from studying or searching for the word pairs learned throughout the week. Participants returned 7-days after their first visit for memory testing to measure possible long-term effects on associative memory performance, but did not receive ON-tES, nor were they able to review word-pairs. A third researcher who was not responsible for the task or ON-tES conducted the second visit.

2.9. Statistical analysis

1. Primary outcome measures.

For Day 1, a comparison was made between the four stimulation groups (active ON-tDCS vs. sham ON-tDCS vs. 40 Hz ON-tACS vs. 1 Hz ON-tACS) on the cumulative percentage of correctly recalled words (i.e., learning rate) using a one-way analysis of variance (ANOVA) with group as the between-subjects variable and the percentage of correctly recalled words after Block 3 was used as the within-subjects variable.

For Day 7, a comparison was made between the four stimulation groups (active ON-tDCS vs. sham ON-tDCS vs. 40 Hz ON-tACS vs. 1 Hz ON-tACS) on how many words participants correctly recalled using a one-way ANOVA. In this analysis, groups were the between-subjects variable and the percentage of correctly recalled words (corrected for how many words they learned on Day 1) was used as the dependent variable. Additionally, a one-way ANOVA was run to assess the percentage of forgotten words from Day 1 to Day 7, whereby groups were the between-subjects variable and the subtraction of forgotten words was used as the dependent variable. For all analyses, if significance was obtained, a Tukey's HSD test was applied post hoc to detect group differences.

Moreover, a repeated measures ANOVA was run to test the effect of ON-tES on the LC-NA pathway, whereby the four stimulation groups (active ON-tDCS vs. sham ON-tDCS vs. 40 Hz ON-tACS vs. 1 Hz ON-tACS) were the between-subjects variable and salivary α amylase (before and after stimulation) was used as the within-subjects variables. A simple contrast analysis was applied to compare the difference conditions using a Bonferroni correction.

2. Secondary outcome measures.

A repeated measures ANOVA was used to analyze the effect of ON-tES on alertness (VAS) with group (active ON-tDCS vs. sham ON-tDCS vs. 40 Hz ON-tACS vs. 1 Hz ON-tACS) as the between-subject variables and VAS (pre-vs. post-measurement) as the within-subjects variable.

A multivariate analysis of variance (MANOVA) was used to assess the differences between scores on side effects marked on the tES exit questionnaire between active ON-tDCS, sham ON-tDCS, 40 Hz ON-tACS, or 1 Hz ON-tACS groups. Following, a chi-square analysis was run to assess if participants in the four stimulation groups were well blinded during the stimulation session. Statistical analysis was performed using IBM SPSS (version 24) software.

3. Results

3.1. Word association task

1. Session on Day 1: learning the word-association task.

To compare the cumulative learning rate (%) amongst the four stimulation groups on Day 1, a one-way ANOVA was utilized. Results yielded a significant difference in the cumulative number of words learned ($F_{3,81} = 4.37, p = .007, \eta^2 = 0.14$; Fig. 3a). A post hoc analysis using Tukey's HSD test was applied for closer inspection. Results revealed that participants who received 40 Hz ON-tACS learned more words ($M = 82.24, Sd = 16.39$) in comparison to participants who received 1 Hz ON-tACS ($M = 59.82, Sd = 22.71$), active ON-tDCS ($M = 66.03, Sd = 24.22$), and sham ON-tDCS ($M = 66.08, Sd = 19.99$). Accordingly, 40 Hz ON-tACS was highly statistically significantly different than 1 Hz ON-tACS ($p = .01$), active ON-tDCS ($p = .03$), and sham ON-tDCS ($p = .02$). The

percentage of words learned during the study phases in the 1 Hz ON-tACS, active ON-tDCS, and sham ON-tDCS groups did not differ significantly.

2. Session on Day 7: recall.

To assess the difference between the four stimulation groups on associative memory recall 7-days after initial learning, a one-way ANOVA was used. Results indicated that the effect of stimulation group significantly influenced the numbers of words recalled ($F_{3,81} = 11.08, p < .001, \eta^2 = 0.29$; Fig. 3b). A post hoc Tukey's HSD test further revealed active ON-tDCS ($M = 51.47, Sd = 15.79$) was highly significantly superior to sham ON-tDCS ($M = 25.75, Sd = 15.42$) ($p < .001$) and 1 Hz ON-tACS ($M = 28.64, Sd = 15.19$) ($p = .004$), but not significantly different from 40 Hz ON-tACS ($M = 46.15, Sd = 22.25$) ($p = .72$). In addition, 40 Hz ON-tACS was statistically significantly more effective than 1 Hz ON-tACS ($p = .04$) and sham ON-tDCS ($p = .001$), but not significantly different from active ON-tDCS. Moreover, a one-way ANOVA reported a significant difference when comparing stimulation groups and the percentages of words forgotten from Day 1 to Day 7 ($F_{3,81} = 4.11, p = .009, \eta^2 = 0.13$; Fig. 3c). A Tukey's HSD post hoc analysis revealed participants who received active ON-tDCS forgot significantly fewer words than those who received 40 Hz ON-tACS ($M = 36.09, Sd = 30.33$) ($p = .04$) and sham ON-tDCS ($M = 40.33, Sd = 24.70$) ($p = .009$).

3.2. Saliva collection

A repeated-measures ANOVA revealed an interaction effect between stimulation groups (active ON-tDCS vs. sham ON-tDCS vs. 40 Hz ON-tACS vs. 1 Hz ON-tACS) and salivary α amylase (before and after stimulation) ($F_{3,62} = 2.98, p = .038, \eta^2 = 0.13$; Fig. 4). A simple contrast analysis revealed a significant effect for both active ON-tDCS ($F_{1,62} = 19.32, p < .001, \eta^2 = 0.23$) and 40 Hz ON-tACS ($F_{1,62} = 5.71, p = .02, \eta^2 = 0.08$) indicating an increase in salivary α amylase after stimulation in comparison to before stimulation. No effect was obtained for sham ON-tDCS ($F_{1,62} = 0.71, p = .55, \eta^2 = 0.033$) and 1 Hz ON-tACS ($F_{1,62} = 0.17, p = .68, \eta^2 = 0.003$). A direct comparison between active ON-tDCS and 40 Hz ON-tACS revealed no effect ($F_{1,32} = 0.11, p = .71, \eta^2 = 0.003$).

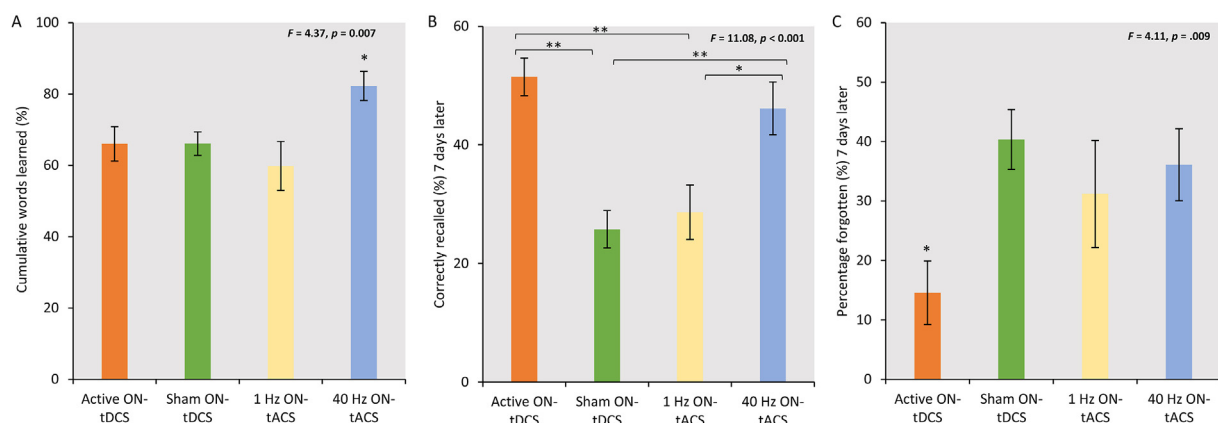


Fig. 3. Behavior effects of ON-tDCS and ON-tACS

Correctly recalled/learned curve of the percentage of words learned **A**: cumulative words learned after Block 3 on Day 1 for each of the stimulation groups. **B**: A comparison on how many words participants correctly recalled during Day 7 between participants that received either 40 Hz ON-tACS, 1 Hz ON-tDCS, active ON-tDCS, or sham ON-tDCS based on total number of words-pairs learned during Day 1. **C**: A comparison on how many words participants forgot during Day 7 between participants that received either 40 Hz ON-tACS, 1 Hz ON-tDCS, ACTIVE ON-tDCS, or sham ON-tDCS based on total number of word-pairs learned during Day 1. Error bars represent standard errors of the mean. * p -value < 0.05 , ** p -value < 0.01 , *** p -value < 0.001 .

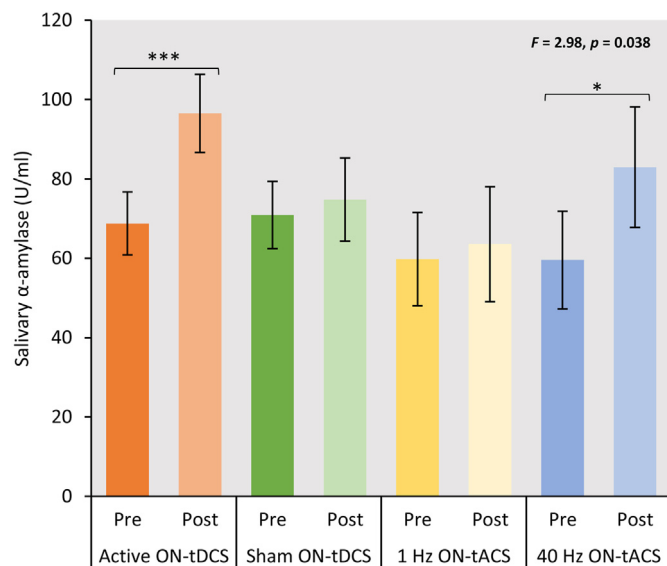


Fig. 4. Salivary α amylase

Saliva α amylase analysis of the four stimulation groups (40 Hz ON-tACS, 1 Hz ON-tDCS, active ON-tDCS, or sham ON-tDCS) to test the effect of ON-tES on the LC-NA pathway. Error bars represent standard errors of the mean. * p -value < 0.05, ** p -value < 0.01, *** p < 0.001.

3.3. Alertness

A repeated-measures ANOVA detected a non-significant interaction effect between VAS – Alert (pre- and post-measurement) and stimulation group (active ON-tDCS, 40 Hz ON-tACS, 1 Hz ON-tACS, and sham ON-tDCS) ($F_{3,81} = 0.59$, $p = .62$, $\eta^2 = 0.02$; Fig. 5a), indicative of participants' alertness not changing because of stimulation application.

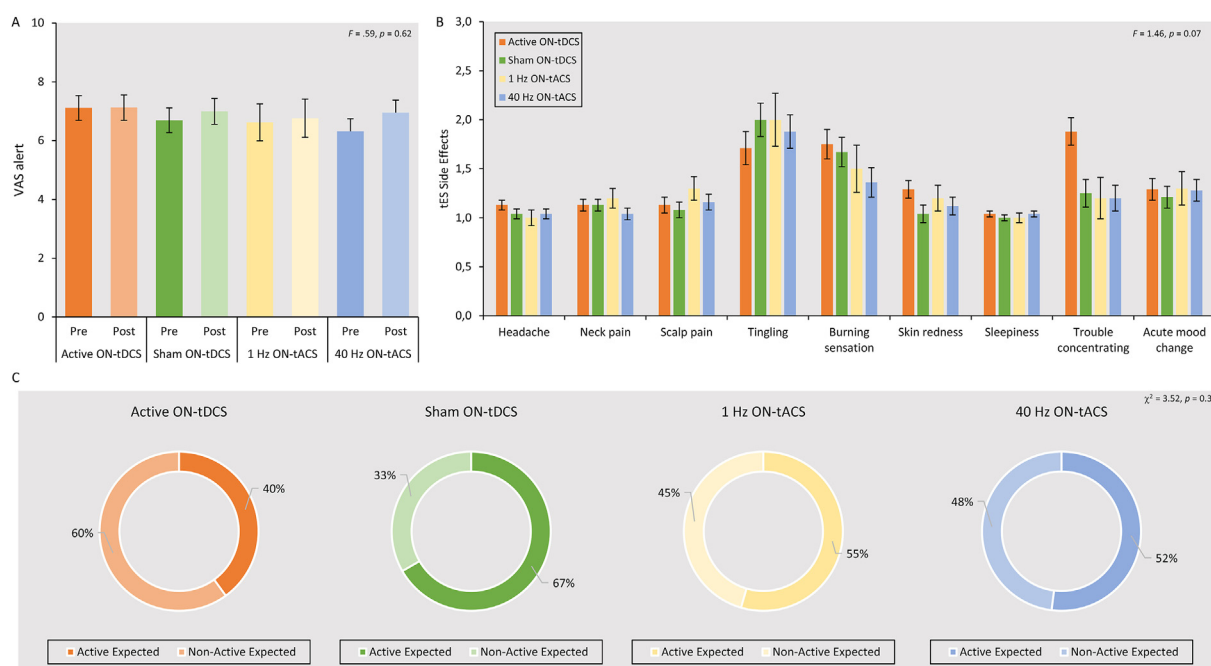


Fig. 5. ON-tES related side effects & Blinding

Analysis pertaining to the four stimulation groups (40 Hz ON-tACS, 1 Hz ON-tDCS, active ON-tDCS, or sham ON-tDCS) are represented in the **A**: bar chart displaying the pre-VAS vs post-VAS scores, **B**: the bar chart depicting the mean scores on different tES related side effects experienced, error bars represent standard errors of the mean, and **C**: the pie charts illustrating the participants' guess of receiving active or non-active stimulation.

3.4. tES exit questionnaire

Using Pillai's Trace, a MANOVA revealed a non-significant main effect on stimulation group for the various tES side effects ($F_{30, 216} = 1.41$, $p = .09$, $\eta^2 = 0.16$; Fig. 5b). All stimulation sessions were well tolerated, and no major stimulation related complications were noted. However, after closer inspection, a one-way ANOVA revealed a significant difference amongst the participant-reported levels of trouble concentrating between stimulation groups ($F_{3,81} = 5.04$, $p = .003$, $\eta^2 = 0.157$). A Tukey's HSD post hoc analysis revealed participants who received active ON-tDCS ($M = 1.92$, $Sd = 0.86$) reported greater difficulty concentrating compared to those who received 40 Hz ON-tACS ($M = 1.20$, $Sd = 0.50$) ($p = .004$) and sham ON-tDCS ($M = 1.25$, $Sd = 0.68$) ($p = .01$), but not those who received 1 Hz ON-tACS ($M = 1.45$, $Sd = 0.93$) ($p = .29$).

3.5. Stimulation condition blindness

A χ^2 analysis performed found no significant relation between stimulation administered and the participant's assumed stimulation they received ($\chi^2(1) = 3.52$, $p = .32$; Fig. 5c), thus suggesting that participants were well-blinded.

4. Discussion

Our study aimed to compare the effects of 40 Hz ON-tACS, active ON-tDCS, and their control counterparts during a verbal associative memory task. Interestingly, only 40 Hz ON-tACS enhanced participants' cumulative learning rate on Day 1 in comparison to the other three groups. The learning rates for 1 Hz ON-tACS, active ON-tDCS, and sham ON-tDCS were similar. Furthermore, participants who received active ON-tDCS or 40 Hz ON-tACS during visit one displayed increased memory recall 7-days after initial learning in comparison to those who received sham ON-tDCS and 1 Hz ON-tACS. Although the amount of correctly recalled words did not

differ between the active ON-tDCS and 40 Hz ON-tACS groups, we do observe that participants assigned to the 40 Hz ON-tACS group forgot significantly more words in comparison to the active ON-tDCS group. These findings provide support for the hypothesis that active ON-tDCS and 40 Hz ON-tACS induce distinct behavioral effects, whereby 40 Hz ON-tACS manifested the greater effect on Day 1, online during encoding with a lesser effect seen offline on Day 7, whereas active ON-tDCS elicited an effect offline during consolidation thereby exhibiting a more influential impact on Day 7 during recall.

The present study provides a replication of ON-tDCS having no direct effect on learning, but does have an effect on memory being observed on Day 7, further depicting active ON-tDCS' viability for upregulating the LC-NA pathway to modulate memory, and corroborating that the effect transpires offline, during consolidation [16,17]. Despite no overall effect of tES side effects in the current study, we note that participants who received active ON-tDCS reported difficulty concentrating when this variable was taken separately. Although this is an unusual observation that has not been seen in our previous studies, we acknowledge that the improvement in word recall may be due to increased effort invested during learning and thus advise future studies to take into consideration. Nevertheless, considering this is the third replication of these results, we presume that our findings suggest the effects of active ON-tDCS occur and influence neuronal plasticity beyond the stimulation timeframe, thus potentially giving rise to a novel channel of stimulation via the LC, which may be exploited to foster initial (cellular) memory consolidation.

At a cellular level, tDCS has been deemed to potentially modify the long-term potentiation (LTP) essential for memory stabilization, thus gaining momentum as an explanation of cognitive enhancement when tasks are aided by tDCS [49,50]. We hypothesize that active ON-tDCS' neurophysiological mechanism reflects the synaptic tag and capture (STC) hypothesis, thus behavioral effects of active ON-tDCS during an associative memory task mimics the behavioral tagging (BT) hypothesis, such that a learning event within a short time period of an additional independent novel event or stimuli will be more likely to convert to long-term memory [51,52]. Recent investigations of STC/BT hypotheses revealed evidence for LC-driven discharge of noradrenaline and dopamine being fundamental for the formation of novelty-induced memories [51], ascribable to our non-invasive approach designed to elevate the novelty response of the LC. Moreover, transforming early-LTP to late-LTP via similar effects of electrical stimulation has been identified in other brain regions, such as the basolateral amygdala [53,54], thus supporting future investigations continue to employ tDCS as a tool to ameliorate impaired synaptic plasticity to improve learning and memory in neuropsychiatric populations [55,56].

In comparison to ON-tDCS' exclusive memory improvement on Day 7, 40 Hz ON-tACS exhibited memory improvement on Day 1 and Day 7. In contrast, the 1 Hz ON-tACS active-control group produced no memory enhancement on either day, thus suggesting the difference of effects is most likely caused by the significant variance in the administered frequencies. Moreover, lower-intensity frequencies have failed to reliably entrain neural oscillations, thus proposing greater intensities of stimulation are required for immediate and long-term effects [57]. The memory performance effects of 40 Hz ON-tACS seen on Day 7 attest to gamma frequencies' association with long-term memory processes [36,37]. The observed increase in memory recall could be attributed to the STC-BT hypothesis given the similarities in timing of stimulation that resulted in post-encoding consolidation seen on Day 7. However, the two noteworthy findings of (1) 40 Hz ON-tACS learning significantly more words on Day 1 and (2) ON-tACS forgetting

significantly more words from Day 1 to Day 7 when comparing the effects of the two techniques suggest that the effects of ON-tACS are a result of immediate memory benefits owing to a potential enhancement of enhanced attentional mechanisms.

We propose a stimulation pathway upregulating the LC via rhythmic activity in the greater occipital nerve would shift the LC into a phasic state. Given that the LC responds to novel/salient stimuli via phasic activity makes the LC a vital component for alterations in attention and cognitive flexibility needed for learning [58–60]. Existing literature has linked gamma oscillations with focused attention, a requisite for the processing of incoming stimuli and memorization [61–63]. Furthermore, a more specific study has associated gamma tACS with voluntary disengagement and reorienting of attention in young, healthy adults [64], further endorsing the proposal that 40 Hz ON-tACS may induce increased attention via a phasic state. Moreover, LC phasic activity facilitates filtering out irrelevant information and increasing attention, reflective of behaviors amid focused attention tasks [58,65,66]. Furthermore, a recent study indicated a causal link exists between activation of the LC-NA pathway and attention control [67]. An operant test of attention and response inhibition in a mouse model demonstrated stimulation of LC neuronal projections to the ventrolateral orbitofrontal cortex resulted in decreased distractibility, whereas stimulation of LC neuronal projections to the dorsomedial prefrontal cortex subsequently resulted in increased goal-directed attention [67]. Therefore, given the present study's and prior research's suggestive evidence of attention being highly driven by the LC, we propose that participants learned more words on Day 1 in comparison to active ON-tDCS and control groups due to 40 Hz ON-tACS producing enhanced attention when completing the task, thus inferring improving attention will improve learning and memory, thereby offering an alternative approach to boost memory performance. We suggest that future research in the pursuance of tACS application to upregulate gamma activity to improve cognitive performance should be continued.

When considering the present results, it must be considered that some limitations exist within the research and warrant additional investigations. Since the current study explicitly focused on behavioral data, future studies using neuroimaging are required to determine the exact mechanism that explains our behavioral effects shown here. The non-invasive technique used here gives rise to the potential of activating other brain areas (e.g., visual or parietal cortices, or other neuromodulatory nuclei) which could influence behavior [68–70], as opposed to more precise invasive mechanism techniques that directly stimulate the ON. However, a previous rodent study from our laboratory revealed that animals that received direct ON stimulation showed increased inhibitory avoidance and object recognition [17]; thus, indicating that both invasive and non-invasive ON stimulation has a marked effect on memory. Additionally, replication studies with additional EEG analysis and/or fMRI measurements will need to be conducted to further examine the physiological mechanism within brain regions. Given higher-order cognition's strong link to an extended range of communication between different brain regions and gamma frequency's inability to travel long distances, it is feasible for tACS to not only be contingent on oscillatory frequency but also gamma oscillations being driven by a lower frequency in nested oscillations; such replications studies will help establish tACS' interaction between brain regions.

Prior laboratory evidence suggests that the left/right positioning of the anodal/cathodal electrodes generates similar behavioral effects [17]. However, there is an ongoing discussion about the conventional anodal-excitation cathodal-inhibition hypothesis from the motor domain and multiple reports have shown that this does not translate to cognitive studies [71–73]. Therefore, there is still a

need to understand the exact mechanism of action of tDCS in the cognitive domain in general. Moreover, future research could usefully explore how stimulation-induced changes of the LC influence other non-neuronal cells and the inflammation processes related to AD. Recent rodent studies spotlight the use of sensory evoked 40 Hz gamma entrainment [74] to induce synchronization across the forebrain resulting in improved cognitive functions [75,76], as well as preserving neuronal and synaptic density, and exhibiting neuroprotective effects against AD-associated neuropathology and neurodegeneration [75–77]. Discussions of tDCS' potential to affect non-neuronal tissues [78] have also transpired; however, use of tDCS in this manner as well as gamma entrainment to enhance cognition while providing therapeutic relief in humans with or without neurological disorders has yet to be examined.

The importance and originality of this study derive from its comparison and exploration of the use of both non-invasive ON-tDCS and ON-tACS to improve memory performance on a word association task. Overall, these findings help to decipher when to stimulate and with what type of stimulation to induce meaningful effects. The present study identifies 40 Hz ON-tACS having more of an impact when administered during encoding by inducing an immediate, online effect owing to an increase in attention, and application of active ON-tDCS during encoding generating a greater effect offline during memory consolidation that brings about more robust long-term memory processes. This study adds to and will be of interest to the growing body of non-invasive stimulation research aiming to enhance cognitive functions.

Author contributions

Alison M. Luckey: Data curation, Data collection; Formal analysis; Project administration; Software; Writing – original draft; Visualization. **S. Lauren McLeod:** Data curation, Data collection; Writing – review & editing. **Anusha Mohan:** Validation – Formal analysis. **Sven Vanneste:** Conceptualization, Supervision, Methodology.

Funding

Alison Luckey is funded through the Trinity College Dublin Ussher Award.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank D. Pruitt for writing the software program related to the behavioral task, and Y. Huang for contributing to the data collection.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.brs.2022.03.001>.

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